Ataxia-telangiectasia and other Primary Immunodeficiency Diseases

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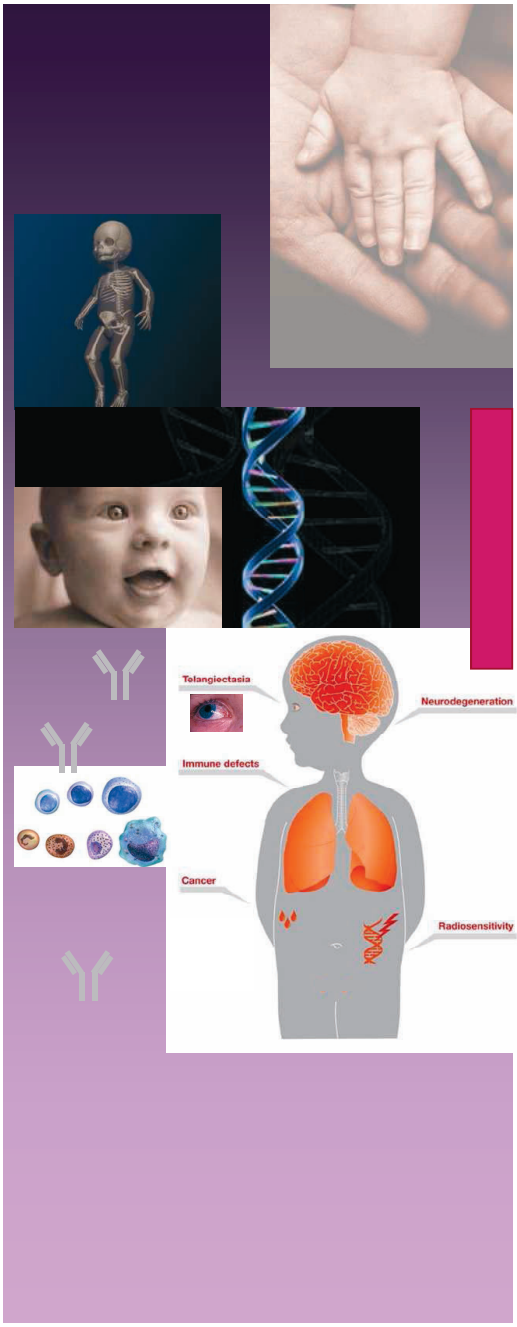

Have faith in the future
Hope for a cure
Love the patients

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To base research on clinical work is to be dependent on others. The studies in paper III, IV, V and VI have been accomplished in collaboration with a variety of professionals from different hospital departments who represent an orchestra of players who allowed themselves to be conducted by the first author. These studies could not have been carried out without help from many others. Thanks to the following people for excellent technical support: Laila Jansen, Anne-Cathrine (Tine) Kristoffersen, Marianne Svendsen, Louise Tunge, Bitte Eriksen, Nina Ørvim and Kari Leone. Thanks to the nurses and secretaries at the pediatric outpatient clinic, Pediatric Department, Rikshospitalet, represented by Heidi Sandersen, Kathleen Halvorsen, Alfhild Bustnes, Kari Gjersum, Ellen Johanne Tung, and Anne Mette Aamodt. Thanks to collaborators at Children Habilitation Unit Hedmark, Center for Rare Disorders Rikshospitalet and Frambu. Thanks to colleagues and collaborators at the Departments of Medical Genetics Rikshospitalet and Ullevål University Hospital for patience and support during the final phase of this thesis. Thanks to Trine Prescott, both a clinical geneticist and a pediatrician, for her valuable comments on the summary.

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List of Papers

Paper I
Stray-Pedersen A, Abrahamsen TG, Froland SS. **Primary immunodeficiency diseases in Norway.** *J Clin Immunol. 2000 Nov;20(6):477-85*

Paper II
Sigstad HM, Stray-Pedersen A, Froland SS. **Coping, quality of life, and hope in adults with primary antibody deficiencies.** *Health Qual Life Outcomes. 2005 May 4;3(1):31.*

Paper III
Stray-Pedersen A, Jonsson T, Heiberg A, Lindman CR, Widing E, Aaberge IS, Borresen-Dale AL, Abrahamsen TG. **The impact of an early truncating founder ATM mutation on immunoglobulins, specific antibodies and lymphocyte populations in ataxia-telangiectasia patients and their parents.** *Clin Exp Immunol. 2004 Jul;137(1):179-86.*

Paper IV
Stray-Pedersen A, Aaberge IS, Fruh A, Abrahamsen TG. **Pneumococcal conjugate vaccine followed by pneumococcal polysaccharide vaccine; immunogenicity in patients with ataxia-telangiectasia.** *Clin Exp Immunol. 2005 Jun;140(3):507-16.*

Paper V
Stray-Pedersen A, Borresen-Dale AL, Paus E, Lindman CR, Burgers T, Abrahamsen TG. **Alpha fetoprotein is increasing with age in Ataxia-Telangiectasia** *Eur J Paediatr Neurol. 2007 May 29*

Paper VI
Myhre AG, Stray-Pedersen A, Spangen S, Eide E, Veimo D, Knappskog PM, Abrahamsen TG, Husebye ES. **Chronic mucocutaneous candidiasis and primary hypothyroidism in two families.** *Eur J Pediatr. 2004 Oct;163(10):604-11.*

Paper VII
Stray-Pedersen A, Rodningen O, Garred P, Heggelund L, Heiberg A, Holmskov U, Lindman CR, Lybæk H, Stoltenberg L, Tvedt B, Van der Hagen CB, Vermeesch J, Aaberge IS, Abrahamsen TG. **Choreoathetosis, developmental delay and severe pulmonary infections due to TITF1 haploinsufficiency and homozygosity for MBL2 variant alleles** *Submitted Eur J Pediatr December 2007 (attached)*

General Introduction

Primary immunodeficiency diseases

Primary immunodeficiency diseases (PIDs) are a heterogeneous group of disorders which give rise to increased susceptibility to infections and/or autoimmunity. Some disorders carry an increased risk of malignancy. As of 2007, more than 150 different PIDs have been characterized, and more will be described during the coming years thanks to the ongoing development of molecular genetic techniques. PIDs are often termed "experiments of nature", because they provide unique and valuable insights into the function of the human immune system. The immunological cells including T- and B lymphocytes, NK cells, neutrophils and monocytes, are white blood cells derived from the bone marrow (Figure 2). PIDs can affect components of the adaptive immune system, i.e. T cells and B cells including antibodies (immunoglobulins). Components of the innate immune system may also be compromised, i.e. phagocytes (neutrophils, monocytes), NK cells, antimicrobial peptides, complement and other pattern recognition receptors such as toll-like receptors and collectins.
PIDs can be classified into eight categories based on immunologic phenotype. A modified version of information presented in the IUIS PID ESID-PAGID reports (1;2) is attached here (Supplement Table 1- 9). For an algorithm for the diagnostic workup of severe combined immunodeficiency (SCID), see Supplement Table 2.

Primary immunodeficiencies and infections

Infections are the hallmark of PIDs: recurrent bacterial infections, prolonged infections that are difficult to treat, severe infections or infections with unusual pathogens.
Other signs include: recurrent abscesses in the skin or internal organs, chronic and recurrent diarrhoea, extensive candidiasis, disseminated infection caused by live vaccines such as BCG.

Types of PID - specific pathogens

The type of infections and microbiological findings are often very characteristic for the different categories of immunodeficiencies. Some pathogens are linked exclusively to specific PIDs. For instance, B cell deficiencies (antibody deficiencies) predispose to *Streptococcus pneumoniae* and *Haemophilus influenzae* ear, nose, throat (ENT) infections and pneumonia. Complement deficiencies are characterised by infections with neisseria including gonococci and with capsular microbes. Phagocyte deficiencies predispose to infections with *Staphylococcus aureus, Aspergillus* and *Pseudomonas aerginosa*. T cell deficiencies result in vulnerability to opportunistic infections with *Pneumocystis jerovici* and *Candida albicans* as well as to fulminant viral infections with CMV and *varicella*. Signs of subtle T cell deficiency are disseminated and persistent warts and molluscs, chronic candidiasis and fungal nail infections (onchomycosis). EBV infections in X-linked lymphoproliferative disease can result in fatal disease, and trigger development of immunodeficiency and lymphoproliferation. Atypical mycobacteria can cause disease in inherited interferon gamma receptor deficiency (IFNGR1), but are rarely if ever otherwise pathogenic. The Sword of Damocles in patients with X-linked Hyper IgM syndrome (CD40 ligand defect) is *Cryptosporidium* infection which causes treatment resistant sclerosing cholangitis and severe liver disease(3). Recognition of genetic defects associated with selective susceptibility to specific pathogens has led to the proposal of a new classification scheme for PIDs on the basis of clinical criteria, and to development of diagnostic protocols for PIDs based on presenting clinical signs (4;5).

Types of PID - typical loci for infection

Site of infection is often very characteristic for specific PIDs. Skin infections and recurrent otitis media are frequent in the phagocytic disorders. Sinopulmonary infections and bronchiectasis are typical of the antibody deficiencies. Osteomyelitis is seen in IFNGR1. Lung abscesses with pneumatocele may complicate Hyper IgE syndrome. Abscesses in internal organs including the liver are a feature of chronic granulomatous disease (CGD). Recurrent infection at the same site, however, warrants the search for a localized structural or functional predisposing factor.

Types of PID - characteristic signs and symptoms

PIDs characteristically present in childhood with persistent, recurrent, antibiotic resistant infections. Failure to thrive and developmental delay are nonspecific, but significant clues to serious infections in young children. Older children and adults may experience involuntary weight loss. Many immunodeficient children develop additional signs such as rashes. Many have associated developmental anomalies of the face, skeletal system, heart, or skin. These manifestations may initially be more prominent than their susceptibility to infection. For some PIDs, non-infection related features are good diagnostic "handles" e.g. delayed separation of the umbilical cord in phagocytic disorders, lack of tonsils in Bruton's agammaglobulinemia, telangiectasias in A-T, microcephaly in certain DNA repair defects, changes in the skin/hair/nails/teeth (ectodermal dysplasia) in NEMO, massive generalized scaling erythrodermia in Omenn syndrome, eczema and bruising in Wiskott Aldrich syndrome (WAS), specific skeletal findings in Schimke immunoosseous dysplasia and Chediak-Higashi syndrome, characteristic endocrinopathy such as hypoparathyroidism plus Addison disease in APECED. In some PIDs the clinical pictures is dominated by autoimmunity or immunodysregulation rather than by infections.

Syndromes in which PID may be a part of the phenotype

PID may be a feature of syndromes in which other characteristic findings dominate the clinical picture, i.e. chronic mucocutaneous candidiasis in APECED, the immunodeficiency in Hoyeraal-Hreidarsson syndrome or the immunodeficiency which accompanies various congenital platelet disorders (6;7). Immunodeficiency, as a component of recognizable syndromes, may be a good diagnostic handle. For example, DNA repair disorders may result in a characteristic pattern of immunodeficiency, and point to the specific defect. Reduced immunity is a feature of some monogenetic syndromes e.g. Smith Lemli Opitz syndrome and some dysmorphic syndromes of unknown etiology e.g., Kabuki syndrome (8;9). Immunodeficiency is frequently reported in patients with chromosomal abnormalities (such as 22q11 deletion (10)and 10p14-p13 deletion in DiGeorge (MIM%601362), 4p deletion (11;12), trisomy 10p (13), Jacobsen syndrome (11q deletion syndrome), 15q duplication and 18q deletion syndrome (14). In some of conditions where immunodeficiency may be a feature, the specifics of the immunodeficiency have not been well characterized, e.g. trisomy 21 (Down syndrome). Even if the immunodeficiency has been characterized, the underlying immunological defect has often not been defined, e.g. males with microduplications which encompass the *MECP2* gene (15).

Age of onset

The age of onset of symptoms may aid in elucidating underlying aetiology. Maternal immunoglobulin is present in infants in the first months of life, and may mask antibody deficiency. If ubiquitous opportunistic pathogens cannot be combated, as in SCID and severe congenital neutropenia (SCN), infections start in the first two months of life. When

immunodeficiency develops later in life, infections start later, as in common variable immunodeficiency (CVID), in which infections may start in adolescence or early adulthood.

Family history

In addition to the patient's medical history and physical examination, the family history may be of great diagnostic importance. The presence of other individuals in the family with possible immunodeficiency, or the occurrence in relatives of similar symptoms and/or death in early childhood due to infection, should raise the suspicion of PID. Consanguinity and/or ethnicity may deserve consideration in the diagnostic work-up in some cases. A considerable proportion of the PIDs demonstrate X-linked inheritance e.g., Bruton's agammaglobulinemia, Wiskott Aldrich syndrome, Hyper IgM syndrome caused by CD40 ligand deficiency, X-linked SCID caused by γc deficiency (IL2RG deficiency), X-linked CGD (gp91phox deficiency), Hoyeraal-Hreidarsson syndrome, NEMO, IPEX.

Incidence and prevalence of PID

Previously, we have had rough estimates of PID patients in Norway. We wanted to describe the true incidence and prevalence of the various PIDs and their regional distribution, in order to improve care and treatment and facilitate better healthcare planning.

Treatment of PID

Lack of awareness of PID delays diagnosis and can lead to chronic organ damage and other complications of recurrent infections. Treatment may prevent infections or lessen their sequelae. Bone marrow transplantation may be curative for some severe PIDs.

The history of Bruton's agammaglobulinemia. Antibiotics, antiviral and antifungal agents

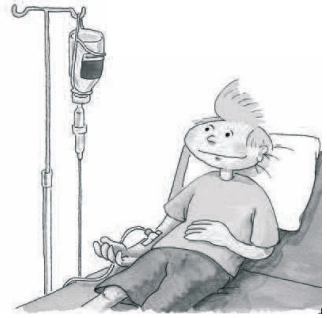
PID as a clinical entity was not discovered until after antibiotics were in widespread use for infections. Patients with an unusual tendency to recurrent infection led physicians to suspect the existence of PID. Antibody deficiency was firstly reported by the physician Colonel Ogden C. Bruton in 1952. Bruton's patient, a four-year-old boy, was first admitted to hospital because of an infected knee. The child recovered well after a course of penicillin, but over the next four years had multiple infections including ten episodes of pneumococccal septicemia. Serum from Bruton's patient was tested with a new laboratory method: electophoresis. Surprisingly, for Bruton the patient had no gammaglobulin fraction. He gave the boy gammaglobulin injections intramuscularly, thereby introduced immunoglobulin replacement therapy.

Over the years, the development of new immunological methods has driven the discovery of new forms of PID. Targeting therapy, replacing the deficient immunological factor, is the ideal therapy when feasible. However, antibiotics are still required to clear intercurrent and recurrent infection in immunocompromised patients. PID patients often need higher doses of anti-infectious agents, intravenous administration and longer treatment time than others. Some PID patients profit from prophylactic antibiotics. Because of the risk of generating resistant microbes, prophylactic therapy is confined to a few selected drugs (such as trimetoprim/sulpha or tobramycin) and to situations without other adequate treatment options. Introduction of antifungal agents such as the lipid formulations of amphotericin B, the second-generation triazoles, and the echinocandins, have increased the options for medical management of severe fungal infections. Antiviral therapy is effective for some severe systemic viral infections in PID patients. However, antibiotics, antifungals and antiviral agents alone are not always able to eradicate the pathogen, because full clearance is often dependent on competence of specific immunological function(s).

Immunoglobulin replacement therapy

Immunoglobulin replacement therapy is the mainstay of treatment in antibody deficiencies. Replacement therapy with immunoglobulin in primary antibody deficiencies increases life expectancy, and reduces infection frequency and severity (16). Immunoglobulins can be administered intravenously (usually once a month) in hospital, or as home based subcutaneous self treatment (usually once a week). Subcutaneous infusion is given by a battery driven electric pump. Subcutaneous infusion, causes fewer systemic reactions than intravenous therapy, and may be the treatment of choice, particularly in individuals requiring small doses of immunoglobulins.(17;18).

Self administered, subcutaneous immunoglobulin therapy; ScIg, administered (weekly) at home



Intravenous immunoglobulin therapy; IVIg, administered (monthly) in hospital.

from "Our immune system" www.octapharma.com

Figure 1 Immunoglobulin replacement therapy

Cytokine and various other adjunctive treatment

Cytokine treatment is used in patients with phagocytic disorders. Interferon gamma injections are given for CGD and autosomal dominant IFNGR1. Granulocyte colony stimulating factor (G-CSF) is used in the treatment of severe congenital neutropenia. Various immune modifying treatments (FK506, cyclosporin, corticosteroids) are helpful in some immunodefiencies with autoimmune manifestations such as IPEX, ALPS and CVID.

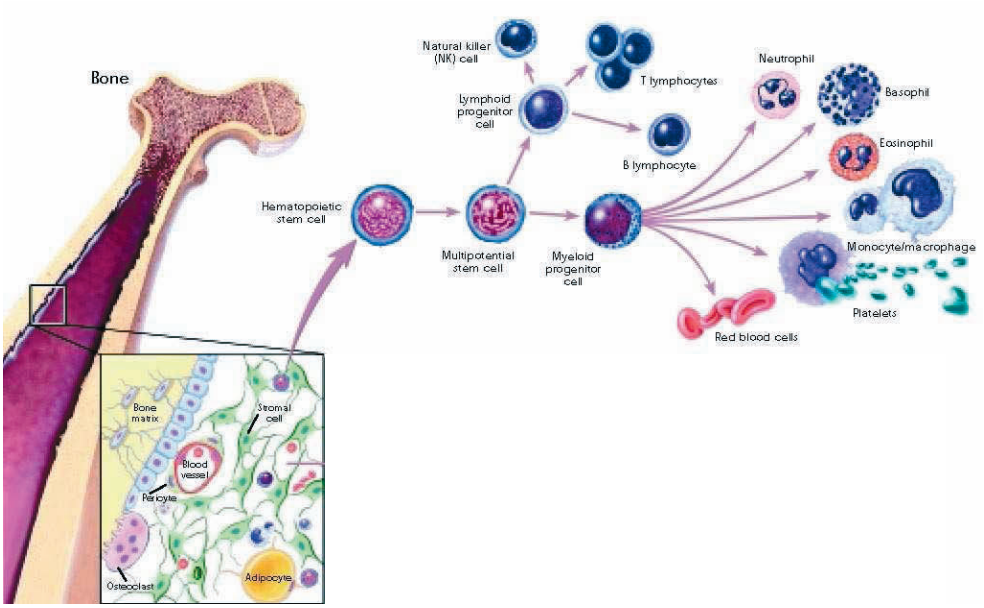
Bone marrow transplantation

Hematopoietic stem cells are produced in the bone marrow (Figure 2). Bone marrow transplantation, i.e. allogenic hematopoietic stem cell transplantation, (Figure 3) has the potential to cure SCID. Recent data support bone marrow transplantation in Wiskott Aldrich syndrome (19), CD40 ligand deficiency and severe CGD (20;21). Non-identical HLA hematopoietic stem cell transplantation may cause severe acute or chronic graft versus host disease (GVHD) where donor T cells (= graft) attack the recipient's (= host's) organs. In PID an HLA identical sibling is the optimal donor, followed by HLA identical registry donor. Haploidentical family donors and mismatched unrelated donors are used less frequently because of poor overall survival.

Preconditioning regimens prior to transplantation may vary from none in T-B-NK+ SCID with HLA identical sibling donor, to reduced or modified regimens in adult PID patients with pre-existing organ damage, to nonmyeloablative conditioning regimens in DNA repair disorders (22), to standard irradiation-based myeloablation. Bone marrow transplantation is associated with potentially life threatening complications, and is reserved for severe PIDs. Survival depends on the specific diagnosis, tolerance of the preconditioning regimen, the patient's age and health, and most importantly, the degree of HLA match between donor and recipient. Generally speaking, treatment by bone marrow transplantation is increasingly successful (23). The EBMT guidelines for hematopoietic stem cell transplantation in primary immunodeficiencies presented in Paris, June 2004 are available at: http://www.esid.org/downloads/BMT_Guidelines_old.doc

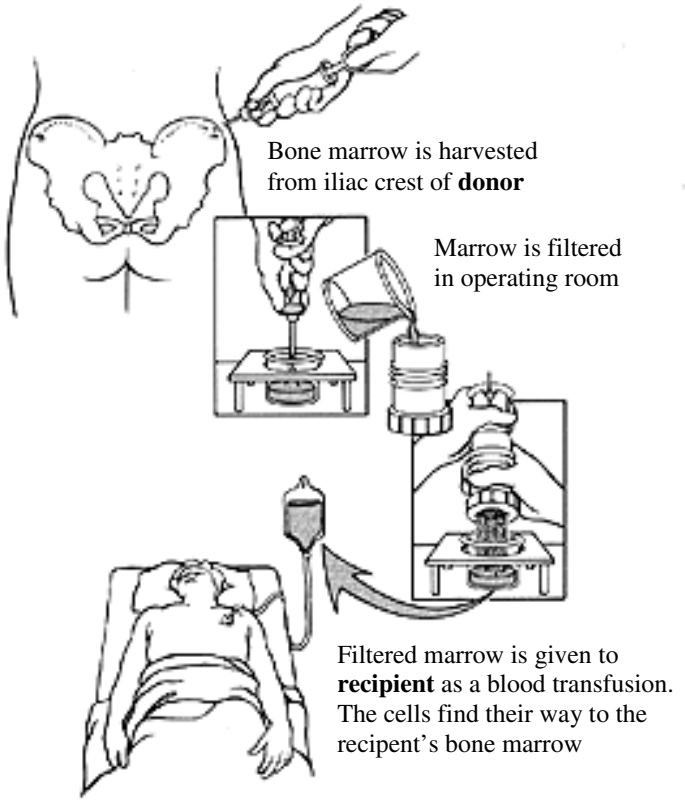
Gene therapy

In the absence of a HLA identical donor, gene therapy has been performed in a few cases internationally (24-29). Retroviral vectors have been successfully used *ex vivo* to transduce hematopoietic precursors from patients with X-linked SCID and ADA deficiency. Trials with gene therapy for other types of SCID and other PIDs such as X-linked CGD are ongoing. PIDs are good targets for hematopoietic stem cell-targeted gene therapy. Treatment of SCID patients was the first example of successful gene therapy based on *ex vivo* retroviral vectors. Advances in gene transfer technology can potentially lead to safe and effective gene therapy for immunodeficiency diseases, primarily for the severe forms.



http://stemcells.nih.gov

Figure 2 Hematopoietic stem cells are produced in the bone marrow



http://www.centerspan.org/pubs/news/art/fig1.gif

Figure 3 Schematic overview of bone marrow transplantation

Quality of life studies in PID patients

- previous studies focused on comparing treatment methods

Despite intensive treatment, patients with PIDs may have problems with infections or other disease manifestations. For example, patients with antibody deficiencies have increased incidence of autoimmune diseases and experience long-term complications of infections and/or treatment. Chronic disease will often have consequences for quality of life. Previous quality-of-life studies in patients with PID have been mainly been limited to the study of different treatment methods in patients with antibody deficiency. As part of the re-introduction of subcutaneous immunoglobulin (ScIg) treatment there have been many studies of quality-of-life status before and after changing from one treatment modality to another e.g. from IVIg to ScIg. After initiation of subcutaneous replacement therapy, increased health-related function and improved self-rated health have been documented in some studies(30-33). It may be that one of the main motives has been to ensure the market of the safety and advantage of the ScIg product rather than to focus on how patients are coping with life in general. We were curious about broader aspects of quality of life among our patients with PIDs. We wanted to know how they are managing their lives which are complicated by persist or recurrent infections and the need for life-long medical treatment. We were interested in how frequently they are on sick-leave because of their disease, if they have missed educational opportunities and so on.

During the late eighties and early nineties (1987-1993) there was a scandal with hepatitis C virus contaminated immunoglobulin preparation for intravenous administration (IVIg)(34;35). Several patients with antibody deficiency who had been treated with IVIg during the interval became infected with HCV and experienced significant morbidity. We wanted to study the possible effect of HCV disease on these patients' quality of life. We also wanted to look closer at other factors that are known to be important for coping, good quality of life, and hope, in general, and see if we could find certain areas of significance for this particular group.

Curative treatment does not always exist

Some immunodeficiencies are accompanied by other genetically determined manifestations, for which there are no cures. This is the case for some of the DNA repair defects which affect multiple organs including the central nervous system (CNS) e.g. ataxia-telangiectasia, radiosensitive SCIDs (*Artemis, DNA ligase IV* and *NHEJ1*deficiency), Hoyeraal-Hreidarsson syndrome, and to a lesser degree Nijmegen breakage syndrome and Bloom syndrome. In radiosensitive SCID, the immunodeficiency is fatal unless treated with bone marrow transplantation, but there is no curative treatment for CNS manifestations. Supportive treatment can, however, sometimes prolong life expectancy and quality of life in such disorders. Precautionary measures may slow disease progression e.g. in DNA-repair disorders with radiosensitivity.

DNA repair and immunodeficiency

Various genetic defects in the DNA repair machinery identified in humans provide insight into consequences of genomic instability for the immune system. Increasingly, immunodeficiency is recognized as a feature of these syndromes. In many conditions with defective DNA repair, the immunodeficiency is clinically variable. Immune function may range from normal to severely impaired. Antibody deficiency is the most common manifestation, and often involves defective antipolysaccharide antigen responses(36).

DNA breaks and repair in development of T cells and B cells:
VDJ recombination

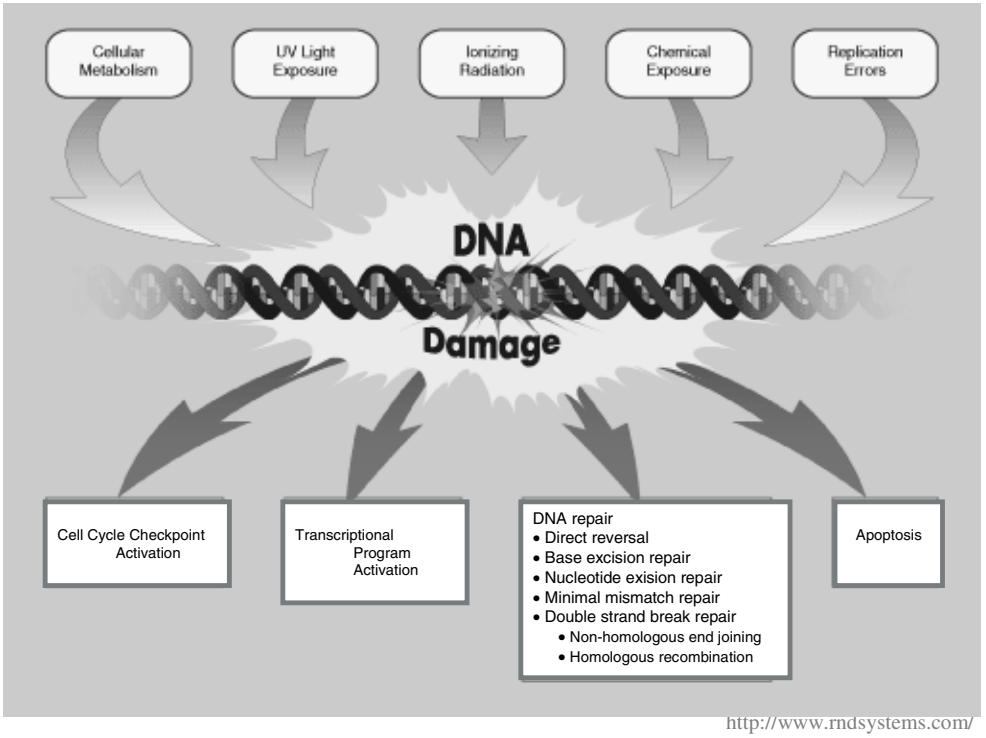
Cellular DNA is constantly exposed to insults that threaten cellular control and replication. The most devastating form of damage is a DNA double-strand break (DSB) which can be caused by exogenous agents such as ionizing radiation. Double-strand breaks also occur in intermediate stages of normal metabolic processes including DNA replication, immune system development and meiotic recombination. Damage response mechanisms maintain genomic stability, and include recognition and repair of damage, assessment at checkpoints that prevent cell cycle progression in the presence of damage, and mechanisms, such as apoptosis, that remove damaged cells (Figure 4).

In contrast, effective immune responses require the generation of >1000 genetically diverse cells bearing a unique receptor capable of recognizing a unique antigen/MHC combination. In higher organisms, these genetically diverse cells are created by breaking, randomly re-sorting/selecting and then joining the DNA sequences coding for antigen receptors by adapting the DNA repair mechanisms normally utilized to maintain genomic stability. This V(D)J recombination of immunoglobulin in B cell precursors and T cell receptor loci in T cell precursors (Figure 5) is a stepwise process during which site-specific DSBs are generated by the endonucleases RAG1/RAG2, followed by DSB repair by nonhomologous end joining (Figure 6). V in V(D)J stands for variable, D for diversity; and J for joining. During V(D)J recombination, one V of the multiple Vs within the TCR and BCR genes is selected and joined with one selected D and one J to form the variable regions of immunoglobulins and T cell receptor, respectively (Figure 7).

The T cell TCR genes with their corresponding chromosomal loci are: TCR beta: 7q35, TCR alpha and TCR delta: 14q11.2, TCR gamma 7p15-p14. B cell immunoglobulin genes and their loci are: Immunoglobulin heavy chain constant region genes (encoding IgM, IgD, IgG1-4, IgA, IgE) and genes for the variable V, D, J regions: 14q32.33, Kappa light chain genes: 2p12, Lambda light chain genes: 22q11.2 (Figure 7). Notice that, in contrast to T and B lymphocytes, no rearranging/recombination take place in NK cells.

Inherited V(D)J recombination defects

Complete defects in V(D)J recombination result in severe combined immunodeficiency (SCID) which is characterized by absence of mature B cells and T cells with preservation of NK cells. As in ataxia-telangiectasia, a subset of T-B-NK+ SCID patients is extremely sensitive to ionizing radiation. In some of these patients mutations in the DNA repair genes *Artemis*, *DNA Ligase IV* or *NHEJ1(cernunnos-XLF)* have been reported. DNA ligase IV and NHEJ1 activity are responsible for the ligation step in nonhomologous DNA end joining and in V(D)J recombination.



http://www.rndsystems.com/

Figure 4 Induction of DNA damage and the intracellular responses to DNA damage

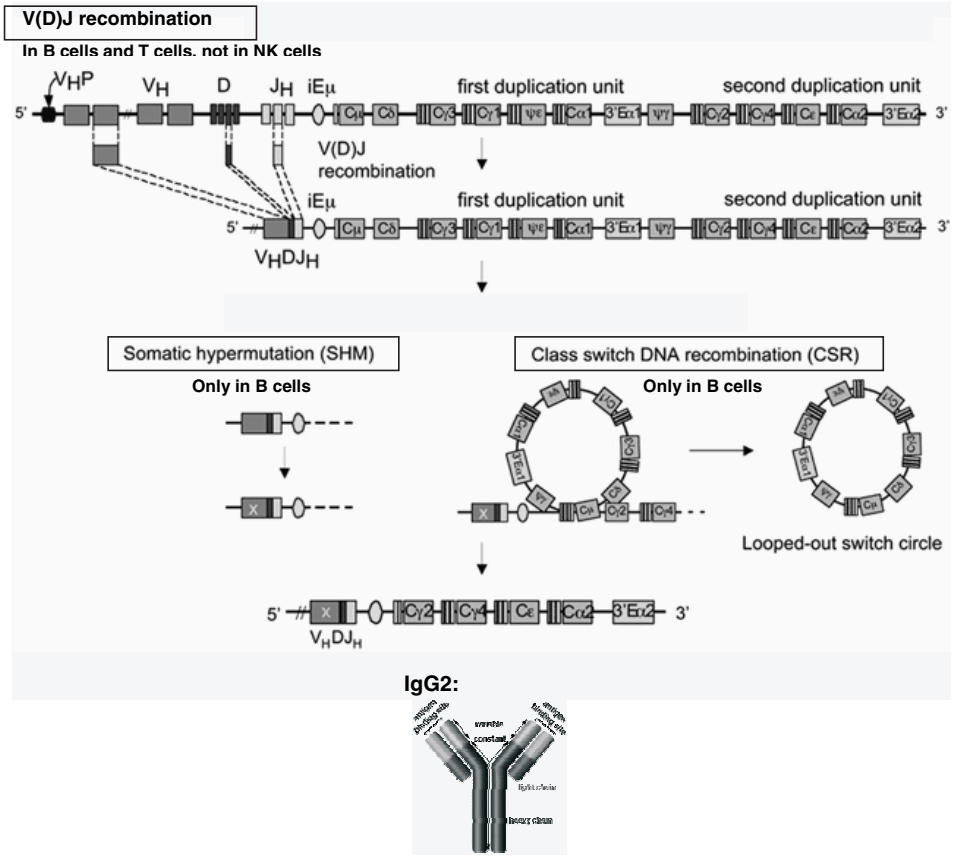


Figure 5 VDJ recombination, class switch recombination and somatic hyper mutation

Genes are rearranged to produce B cell immunglobulins. The VDJ step also takes place in T cell receptor formation. Somatic hypermutation (SHM) and class switch recombination (CSR) are confined to B cells. V in V(D)J stands for variable, D for diversity; and J for joining. During V(D)J recombination, one V of the multiple Vs within the TCR and BCR genes is selected and joined with one selected D and one J to form the variable regions of immunoglobulins and T cell receptor, respectively. VDJ recombination involves DNA double strand breaks and DNA ends are repaired by non-homologous end joining. CSR and SHR are examples of DNA single strand breaks involving base-excision repair processes.

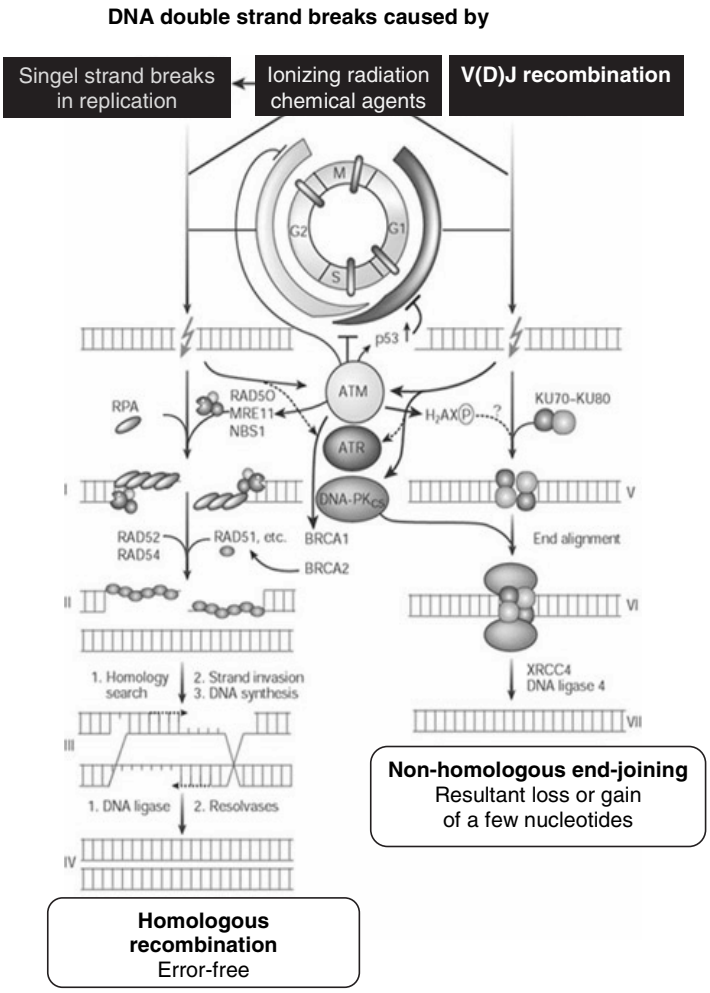


Figure 6 DNA double strand breaks repair: Homologous or non-homologous end joining.

Homologous recombination occurs in cell cycle S phase, while non-homologous end joining takes place in G1 (and G2). ATM (ataxia telangiectasia mutated) kinase arrests cell cycle progression, allowing DNA repair processes to occur.

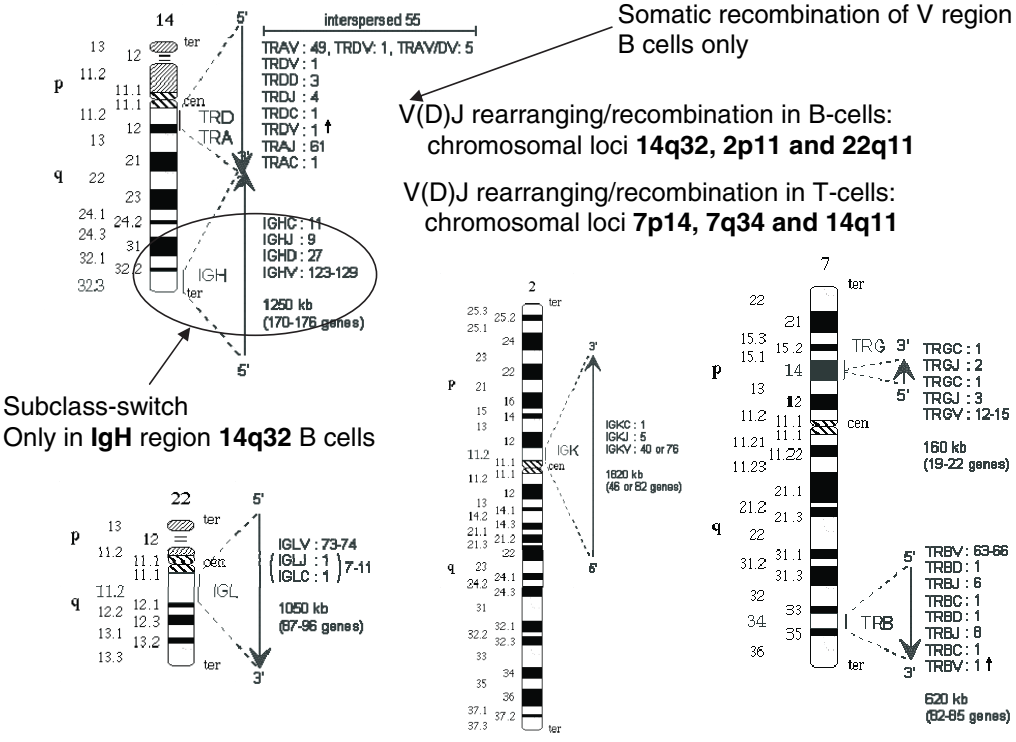


Figure 7 Genes important for a diversified T- and B-cell immune response

T cell TCR genes and B cell immunoglobulin genes and their chromosomal loci on chromosome 2, 7, 14 and 22.
Abbreviations: IGL, Immuoglobulin lambda light chain; IGK, immunoglobulin kappa light chain; IGH immunoglobulin heavy chain, TRA, T cell receptor alpha; TRC, T cell receptor beta; TRD, T cell receptor delta, TRG, T cell receptor gamma genes; V, variable; D, diversity; J, joining; ter, terminal; cer, centromere; p, short arm of the chromosome; q, long arm of the chromosome

Further DNA damage and repair processes in B-lymphocyte development: Somatic hypermutation and class switch recombination

B cell response undergoes a secondary repertoire diversification, which is antigen-triggered and occurs in germinal centres within lymphoid organs. Somatic hypermutations (SHM) of the V region take place (Figure 5) and increase antibody specificity against microbial agents (antigens) Refinement of the antibody response involves changing from IgM isotype to Ig isotypes (IgA, IgE, IgG) during class switch recombination (CSR). CSR is a region-specific DNA recombination reaction that replaces one immunoglobulin (Ig) heavy-chain constant region gene with another. A single variable region gene in conjunction with different downstream heavy-chain constant region genes, can give rise to several gene products, each of which has unique biologic activity. The ability of B cells to express immunoglobulins with identical antigen specificity, but different effector functions is a result of CSR. The constant (C) heavy chain genes are located in reading frame, 5' to 3', as follows: Cμ, Cδ, Cγ3, Cγ1, Cα1, Cγ2, Cγ4, Cε, Cα2 (Figure 5). Both SHM and CSR are dependent upon activation-induced cytidine deaminase (AID), coded for by the *AICDA* gene. Defects in AID or uracil-DNA glycosylase (UNG) result in Hyper IgM syndrome types 2 and 4, respectively, with inability to switch from IgM to other Ig isotypes and reduced antigen specificity (37;38). AID and UNG deficiency are examples of defective *base excision repair* of DNA *single* strand breaks

The four major DNA repair pathways

The four major DNA repair pathways in humans are:

1. base excision repair (BER),
2. nucleotide excision repair (NER),
3. mismatch repair (MMR) and
4. recombinational repair/repair of DNA double strand breaks (DSBs) including
    a) nonhomologous end joining (NHEJ) (Figure 6)
    b) homologous recombination (HR) (Figure 6)

In addition, mechanisms for direct repair of damage exist. For example, methylation of guanine bases may be directly reversed by the protein methyl guanine methyl transferase.

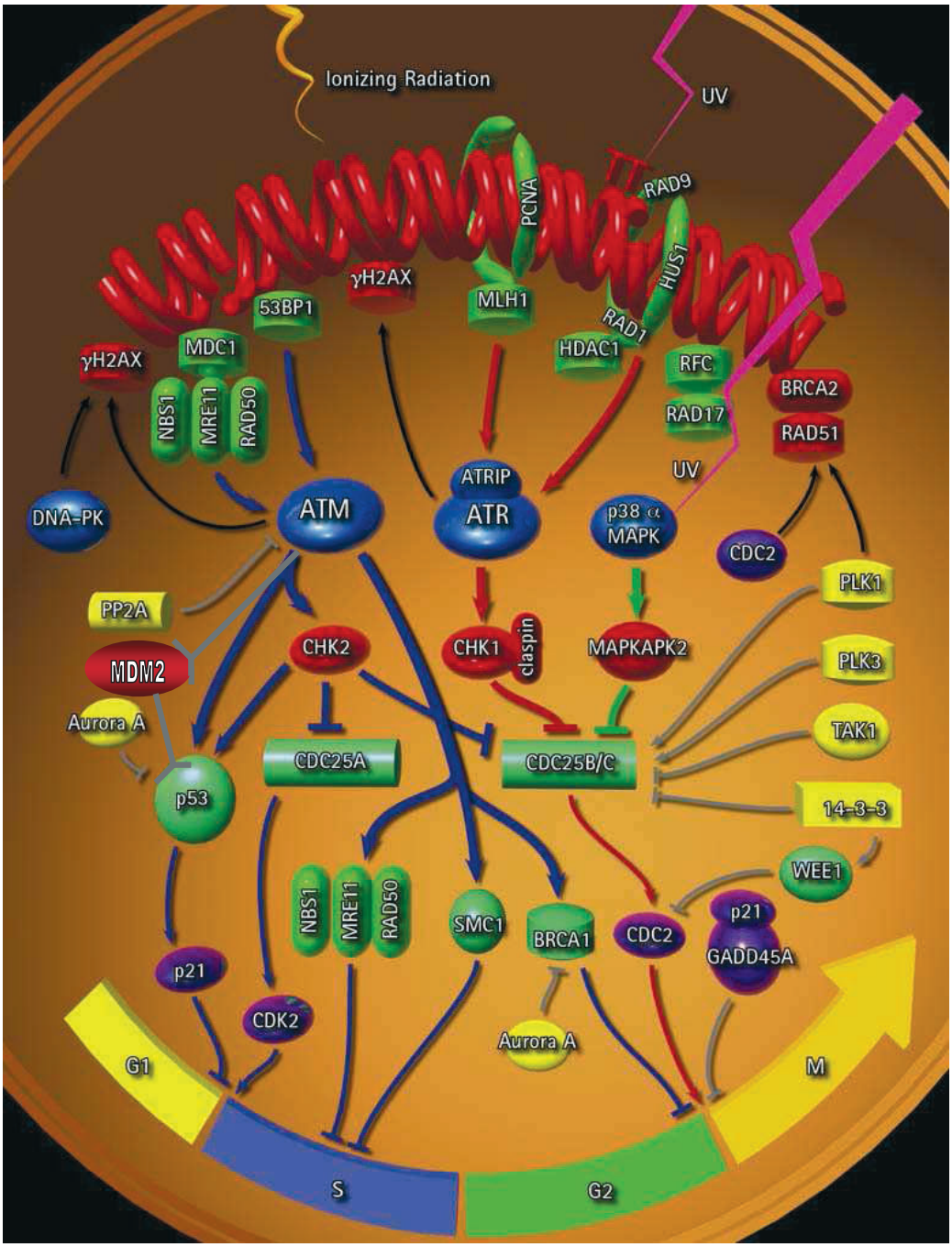
Examples of diseases caused by defects in various DNA repair mechanisms

Diseases caused by defects in BER include Hyper IgM syndrome type 2 (AID deficiency), type 5 (UNG deficiency), and familial adenomatous polyposis (FAP). FAP is caused by mutations in the *APC* gene. Patients have numerous colorectal polyps and increased risk of developing colon cancer. Examples of defects in NER are Cockayne syndrome, Xeroderma pigmentosum and trichothiodystrophy. These diseases have in common extreme UV-sensitivity. Examples of defects in MMR are hereditary nonpolyposis colon cancer (HNPCC), recently renamed Lynch syndrome. Inherited mutations in *MLH1* and *MSH2* account for 60% and 35%, respectively, of genetically characterized Lynch syndrome cases. Examples of defects in DNA double strand breaks repair are ataxia-telangiectasia, Nijmegen breakage syndrome, ATR Seckel, and Omenn syndrome or SCID caused by deficiency in *RAG1/RAG2, Artemis, NHEJ* or *DNA ligase IV*. Some of these disorders are characterized by radiation sensitivity, neurological symptoms and/or immunodeficiency.

DNA repair defects cause immunodeficiency, cancer and/or neurodegeneration

Different DNA repair mechanisms are associated with immunodeficiencies, cancer and neurodegenerative disorders

DSBs occurring at random upon genotoxic stress are preferentially repaired by nonhomologous end-joining (NHEJ) in higher eukaryotes (Figure 6). Homologous recombination (HR) functions only in cell cycle S phase. In mammalian cells, the checkpoint response to DNA damage, or replication stress, regulates processes such as cell-cycle progression, apoptosis, and DNA replication. Damaged DNA is detected by sensor proteins and is relayed to downstream effectors leading to cell cycle arrest, activation of DNA repair processes or apoptotic cell death depending on context. DSBs, induced by *ionizing radiation,* for example, are detected by a complex of sensor proteins including γ-H2AX, 53BP1, MDC1, BRCA1, and the MRN complex which is composed of MRE11, RAD50, and NBS1 (Figure 8). Damage signals are then relayed to the central checkpoint mediator, ATM (ataxia-telangiectasia mutated), which in turn activates CHK2 via phosphorylation resulting in G2 cell cycle arrest, (Figure 6) allowing the DNA damage to be repaired. *UV-induced DNA damage* and replication stress are detected by an alternative set of sensor proteins including the 911 complex (composed of HUS1, RAD1, RAD9), RAD17 and RAD26 (Figure 8). Signals are subsequently relayed to the central checkpoint mediator, ATR, which in turn activates CHK1 and either inter S phase (leading to HR) or G2 cell cycle arrest (leading to NHEJ)(Figure 6). Cancer cells often adapt to DNA damaging chemotherapeutic agents and escape apoptosis. This adaptive mechanism may include cell cycle arrest and repair of damaged DNA. The checkpoint proteins are important in oncogenesis and, thus, a potential target for cancer therapy. Failure to properly repair DSBs results in genomic instability, aberrant CNS development, and various forms of immunodeficiency. Reduced repair capacity caused by mutations in one of the DNA repair genes is linked to several human syndromes which feature cancer predisposition, developmental abnormalities, neurological abnormalities, and/or premature aging.



http://biochemistry.unh.edu/

Figure 8 Current view of the ATM-dependent DSB response network.
When the MRN complex (MRE11, RAD50, NBS1) sense DNA double strand breaks (DSB), ATM is phosphorylated from the inactive dimer to the active monomer which activates multiple processes through phosphorylation, and coordinates further events leading to DSB repair. The MRN complex acts both upstream and downstream of ATM. ATR are able to phosphorylate some of ATM's substrates in response to these DNA lesions at later time points and slower kinetics

Ataxia-telangiectasia

Ataxia-telangiectasia (A-T) is an autosomal recessively inherited disorder caused by mutations in the *ATM* gene. The condition is pleiotropic with manifestations from many different body systems including: progressive cerebellar ataxia, oculomotor apraxia, dysarthria, immunodeficiency, progressive oculocutaneous telangiectasias, progeric hair and skin changes, endocrine abnormalities, growth retardation, chromosomal instability, radiation sensitivity and cancer susceptibility particularly for lymphomas and leukemia (MIM#208900).

Life expectancy

Malignancies or chronic lung failure with pulmonary infections cause death in early adulthood. The role of the immunodeficiency has not been clearly defined. Median survival in two large cohorts of patients with this disease, one prospective and one retrospective was 25 and 19 years, with a wide range. Life expectancy did not correlate well with severity of neurological impairment in one study (39).

Diagnosis

Elevated serum alpha fetoprotein (AFP) and reduced serum IgA are characteristic and support the diagnosis (40-42) (Table 1), given a clinical suspicion of A-T. Identification of mutations in both the patient's *ATM* genes is diagnostic. A characteristic finding in lymphoblastoid cell lines derived from A-T patients is the absence of ATM protein or, when residual protein is present, reduced kinase activity (43). ATM protein kinase is involved in DSB response and repair (44), and in maintenance of cell homeostasis after oxidative damage (45-48). Classic A-T is the result of two truncating mutations leading to total loss of function of the ATM protein. Milder disease is associated with leaky splice site or missense mutations (49-52).

Table 1 Diagnostic criteria for A-T, from the guidelines developed by ESID http://www.esid.org/

Definitive:
Male or female patient with either increased radiation induced chromosomal breakage in cultured cells, or progressive cerebellar ataxia, who has disabling mutations on both alleles of ATM.

Probable:
Male or female patient with progressive cerebellar ataxia and three out of the following four findings:
1) Ocular or facial telangiectasia
2) Serum IgA at least 2 SD below normal for age
3) Alpha fetoprotein at least 2 SD above normal for age
4) Increased chromosomal breakage after exposure to irradiation

Possible:
Male or female patient with progressive cerebellar ataxia and at least one of the above mentioned four findings.

History

Syllaba and Henner published the first descriptions of patients with A-T in 1926 (53) after observing progressive choreoathetosis and ocular telangiectasia in three members of a single family. The next report describing patients with A-T came in 1941 when Louis-Bar described progressive cerebellar ataxia and cutaneous telangiectasia in a Belgian child (54). This condition was subsequently named Louis-Bar syndrome. A-T was not described as a distinct clinical entity for another two decades when Boder and Sedgwick as well as Biemond and another Belgian pediatrician, Pelc, reviewed autopsy findings and reported abnormal organ

development, cerebellar atrophy, neurologic manifestations and two additional major features, immunodeficiency and increased risk of lymphoreticular malignancies (55-60). In Norway, five cases were described by Smeby in 1966 (61). The Norwegian immunologists Natvig, Harboe et al reported IgA deficiency in 5 out of 8 A-T patients (62). Assays of the other isotypes IgM, IgE, IgD, IgG including IgG subclasses were not available clinically until 80s.

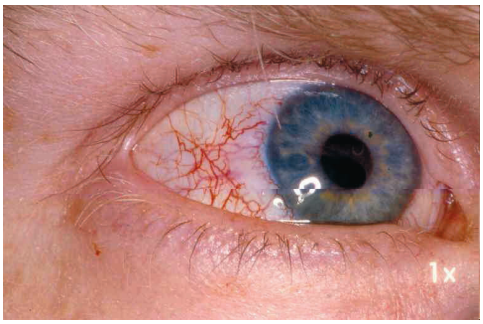
Clinical manifestations of A-T

Progressive cerebellar ataxia

The earliest manifestation of A-T is truncal ataxia, usually noticed when the child begins to walk between 1-1 ½ years of age. The child's head and trunk sway on standing and even on sitting. In contrast to other cerebellar ataxias the gait is narrow, not broad-based. The child is often a better runner than walker, and finds walking in uneven terrain easier than on level surfaces. Standing upright without support is the biggest challenge. Cerebellar ataxia progresses with age and is accompanied by drooling and dysarthria. Later loss of deep tendon reflexes and dystonia can be present. After age 5 years, ataxia is increasingly apparent and the child needs support for walking. The child usually prefers a wheel chair instead to walking by age 10 or 11 years. Electric wheel chairs are introduced early so that the child can rest as needed, not use all his or her strength on ambulating, and have energy to play with the other children. The all terrain electric wheelchairs allow individuals with A-T independent mobility. Joystick and remote controls are easy to manage for most children with A-T.

Conjunctival telangiectasis

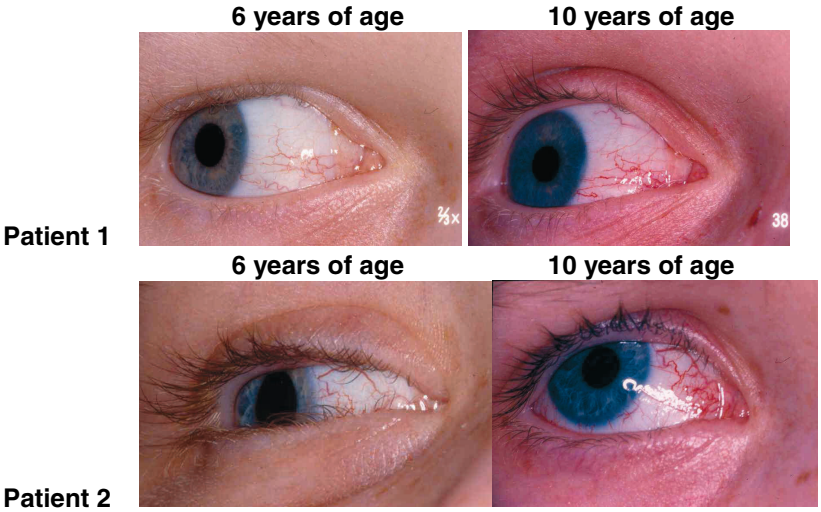
Oculocutaneous telangiectasia, the second diagnostic hallmark of A-T, usually manifests later than ataxia and oculomotor apraxia. The name ataxia-**telangiectasia** can be misinterpreted to mean that telangiectasis is a mandatory feature and delay diagnosis. The mean age of onset for telangiectasias is 5 years with a range from 4 to 10 years (63). Conjunctival telangiectasias are first visible in the palpebral fissure, later they can also be seen in the conjunctival fornix (Figure 9). Dilatation and tortuosity of blood vessels increases gradually with age (Figure 10). With time cutaneous telangiectasias can also typically be observed on the backs of the hands, neck and ear lobes (Figure 11).



(Photo: Dr.Ruth Riise)

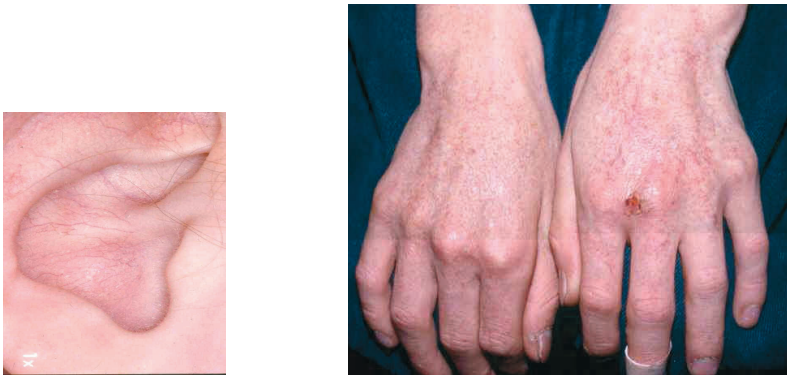
Figure 9 Conjunctival telangiectasias

Conjunctival telangiectasias in a 19-year old individual with A-T



(Photos: Dr.Ruth Riise)

Figure 10 Conjunctival telangiectasias increasing with age in A-T



A **B** (Photos: Dr. Ruth Riise)

Figure 11 Cutaneous telangiectasias

Ear lobe of a child with A-T (A). Hands of an adult with A-T (B).

Oculomotor apraxia/ataxia

The onset of ocular movement abnormalities, especially saccades, precedes the advent of telangiectasias. The four components of ocular stability and motility: ocular fixation, smooth pursuit movements, saccades and optokinetic nystagmus, are affected from early childhood in A-T, and the changes are progressive and irreversible. The oculomotor disturbance in A-T has been described as oculomotor apraxia, but oculomotor **ataxia** is a more appropriate term. Ocular fixation becomes unstable with small saccades and a tendency to ocular flutter. Normal fixation is universally lost after the age of six years. Smooth pursuit movements are affected from the age of four years, when small catch-up saccades can be seen. The eyes follow a moving object in a step-wise/staccato manner, sometimes with over-shooting. The ocular movement abnormalities are characteristic for cerebellar abnormalities, but also point to brainstem abnormalities (64).

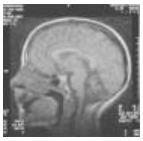
In A-T there is increased delay time for the initiation of visually and verbally induced saccades. There are many small saccades with low gain (under-shooting), both horizontally and vertically (oculomotor apraxia). Compensating head thrusts as in Cogans' congenital ocular motor apraxia have been reported previously, but were not seen in the Norwegian A-T patients (63). Optokinetic nystagmus is lost before three years of age in classical A-T patients. To read along lines becomes increasingly difficult. Instead of learning to read by spelling the letters to form words, children are better off learning to recognize whole words. Children with A-T should have the opportunity to learn to read as early as possible, before the oculomotoric problems progress. Visual acuity and retinal appearance are usually normal in patients with A-T.

Cerebral and cerebellar MRI findings

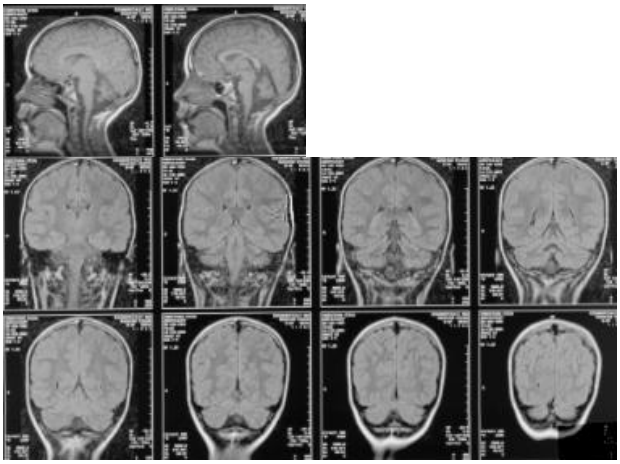
Typical MRI findings in A-T are diffuse cerebellar atrophy, particularly affecting the vermis and the superior cerebellar cortex (65;66) (Figure 12). Head circumference is usually within normal range. A small proportion of A-T patients are microcephalic. Head circumference is not related to severity of neurological impairment in a linear manner (Personal communication dr.Tom Crawford, A-T Clinical Center John Hopkins Hospital). Proton MRI spectroscopic features in A-T correlate closely with the structural neuroimaging finding of posterior fossa atrophy. Older A-T patients exhibit profound loss of all metabolites in the cerebellar vermis and a trend towards decreased metabolite content within the cerebellar hemispheres. Although symptoms suggesting extrapyramidal dysfunction are part of the A-T phenotype, these are not associated with altered metabolite levels in the basal ganglia. A few older A-T patients tested had multiple small foci of hypointensity on T2*-weighted images suggestive of capillary telangiectasia throughout the brain (67;68)

Degeneration of cerebellar Purkinje cells

Cortical cerebellar degeneration in A-T involves primarily Purkinje and granular cells, while adjacent basket cells remain unaffected. Although degenerative changes in the central nervous system are seen predominantly in the cerebellum, changes in other parts of the CNS have also been described, and are clinically demonstrated by the characteristic oculomotor abnormalities and by additional neurological signs in older patients.



MRI picture of a 20 year old patient with A-T



These cerebral MRI pictures of a 10 year old child with A-T show cerebellar atrophy, especially of the vermis. The fourth ventricle is secondarily enlarged. The brain stem and cerebrum appear structurally normal.

Figure 12 MRI images showing cerebellar degeneration in A-T

Other clinical neurological findings

- *Dysarthria*, slow and slurred speech becomes a prominent feature with age and is caused by cerebellar degeneration.

- *Drooling* and hypersalivation are usually present at the age of diagnosis but often become less of a problem with age. Most patients are not treated for drooling.

- Individuals with A-T are *not mentally retarded*. A modest *decline in short-term memory* and cognitive functioning has been observed as has a reduction in age specific skills, when the tests of cognitive functions are adjusted so that time expenditure or ataxia do not affect results (Reported by Oril Johnsen, Children Habilitation Unit, Hamar).

- In contrast to other diseases with cerebellar damage, individuals with A-T generally have *normal or increased muscle tone*. Increasing *rigidity* is present in older patients. Exercise and physiotherapy are recommended, do not cause harm and will eventually improve muscle strength, and may help to compensate for loss of balance.

- *Extrapyramidal signs* such as rigidity, bradykinesia, mask-like facies, adventitious movements, chorea, athetosis and tremor have been reported in older A-T patients and reflect more global neurodegeneration of the brain involving areas such as the basal ganglia.

- Proprioception is good in individuals with A-T until late in the disease. A p*eripheral neuropathy*, large fiber sensory neuropathy, can be observed in older A-T patients. Plantar responses become inverted or disappear. Deep *tendon reflexes are lost* with age. In very late disease, *muscle weakness and distal spinal muscular atrophy* can be seen (69;70).

- Clinical data including autopsy reports of patients older than 25 with classical A-T point to vascular abnormalities/microangiopathy in the brain parenchyma with an increased risk of intracerebral haemorrhage. The vascular changes are similar to those seen in the long term in post-radiation brain injury (71-73).

Other non-neurological clinical manifestations

High incidence of lung problems in A-T, risk of chronic lung failure

Chronic neuromuscular diseases affect the respiratory muscles in varying patterns and to different degrees. As a result, patients with such disorders develop restrictive pulmonary disease, ineffective coughing, atelectases and pneumonia, and chronic respiratory insufficiency leading to respiratory failure. Therapeutic strategies are under development to augment coughing and airway clearance, improve lung volume, and generally support the patient with progressive ventilatory failure. These techniques have improved longevity and quality of life for many patients with neuromuscular disease.

Pulmonary infections are reported in older A-T patients. Recurrent respiratory infections have previously been linked to the immunodeficiency. Pulmonary status is a prognostic factor for A-T patients. Older patients tend to have a bell-shaped chest, chronic lung failure and

insufficient oxygenation. There is also a risk of severe rare interstitial lung disease in A-T (74).

Endocrine and autoimmune abnormalities. Progeria

Growth retardation

Patients with A-T tend to be short as adults. Short stature and a slender habitus (cachectic) are also observed in other DNA repair disorders such as Bloom's syndrome, Fanconi's anemia and Nijmegen breakage syndrome. There are reports of growth hormone deficiency in A-T (75;76). Progressive difficulties with chewing and swallowing as well as malnutrition may influence growth in A-T.

Endocrine abnormalities

Laboratory and clinical findings in A-T patients indicate that primary gonadal failure is an integral part of the disease (77). A link between the gonadal dysfunction in males and chromosomal instability may also exist. In addition to hypogonadism, other endocrine abnormalities are frequent (78). *Diabetes mellitus* is often observed in older A-T patients.

Autoimmunity

Various autoimmune phenomena are frequently observed in A-T. Coombs positive transient erythroblastopenia has been reported. Autoimmune signs of the skin and hair may include alopecia, café au lait spots and vitilligo. The patients have signs of *premature aging* (progeria) of the skin and hair.

Immunodeficiency in A-T

Immunodeficiency has been highlighted as one of the major health issues in A-T. Several reports list recurrent sinopulmunary infections as a prominent feature and the cause of age-related respiratory failure (79). In early reports 80-90 % of A-T patients were noted to be immunologically impaired: IgA deficiency (60-80%)(62), IgE deficiency (23-80%), IgG2 deficiency (50%), IgG4 deficiency and hypogammaglobulinemia (10-20%). At least 10% were on intravenous Ig treatment. Both humoral and cellular immunodeficiency has been reported previously (80-83). Underdevelopment of the thymus was emphasized as an important diagnostic sign as well as a sign of the severe immunodeficiency. There have been reports describing a reduced number of T cells, increased proportions of HLA-DR+ activated T cells, double negative gamma delta T cells, and memory T cells compared to alpha beta T cells and naïve T cells (81). However, there have been no reports of *Pneumocystis jerovicii* infection, invasive fungal infection or other clinical signs of severe T cell deficiency in A-T. High IgM levels have been reported frequently, but in contrast to patients with hyper IgM syndrome, most patients with A-T have had IgG1 levels within the reference range. There are some reports which describe inter-personal variation in type and degree of immunodeficiency, including variation between siblings (84). No *ATM* genotype- immunological phenotype studies had been performed previously.

A-T patients were previously advised to avoid exposure to chickenpox and live vaccines such as MMR, BCG and oral polio. It was thought that because of the immunodeficiency other vaccines would be less effective or only have negligible effect. In some countries vaccines were not recommended in A-T. Low levels of pneumococcal antibodies were documented in A-T patients. Other researchers found A-T patients did not mount an antibody response after vaccination with pneumococcal **polysaccharide** vaccine (85). The conjugate pneumococcal vaccine had previously not been tested on A-T patients. We wanted to test our patients'

response to this vaccine, in an attempt to potentially severe invasive pneumococcal disease. See the end of this introductory chapter for a more detailed description of *Streptococcoccus pneumonia* and immune defence.

Spontaneous and irradiation induced chromosomal changes in A-T lymphocytes

A-T patients may have spontaneous chromosomal breakage in peripheral lymphocytes on standard karyotyping after PHA stimulation. Chromosome 14 is frequently involved and chromosome 7;14 translocation, is seen in 5-15% of cells (Aurias 1980). The spontaneous chromosomal rearrangements in lymphocytes from A-T patients harbour breakpoints which consistently involve the TCR and BCR loci, including the immunoglobulin heavy chain genes, on chromosomes 7 and 14, respectively (86) (Figure 7). These clones may undergo expansion as part of the evolving malignancy process and precede overt lymphocytic malignancies (87-89)

Increased risk of cancer in A-T

Patients with A-T are predisposed to cancer. The lifelong cancer risk is estimated to lie between 20-30%, but estimates vary in different reports (90). This incidence is approximately 100-fold greater than expected for an age-matched population. Lymphomas and acute promyelocytic leukemias constitute the majority of childhood cancers in A-T and half of cancers after age 20 years. Older patients have been reported to have an increased risk of other malignancies, such as epithelial tumors including breast cancer (91-93). Although A-T patients develop both B cell and T cell malignancies, the relative proportions are quite different than in the non–A-T population, malignancies of pro-T cell origin dominate in A-T (90){Taylor, 1996 194 /id}. A-T disease is caused by germline (constitutional) ATM mutations. Somatic (acquired) mutations in ATM have been found in lymphoid cells from non-A-T patients with leukemia and lymphoma of T cell or B cell origin, and in T cell preleukemic clonal proliferations. The mutational spectrum includes missense mutations, in-frame deletions, and null mutations, scattered across the *ATM* gene. Thus, ATM is directly linked to the development of leukemias/lymphomas, but no single location within the *ATM* coding sequence predisposes to a specific type of lymphoid malignancy. Recently, Matei *et al.* have shown that ATM deficiency increases the frequency of T cell receptor alpha deletion events, compromising T cell maturation, and may be responsible for oncogenic T cell receptor translocations (94). The risk of leukemia and lymphoma need to be kept in mind.

Radiation sensitivity

A-T patients are extremely sensitive to ionizing radiation. Radiotherapy is contraindicated because it can cause acute as well as chronic damage to various organs. Cytostatic drugs containing radiomimetic and DNA damaging agents may have similar effects. X-rays and CT scans should be reduced to a minimum and replaced by MRI or ultrasound scans whenever possible.

***In vitro* sensitivity to radiation**

The abnormal response manifests *in vitro* as hypersensitivity of cultured fibroblasts to ionizing radiation and radiomimetic chemicals (95). Radiosensitivity assays of lymphoblastoid cell lines show abnormal cell survival in patients and carriers, and has been used diagnostically in some countries (Table 1). By and large, mutation analyses have replaced radiosensitivity assays diagnostically.

Treatment of A-T

At present there is no cure for the progressive neurodegeneration in A-T. Little progress has been made in treating the progressive ataxia. The only therapeutic options are medical management of the patient's problems such as immunodeficiency, sinopulmonary infections, reduced lung function, malignancy, various neurologic dysfunctions, and rehabilitation for physical and social disabilities.

Treatment of the *immunodeficiency:*

Immunoglobulin treatment is indicated whenever hypogammaglobulinemia causes symptoms that likely will be ameliorated by replacement therapy. Antibiotics should be given promptly when pneumonia is suspected. Respiratory infections respond well to antibiotics. Fungal infections of the skin and/or nails are treated with standard doses of antifungal therapy. Local antiviral therapy can be used in herpes simplex virus (HSV) relapses. Usage of vaccines in A-T has long been debated. Avoidance of live vaccines has been advised previously. Bone marrow transplantation suggested by Matei (89) and Bagley (96) should not be done in A-T patients because the typical A-T immunodeficiency is far from classical SCID, and because preconditioning cytostatics may worsen neurological defects, as well as confer an increased risk of secondary cancer.

Treatment of *cancer* – avoid radiomimetic agents and radiation therapy

If a child with A-T develops cancer, special protocols for treatment should be followed, and the child must be cared for by a pediatric oncologist experienced in cancer therapy in DNA repair disorders. In addition to the extreme sensitivity to cytostatic agents with radiomimetic properties, patients with A-T are also particularly sensitive to agents with neurotoxic effects such as bleomycine and vincristine.

Treatment of neurological symptoms

Modest improvement in associated *neurological symptoms* can sometimes be achieved with treatment:

Basal ganglia dysfunction may respond to L-DOPA derivatives, dopamine agonists and, occasionally, to anticholinergics. Anticholinergics may also reduce drooling. None of our patients have been treated with dopaminergics.

Treatments for *drooling* include 1) oro-facial training or massage 2) prochlorperazine (stemetil®), an antipsychotic drug with mild sedative effect. The drug is widely used for its antiemetic effect. It produces hyposalivation and has been used widely in the treatment of drooling. 3) Trimonthly botox injections in the submandibular and parotid glands.

Loss of balance may respond to amantadine, fluoxetine or buspiron. These may also improve speech and coordination. None of our patients have been treated with these compounds. *Tremor* may be controlled with gabapentin, clonazepan or propanalol. None of our patients are treated with these compounds, since it is not a big problem and because of the potential side effects.

The most debilitating feature of this disorder is progressive neurodegeneration due to the loss of Purkinje cells in the cerebellum and the malfunctioning of other neuronal cells. Correcting the loss of Purkinje cells is technically very difficult and would require transplantation of embryonic stem cells. Embryonic stem cell transplantations have great promise but delivery to the most affected regions of the brain, is a formidable challenge.

A recent observation shows that treatment with the steroid *bethametasone produces a short-term improvement in ataxia* (97). However, the long-term complications of steroid use are likely to outweigh the short-term benefit.

Some promise exists in the development of methodology designed to target specific prototypes of mutations in the ATM gene. Aminoglycoside antibiotics cause expression of functional ATM protein by reading through the termination codon of the ATM mutation. This is promising. However aminoglycosides are toxic in effective doses and transport across the blood-brain barrier to the Purkinje cells within the cerebellum is difficult to achieve.

Another treatment strategy is to replace ATM functions or circumvent the ATM kinase deficiency in the DSB response network within the Purkinje cells. ATR and ATM are close siblings within the PIKK family (Figure 9), having similar structure and functions. Increased ATR activity may be able to act as an ATM backup system. Topoisomerase increases ATR expression. ATR phosphorylates some ATM substrates and restores partial ATM function.

Although deficiencies of thiamine, vitamin B_{12} and vitamin E can cause ataxia, ordinary multivitamin supplements do **not** correct ataxia in A-T patients.

New treatment strategies should focus on slowing the progress of neurodegeneration. Since it seems likely that oxidative stress contributes to neurodegeneration, potential therapies based on the use of antioxidants (98) offer some hope(97;99-102). Antioxidants may reduce reactive oxygen species (ROS) which cause DNA damage. Antioxidants may concomitantly slow progression of the neurological phenotype and reduce the risk of cancer. Antioxidant treatment and effects on immunological functions in A-T patients is more uncertain (Lederman, Crawford et al) (103).

While the number of treatment options for A-T patients is limited at present, the great advances achieved over the past decade in identifying mutations and understanding how ATM functions in response to DNA damage, provide additional hope for the future therapies.

A-T carriers

Immunological findings in A-T carriers

Low serum IgE levels (104) and IgA deficiency (105)have been reported in relatives of A-T patients (105). The clinical implications of the findings were not reported.

A-T carriers and cancer risk

Several studies cite a three-to five-fold risk compared to normal for developing breast cancer in A-T carriers (93;106-109). The Nordic follow-up study (83) of 1445 blood relatives of 75 A-T patients, comprising 66 families of Danish, Finnish, Swedish or Norwegian origin, showed an increased risk of breast cancer among female carriers, especially among mothers < 55 years. The standardised incidence rate (SIR) was 2,9 (95% confidence interval, range 1.8-4.5) for all female carriers, and a SIR of 8.1 (95% CI, 3.3-17) was found for mothers < 55 years (110) (Olsen 2005). Cancer incidence and mortality information for 1160 relatives of 169 UK A-T patients showed that the overall relative risk (RR) of breast cancer in carriers was 2.23 (95% CI, 1.16- 4.28) compared with the general population, but was 4.94 (95% CI,1.90-12.9) in those younger than age 50 years (111).

Mutations in genes identified to date explain approximately 20% of the familial aggregation of breast cancer. In the context of multiple-case families, mutations in *BRCA1* and *BRCA2* confer high lifetime risks of breast cancer and ovarian cancer, and more moderate risks of prostate cancer and some other cancer types. Mutations in the *CHEK2* and *ATM* genes, but not in the *ATR* gene, cause more modest, but important, risks of breast cancer. Female carriers of *NBS1* mutations are also susceptible to breast cancer (112;113). Interactions between ATM, ATR, NBS, CHEK2, BRCA1 and BRCA2 are illustrated in Figure 8.

Further studies to explain the differences in cancer occurrence between the A-T carriers are needed. It appears that specific mutant ATM protein can mitigate the neurological aspects of the clinical phenotype, as well as cellular features measured in vitro, but, at the same time, may promote a tumor phenotype. Other expressed in-frame deletions and missense mutations appear to contribute to the development of tumors, without the same mitigation of other aspects of the phenotype. In some A-T families with truncating mutations in which there is no ATM expression, however, the relationship of *ATM* mutations to tumor development appears to be more complex, and additional factors may be involved. Variation in breast cancer risk has been suggested to be due to exogenous/environmental factors (114) and to endogenous factors such as modifier gene variants. ATM gene variants/polymorphisms that are not linked to A-T disease are currently under study as are other factors which modulate ATM protein responses, such as MDM2 (Figure 8).

Incidence of A-T

Disease incidence and carrier frequency - international data:

A-T has been reported in all regions of the world. The incidence of A-T is about 1 per 100.000 livebirths (115). The frequency of A-T mutant heterozygous alleles has been estimated to be 1.4-2% in the general population (115;116).

The *ATM* gene and mutation spectrum

The *ATM* (A-T mutated) gene, maps to chromosome 11q22.23 (117), spans~150 kb of genomic DNA, contains 66 exons and was identified in 1995 (118). GDB:593364 [http://gdbwww.gdb.org/]; (119). ATM protein kinase is involved in DSB response and repair (44), and in maintenance of cell homeostasis in response to oxidative damage (45-48). Mutations in *ATM* are spread widely across the gene's 66 exons (Figure 13). As of year 2007, more than 400 different A-T disease causing sequence alterations in ATM have been reported (http://chromium.liacs.nl/lovd/).

ATM databases:

- The former A-T mutation database was last updated May 27, 2004: http://benaroyaresearch.org/investigators/concannon_patrick/atmut-t.htm.
- The new international A-T mutation database is electronically available at http://chromium.liacs.nl/lovd/
- ATM protein kinase database: "Showcase for ATM Related Pathways" (SHARP) is designed to assist researchers in integrating, visualizing and interpreting pre-existing and new information on the ever-growing ATM-mediated network. SHARP is available on internet address: http://www.cs.tau.ac.il/~sharp.

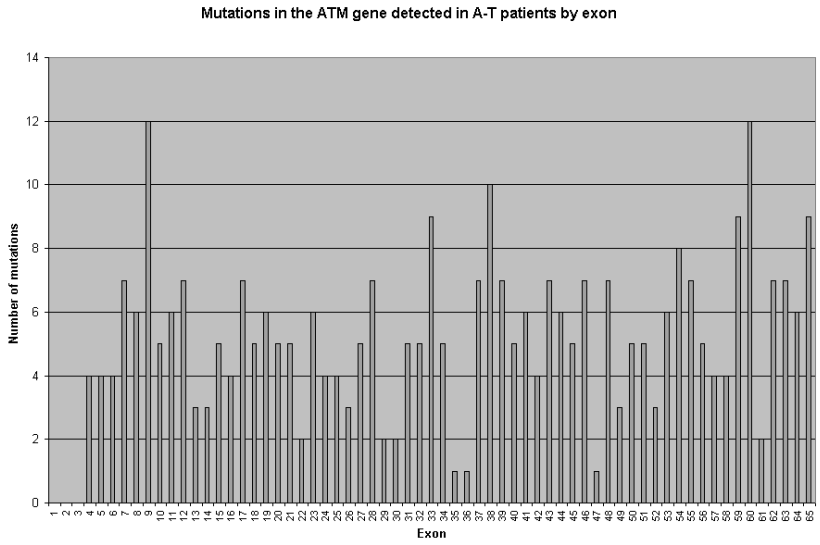


Figure 13 Mutations in the ATM gene detected in A-T patients by exons

Picture from the ATM mutation database year 2004 at Virginia Mason Research Center, USA: http://benaroyaresearch.org/investigators/concannon_patrick/images/atgraph.gif

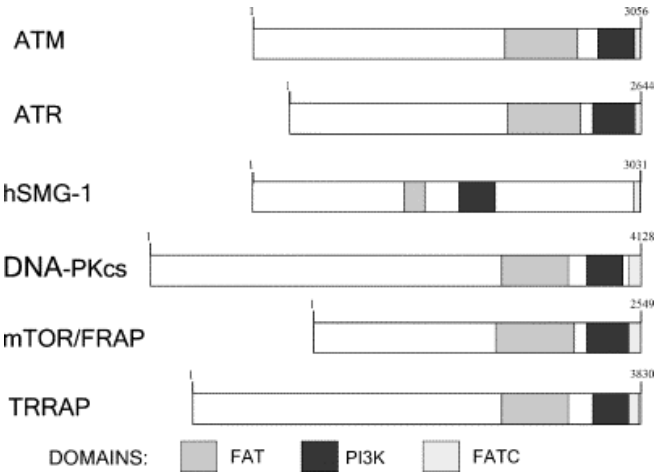


Figure 14 Schematic figure of the human PIKK proteins.

The number of residues is indicated for each protein. All of these proteins with the exception of TRRAP possess protein kinase activity. Three motifs are common to all: the FAT and FATC domains are of unknown functional significance, but the FAT domain of ATM contains serine 1981—the site that is autophosphorylated during ATM activation. The PI3K domain, which contains the phosphatidylinositol 3-kinase motifs, harbors the catalytic site in the active kinases of the family.

The gene product – the ATM protein kinase and its functions

The deduced amino acid sequence of the ATM gene contains 3056 residues and, at its carboxyl-terminal end, shows similarity to the catalytic domain of phosphatidylinositol-3 kinases (Figure 14). The PI-3 kinase motif is common to a group of proteins that are involved in cell-cycle regulation, response to DNA damage, interlocus recombination, and control of telomere length. The gene product, the ATM protein kinase, is confined to the nucleus and has multiple functions. ATM has a central role in DSBs (DNA double strand breaks) response and DSBs repair processes and in intracellular signalling pathways (44) (Figure 8) and also in maintenance of the cell homeostasis in response to oxidative damage(45-47;47;48). The MRN complex sense DSB, ATM is phosphorylated from the inactive dimer to the active monomer which activates multiple processes through phosphorylation, and coordinates further events leading to DSB repair. MRN complex acts both upstream and downstream of ATM. ATM has a central and coordinating function both in HR and NHEJ. ATM is involved in VDJ recombination (in T and B cells) and isotype class switch (in B cells), but not in somatic hypermutation (120). ATM plays an important regulatory role in phosphorylation of TP53 (p53), a tumor suppressor, thereby abolishing TP53–MDM2 interaction and allowing TP53 to accumulate. ATM also phosphorylates MDM2 which interferes with the nucleo-cytoplasmic shuttling of the MDM2–TP53 complex and contributes to TP53 stabilization. In addition, ATM functions as a regulator of a wide variety of downstream proteins, including tumor suppressor BRCA1, checkpoint kinase CHK2, checkpoint protein RAD50 and DNA repair protein NBS1.

Alpha fetoprotein in A-T

The finding of elevated serum alpha fetoprotein (AFP) is characteristic of A-T and supportive of the diagnosis (40-42). The AFP elevation in A-T was initially recognized because it was evident on the serum protein electrophoresis when serum albumin and immunoglobulin levels were measured. It was postulated to be related to the immunodeficiency and immunoglobulin levels or caused by a common defect in tissue differentiation. Although the elevation of serum AFP in A-T has been known for decades, the underlying mechanism including the link to ATM protein kinase deficiency has not been defined.

Elevated serum levels of AFP have proven to be a reliable marker of A-T after the age of two years. An age-dependent elevation was commented upon in some of the first reports and reviews of A-T (121). But in a large cross-sectional study from the UK, the AFP levels of 48 A-T patients were measured on one occasion and no correlation between age and serum AFP level was found (42). This finding has influenced practice over the last 15 years, and serum AFP levels have not been followed longitudinally as a routine in A-T patients.

AFP in other neurodegenerative diseases

Other similar and rare neurodegenerative diseases with increased levels of AFP are ataxia oculomotor apraxia syndrome 2 (AOA2 = SCAR1) and ataxia with vitamin E deficiency (AVED caused by mutations in the alpha-tocopherol transfer protein, TTPA)(122-124). But remarkably, given the clinical similarity of these diseases, AFP is not increased in the A-T like disorder (ATLD=MRE11 deficiency) or in Nijmegen Breakage Syndrome, AOA1 and autosomal recessive spinocerebellar ataxia with axonal neuropathy (SCAN1)(125). Table 2 summarizes serum AFP levels in A-T and A-T related disorders.

Differential diagnoses to A-T

The differential diagnosis of AT includes a variety of conditions: infectious encephalitis, Friedreich's ataxia which has a later onset, ataxia associated with a number of metabolic diseases of infancy and childhood, ATLD and the autosomal recessive syndromes with ataxia ATLD, AOA1 and AOA2. Figure 12 is a diagnostic algorithm of progressive ataxia in childhood.

Table 2 Gene, loci and Alpha fetoprotein in A-T and A-T related syndromes

Diagnosis	Gene	Locus	OMIM	Inheritance	Elevated AFP
Ataxia-Telangiectasia (A-T) classical	*ATM*	11q22.3	208900	AR	+
Vitamin E deficiency, AVED caused by mutations in *TTPA*	alpha-tocopherol transfer protein (*TTPA*)	8q13.1-q13.3	277460	AR	+
Ataxia-oculomotor apraxia 2 (AOA2), Spinocerebellar ataxia recessive of non-Friedreich type 1 (SCAR1)	*senataxin*	9q34	606002	AR	+
A-T like disorder (ATLD)	*MRE11*	11q21	604391	AR	-
Nijmegen Breakage Syndrome (*NBS1*)	nibrin, p95 protein of the MRE11/RAD50 complex	8q21	251260	AR	-
Ataxia-oculomotor apraxia 1 (AOA1)	*aprataxin*	9p13.3	208920	AR	-
Autosomal recessive spinocerebellar ataxia with axonal neuropathy (SCAN1)	Tyrosyl-DNA phosphodiesterase 1 (*TDP1*)	14q31-q32	607250	AR	-

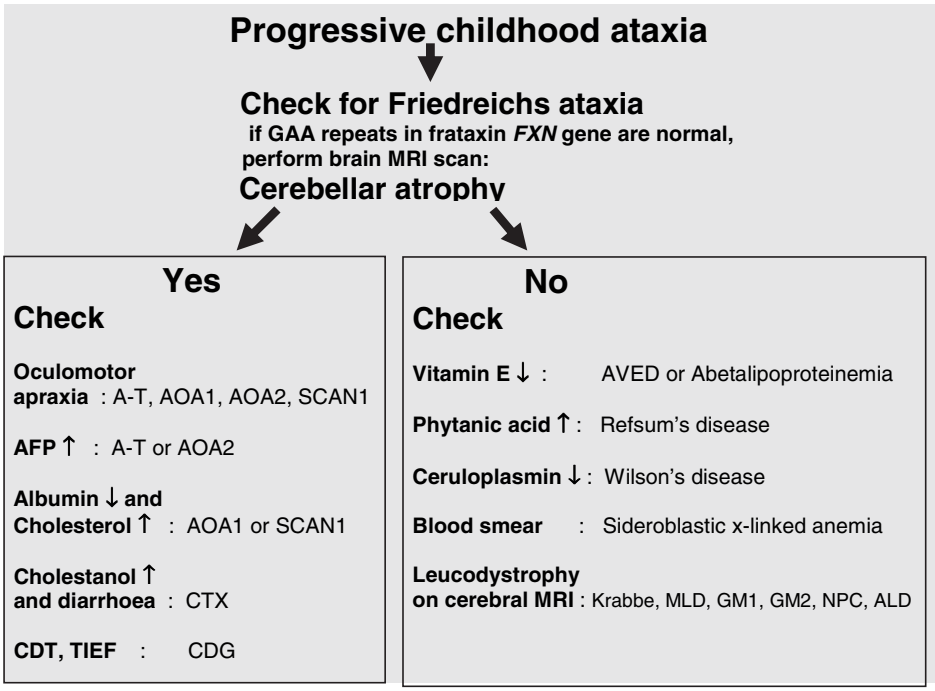


Figure 15 Algorithm for the work-up of progressive childhood ataxia

Abbreviations: AFP, alpha fetoprotein; AOA, Ataxia oculomotoric apraxia; A-T, Ataxia telangeictasia; AVED, Fredreich-like ataxia with selective vitamin E deficiency; ALD, Adrenoleukodystrophy; CTX, Cerebrotendinous xanthomatosis; CDG, Congenital disorder of glycosylation; CDT, Carbohydrate deficient transferring; TIEF, Transferrin isoelectric focusing; GM1, Gangliosidosis with defect beta-galactosidase; GM2, Gangliosidosis with defect beta-hexosaminidase; MLD, Metachromatic leucodystrophy; NPC, Niemann Pick type C disease; SCAN1, Spinocerebellar ataxia with axonal neuropathy type 1.

Other DNA repair disorders with immunodeficiency

DNA repair disorders similar to A-T with defect DNA double strand breaks repair

Mutations in *NBS1* and *MRE11* give rise to Nijmegen breakage syndrome (NBS) and ataxia-telangiectasia-like disorder (ATLD), respectively, the clinical and cellular phenotypes of which overlap considerably with ataxia-telangiectasia

Nijmegen breakage syndrome (NBS)

The Nijmegen breakage syndrome (NBS1; OMIM 251260), is an autosomal recessive disease characterized by microcephaly, characteristic facial features, growth retardation, immunodeficiency and cancer predisposition (high risk of lymphoma/leukaemia). NBS cells show spontaneous chromosomal instability and hypersensitivity to ionizing radiation in combination with radioresistant DNA synthesis. At the cellular level, NBS has some features in common with A-T. NBS is caused by a hypomorphic mutation of the *NBS1* gene 8q21, encoding nibrin, which forms a protein complex with Mre11 and Rad50, both involved in DNA repair. Nibrin participate in the repair of gamma-irradiation damage and maintenance of chromosomal stability. Unlike A-T there is a limited spectrum of different mutations. Over 90% of NBS patients are homozygous for the hypomorphic 657del5 mutation and are of Slavic origin. A couple of other truncating mutations have been identified in patients of other ethnic origin. Partially functional proteins produced by alternative initiation of translation, reducing the severity of the NBS phenotype, have been described for some of these NBS1 mutations (126). And homozygozity for 1089C>A has been linked to a Fanconi anemia phenotype. Incidence of NBS is 1: 100.000 live births, with an estimated carrier frequency 1/150 in the Eastern Europe.

NBS and cancer

In 40% of the NBS patients cancer are noted before the age of 21 years, mainly lymphomas. Thus, they seem to be more at risk for cancer than in A-T (127). Compared to A-T, they have earlier sign of growth deficiency, with lower than normal birth weight and small for gestational age. The microcephaly is progressive but usually not accompanied by severe mental retardation or ataxia. Important additional features are the skin abnormalities, particularly cafe au lait spots and vitiligo.

NBS carriers and increased risk of breast cancer

Heterozygous carriers are healthy, but may have an increased risk of breast cancer (112;113).

NBS and immunodeficiency

As part of the Mre11/Rad50/NBS complex Nibrin plays a role in the recombination of Ig constant region genes and in B lymphocytes and in Ig class-switch recombination. Nibrin localizes to chromosomal sites of class switching, and B cells from NBS patients show an enhanced presence of microhomologies at the sites of switch recombination. nibrin acts downstream of switch transcript-induced targeting of switch regions and activation-induced cytidine deaminase (AID)-induced DNA breaks in the targeted switch regions (128). Similar to A-T a characteristic, **variable deficiency of serum IgG and IgA with normal IgM levels** is observed. Individual Sμ–Sα switch-recombination junctions of Ig class-switched B lymphocytes from NBS and ATLD patients show a preponderance of microhomologies at the site of recombination which is also seen in A-T. In addition to the important role that nibrin plays in CSR, nibrin is also crucial for the survival of B lymphocytes. Agammaglobulinemia has been found in 35% and IgA deficiency in 20% of affected individuals. Deficiencies in IgG2 and IgG4 are frequent even when the IgG serum concentration is normal. The most commonly reported defects in cellular immunity are reduced percentages of total CD3+ T cells and CD4+ cells. An increased frequency of memory T cells and a concomitant decrease in naive T cells has been reported. (129).

NBS interacts with ATM

NBS has an important role within the Mre11-Rad50-Nbs1 complex. This Mre11-complex acts both upstream and downstream of ATM as a sensor of DSBs, in the cell cycle control and DSBs repair machinery.

MRE11 A-T like disease

MRE11 mutations are the underlying cause of the Ataxia-telangiectasia-like disorder (ATLD), also a rare autosomal recessive disorder (locus 11q21, OMIM 604391). The clinical features of patients with ATLD are very similar to those with A-T, with ATLD patients showing progressive cerebellar ataxia plus ocular apraxia. However, the neurological features have a later onset and slower progression. As of 2006, less than 20 ATLD patients have been reported internationally. No elevation of serum alpha fetoprotein has been observed, and most of the patients reported do not have telangiectasia. Chromosomal instability, increased sensitivity to ionizing

radiation, defective induction of stress-activated signal transduction pathways, and radioresistant DNA synthesis are observed at the cellular level. **Clinical immunodeficiency and cancer risk may be part of the syndrome**. The mutations identified in ATLD are hypomorphic. The severity of the disease may be dependent on the residual activity of the mutated *MRE11* alleles

MRE11 interacts with ATM
Mre11 has an important role within the Mre11-Rad50-Nbs1 complex (130). This Mre11-complex acts both upstream and downstream of ATM as a sensor of DSBs, in the cell cycle control and DSBs repair machinery.

DNA repair disorders causing either T-B-NK+ SCID or Omenn syndrome T(+)B-NK+: defective DNA double strand breaks repair including defect NHEJ

RAG1/RAG2

Mutations in the recombinase activating genes *RAG1* (MIM*179615) or *RAG2* (MIM*179616), both located to 11p13 and containing only one exon, cause **T-B-NK+ SCID without radiosensitivity**. The inheritance is autosomal recessive. Omenn syndrome (MIM#603554), a milder SCID variant with massive eosinophilia and generalized erytrodermia/ eczema is also due to mutations in the RAG1 and RAG2 genes. The patients have no increased sensitivity to irradiation while this is a feature of the other DNA repair disorders causing SCID or Omenn, such as Artemis or DNA Ligase IV

Artemis

Null mutations of the Artemis gene (10p, OMIM 605988) result in a complete absence of T and B lymphocytes and increased cellular sensitivity to ionizing radiations, causing **radiosensitive-SCID.** Hypomorphic mutations in *Artemis* resulting in partial SCID/Omenn syndrome with radiosensitivity have been observed. Both T-B-NK+SCID and Omenn syndrome have been observed within the same family (131).
Hypomorphic mutations provide residual protein function often causing a heterogeneous phenotype, like hypomorphic Artemis mutations, which have been found to cause partial T and B lymphocyte immunodeficiency associated with lymphoma predisposition or Omenn syndrome. In contrast, null mutations lead to a complete loss of protein function inducing a homogenous phenotype like Artemis RS-SCID.

Artemis interacts with ATM
Atremis is a DNA cross-link repair protein involved in V(D)J recombination by cleavage of the hairpin intermediate generated by RAG1/RAG2, this process seems ATM independent. Artemis is phosphorylated in an ATM dependent manner after irradiation. ATM is specifically required for the repair of a subset of lesions (10%) induced by X or gamma rays that are normally rejoined by slow kinetics in control cells, whilst ATM is dispensable for the more rapidly repaired DSBs after irradiation. The slow kinetics process requires Artemis. ATM regulates the artemis-dependent end-processing prior to rejoining by NHEJ (132)

DNA ligase IV

Ligase IV syndrome is an extremely rare autosomal recessive condition caused by hypomorphic mutations in the *LIG4* gene (13q22-q24, OMIM 601837) the condition closely resembles that of Nijmegen breakage syndrome (NBS), and is characterized by microcephaly, characteristic facial features, growth retardation, developmental delay, pancytopenia and immunodeficiency, extreme radiosensitivity. The patients have normal levels of LIG4 protein with impaired function. Dependent on the type of *LIG4* mutation the phenotype can vary and also be **radiosensitive T-B-NK+ severe combined immunodeficiency** with or without microcephaly and developmental delay. These patients have undetectable levels of LIG4 protein. Hence, different LIG4 mutations can result in either a developmental defect with minor immunological abnormalities or a SCID picture with normal development.(133;134). Hypomorphic mutations provide residual protein function often causing a heterogeneous phenotype, like hypomorphic *Artemis* mutations, which have been found to cause partial T and B lymphocyte immunodeficiency associated with lymphoma predisposition or Omenn syndrome. In contrast, null mutations lead to a complete loss of protein function inducing a homogenous phenotype like Artemis RS-SCID. Lig4 is critically required for murine development, as *Lig4-/-* mice die during embryogenesis. The neuronal defect of LIG4- or XRCC4-deficient mice can be rescued by a homozygous mutation in *TP53* or *ATM* (25-27). However, the V(D)J recombination process cannot be rescued. Cell lines from patients with LIG4 syndrome show pronounced radiosensitivity, however, unlike NBS cell lines, they show normal cell cycle checkpoint responses but impaired DNA double-strand break rejoining in NHEJ

Cernunnos-XLF/ NHEJ1

Radiosensitive T-B-NK+ SCID, +/- microcephaly and developmental delay.
Patients homozygous for mutations in the *Cernunnos* gene have growth retardation, microcephaly, and **T-B-NK+ SCID**(134;135). The patients' features overlap with those described in DNA ligase IV deficiency (36). *Cernunnos; XRCC4-like factor (XLF)* and *NHEJ1* are all names for the same gene. Cernunnos-XLF represents a novel DNA repair factor essential for the NHEJ pathway and is involved in the last step, the DNA religation, of the NHEJ pathway. Cernunnos physically interacts with the XRCC4 & DNA-ligase IV complex.

Bloom syndrome

Bloom syndrome (also called Congenital Telangiectatic Erythema) is caused by mutations in the gene *BLM* encoding DNA helicase RecQ protein-like-3 (15q26.1, OMIM 210900), and is an autosomal recessive disorder characterized by proportionate pre- and postnatal growth deficiency, UV-sensitivity, and predisposition to malignancy. The classical skin changes are erythematous telangiectasias of the face and areas with hypo- and hyperpigmentation. The patients have risk of developing diabetes type II starting already in their second/third decade. **Low IgM and IgA have been reported in a few Bloom patients (136-138).**
Several different mutations in the *BLM* gene have been identified as causing Bloom Syndrome. Bloom disease is most common among the Ashkenazi Jewish population, where 1/100 is a carrier.
Cells from patients with Bloom syndrome show various types of chromosomal instability, including chromosomal breaks, gaps, deletions, and elevated levels of sister chromatid exchanges which can be used for diagnostic reasons. This disorder is the only one that features an increased risk of sister chromatid exchange. In individuals with Bloom syndrome, the chromosomes will show an approximately 10-fold increased rate of sister chromatid exchange.
BLM is a member of the RecQ family of helicases which are enzymes that unwind DNA so that replication, transcription, and DNA repair can occur. Helicases are vital to the life of a cell and aid in the maintenance of genomic stability. Werner Syndrome and Rothmund-Thomson Syndrome are also caused by mutations RecQ helicase genes, *RECQL2* and *RECQ4* respectively. Both of these diseases, along with Bloom Syndrome, exhibit a cancer predisposition.

BLM interacts with ATM:
BLM is linked to the ATM induced pathways; ATM interacts with and directly phosphorylates BLM. LIG4 interacts with BLM and ATR phosphorylates BLM.

Høyeraal Hreidarsson syndrome

Hoyeraal-Hreidarsson (HH) syndrome (Xq28, MIM#300240) is a multisystem disorder affecting boys characterized by aplastic anaemia (AA), immunodeficiency, progressive pancytopenia including thrombocytopenia, microcephaly, cerebellar-hypoplasia and growth failure of prenatal onset. The disorder has been found to be caused by mutation in the *DKC1 (dyskerin)* gene. Mutation in the same gene causes X-linked dyskeratosis congenital. Dyskerin is closely associated with the telomerase, and aid the function and the location of the resulting telomerase complex. Activated telomerase is responsible for the de novo synthesis and the maintenance of telomere ends to extend the lifespan of activated lymphocytes, germline cells and tissue stem cells.
The patients have severe *truncal ataxia,* without telangiectasia and their cells exhibit chromosomal instability. Cerebellum demonstrates reduced cellularity of the molecular and granular layers with relative preservation of Purkinje cells and minimal gliosis(139).
X-linked Hoyeraal-Hreidarsson syndrome (XL-HHS) is the severe infantile variant of X-linked dyskeratosis congenita (XL-DC) and both are due to mutations in the *DKC1* gene within Xq28. **Progressive combined immunodeficiency has become a recognized feature of the syndrome since immunoglobulin deficiency and lymphocyte abnormalities have been documented in half of the published cases (140;141) (142;143)**

More DNA repair genes and DNA repair disorders will be detected in the future

In the near future additional disease causing genes are expected to be found in the pathways of DNA maintenance and repair. The diseases caused by defects within these pathways have a wide spectrum of features including: hair and skin changes, premature aging, immunodeficiency, cancer and neurological disease. These manifestations can facilitate diagnosis.

***ATM* knock-out mice: Knock-out, Knock-in**

Mice knockout studies provide valuable knowledge in the field of DNA repair and immunodeficiency. The *Atm -/-* mice are immunodeficient and develop leukemias and lymphomas, but do not have all of the other features of A-T. Disrupting the ATM protein in mice gives rise to some of the same findings as in A-T-patients, but not neurodegeneration. However, an increased amount of ROS are generated in the cerebellum, particularly in the Purkinje cells from *Atm -/-*mice.
Difference in symptoms and disease manifestations between species is a phenomenon in other inherited disorders as well. *Aire-/-* mice unlike humans with APECED, do not get candidiasis.

Fungal infections in PIDs,

- focusing on candida infections and chronic mucocutaneous candidiasis

Patients with phagocytic (144), cellular, combined and other primary immunodeficiencies exhibit immune deficits that confer increased susceptibility to fungal infections. A number of yeasts and moulds, most commonly *Candida* and *Aspergillus* but also *Cryptococcus*, *Histoplasma*, *Paecilomyces*, *Scedosporium*, *Trichosporon*, *Penicillium* and other, rarely isolated, fungal organisms, have been variably implicated in causing disease in patients with CGD, SCID, hyper-IgE syndrome, myeloperoxidase deficiency, leukocyte adhesion deficiency, defects in the interferon-γ/interleukin-12 axis, DiGeorge syndrome, X-linked hyper-IgM syndrome, Wiskott Aldrich syndrome, CVID and chronic mucocutaneous candidiasis including APECED. Differences in the spectrum of fungal pathogens as well as in the incidence and clinical presentation of infections may be observed among patients with different PIDs, as a function of their various underlying immune disorders.

Candida

Candida is the most common fungal pathogen in humans. It is also a frequent colonizer of human skin and mucous membranes and part of the normal flora of skin, mouth, vagina, and gut. It is present in the environment, particularly on leaves, flowers, water, and soil. The genus *Candida* includes more than 150 species. Among these, six are most frequently isolated in human infections. While *Candida albicans* is the most abundant and significant species, *Candida tropicalis, Candida glabrata* and *Candida parapsilosis* often cause invasive candidiasis. *Candida krusei* and *Candida lusitaniae* can also cause infection. With the introduction of antifungal therapy, azole resistant *C. glabrata* and *C. krusei* have become more prevalent. The diversity of *Candida spp.* that is encountered in infections is expanding and the emergence of other species as infectious agents is likely used.

Chronic mucocutaneous candidiasis (CMC)

Patients with chronic mucocutaneous candidiasis (CMC) are selectively unable to clear *Candida*, and are prone to persistent debilitating infections of the skin, nails, and mucous membranes. *C. albicans* is implicated in most of cases(145). Other *C. species* have also been reported to cause disease in CMC patients(146-148). The exact molecular defect is not known for most forms of primary CMC, with the exception of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome. Patients with primary CMC rarely develop invasive disease or disseminated *Candida* infection.

APECED

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED; MIM#240300, locus 21q22.3), also known as autoimmune polyendocrine syndrome type I (APS I), is an autosomal recessive disorder caused by mutations in the *AIRE* gene. There is progressive loss of tolerance against self which leads to multiple organ failure via immunological destruction of endocrine glands. Functional studies of *AIRE* suggest a role as a transcription factor in antigen presenting cells. APECED may also be inherited as an autosomal dominant disorder (149) due to AIRE mutations resulting in a dominant negative effect. The mutant protein binds to the wild-type AIRE protein, preventing formation of complexes needed for transactivation. APECED patients usually present with CMC in childhood. The most common endocrine manifestations are hypoparathyroidism, Addison disease, and ovarian failure, followed by diabetes mellitus type 1, testicular failure, hypophysitis, and autoimmune thyroiditis. Patients may have autoimmune gastritis, autoimmune hepatitis, and malabsorption. Hyposplenia/asplenia has been reported. Keratoconjunctivitis, vitiligo, alopecia, nail pitting are common.

Autosomal dominant and recessive inheritance of CMC

APECED usually follows autosomal recessive inheritance. Sporadic occurrence or autosomal dominant inheritance has been described in most CMC families where APECED has been excluded.

Treatment of *Candida* in CMC

The persistent immunological defect of chronic mucocutaneous candidiasis requires a long-term approach that is analogous to that used in AIDS patients with rapidly relapsing oropharyngeal candidiasis. Systemic therapy is needed, and all azole antifungal agents (ketoconazole, fluconazole, and itraconazole) have been used successfully. Dosage is as in other forms of mucocutaneous candidiasis. Development of resistance to these fungal agents has been described.

The susceptibility to *Candida* is still a mystery.

Even if the gene responsible for APECED has been found, the immunological defect causing susceptibility to candida is still a mystery. Studies in *Aire-/-* knockout mice highlight the importance of type 1 cytokines in protection against *Candida*, and previous work suggests that CMC patients may have aberrant cytokine production in response to *Candida.*

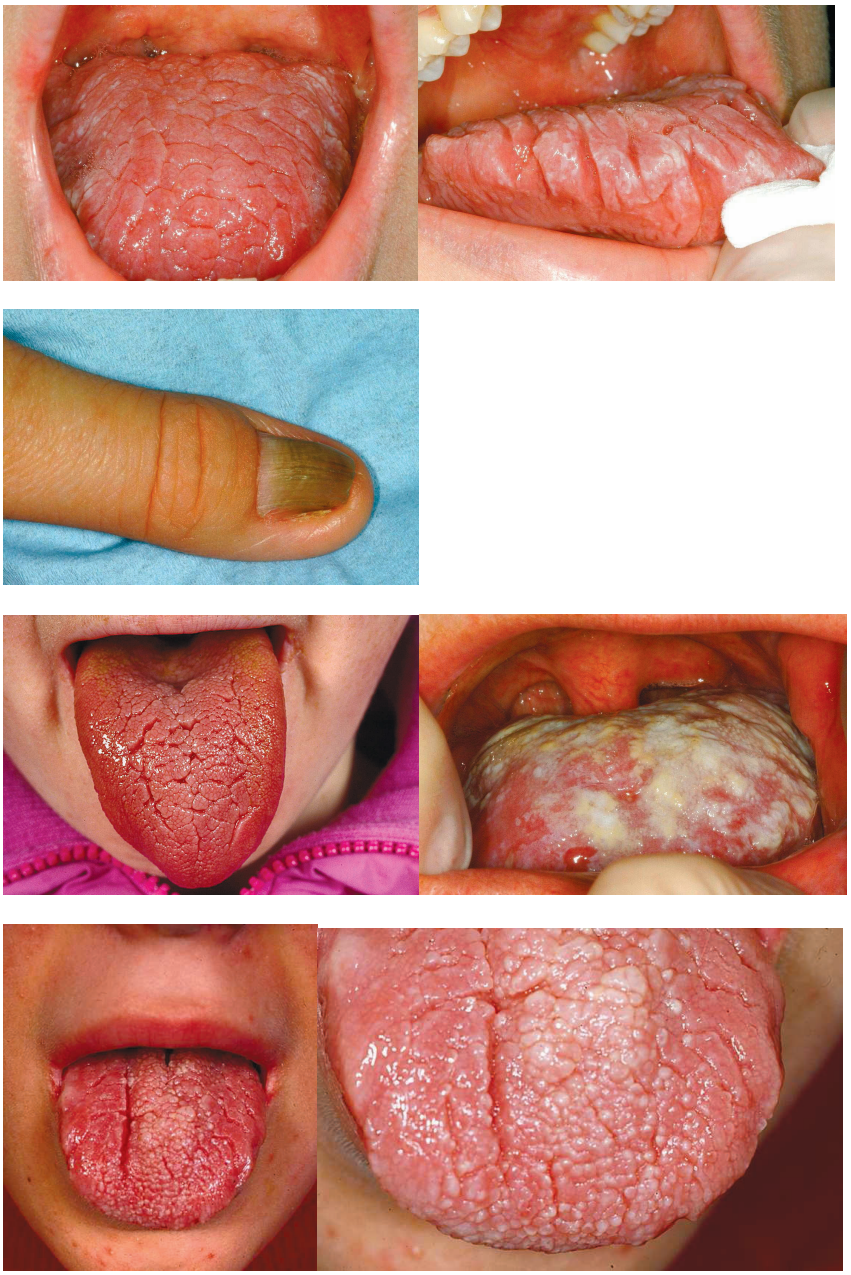
Protection against *C. albicans* has been considered to be T-cell-mediated

When the antigen presenting cells (APCs) interacts with CD4+ T helper cells, the T cell responses induced are classified as Th1 or Th2. In general, Th2 responses are linked to

induction of humoral responses, and Th1 to cellular responses. Protection against *C. albicans* is generally considered to be T cell mediated. Clearance of *Candida* is dependent on an appropriate Th1 response. Th1 type response, characterized by antigen stimulated lymphocyte production of cytokine IL-12 and interferon gamma, is associated with protection against *Candida* in animal studies(150), although the situation in humans is less well understood.

Studies of CMC and autoimmunity in knockout mice

Pathogenesis of the endocrinopathy in APECED has been studied, but remains somewhat unclear. Most patients have autoantibodies against autoantigens that are expressed in affected tissues. Studies in knockout mouse models have highlighted a role for AIRE in regulating expression in the thymus of a subset of autoantigens from peripheral tissues. Normally, potentially autoaggressive T cells that recognize these antigens are deleted, but they are presumed to escape to the periphery in patients with *AIRE* mutations or in *Aire–/–* mice, leading to autoimmune inflammation in selected organs. Aire is expressed in APCs in the thymus, spleen and in peripheral dendritic cells.



(Photos: TAKO centre & A.Stray-Pedersen)

Figure 16 Photos of patients with chronic mucocutaneous candidiasis

Dendritic cells (DCs) are antigen presenting cells (APCs)

Dendritic cells (DCs) form an enormously heterogeneous group of APC with different lineage backgrounds, precursors and various stages of differentiation and maturation (Figure 2 and Figure 17). The lymphoid DCs originate from pre T cells in the thymus and predominantly populate the thymic cortico-medullary junction where they are instrumental in the deletion of deleterious autoreactive T cells. The myeloid DCs originate from a specific CD34+ precursor in peripheral blood or from CD14+ circulating monocytes. The monocyte-derived DCs are closely linked to other classes of APC, such as the veiled macrophages and often to other types of accessory macrophages.

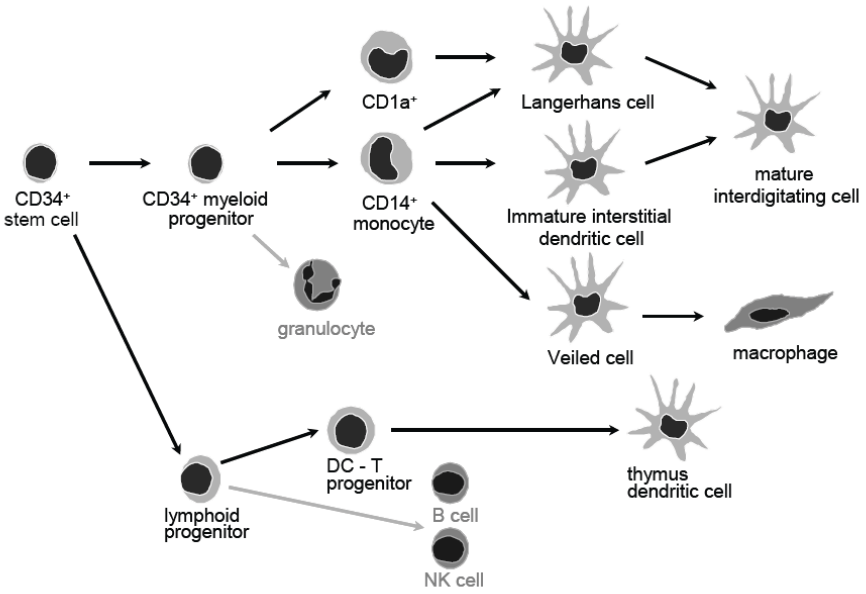


Figure 17 A scheme of origin and maturation of dendritic cells

Human leukocyte antigen (HLA) = major histocompatibility complex (MHC)

Selection of both related and unrelated donors for hematopoietic stem cell transplantation includes matching for alleles of each human leukocyte antigen (HLA) locus within the major histocompatibility complex (MHC) on chromosome 6p21.3. In human populations, multiple blocks of genetic variation in the MHC are strongly associated with each other as extended haplotypes.

HLA haplotypes Class I and Class II

Haplotypes are a set of closely linked alleles on a single chromosome that tend to be inherited *en bloc*, i.e. the alleles do not become separated by recombination at meiosis. The class I gene complex contains three major loci, B, C and A, each coding for an alpha-chain which contains antigenic determinants and is polymorphic (i.e., has many alleles). In the cell membrane, the alpha-chain associates with beta-2 microglobulin (beta-chain), encoded by B2M at locus 15q21-q22. The class II gene complex also contains at least three loci, DP, DQ and DR; each

of these loci codes for one alpha- and one beta-chain polypeptide which associate to form the class II antigens. Like the class I antigens, the class II antigens are polymorphic. HLA specificities are identified by a letter for locus and a number (A1, B5, etc.) and the haplotypes are identified by individual specificities (e.g., A1, B7, Cw4, DP5, DQ10, DR8). Specificities defined by PCR analyses are named with a letter for the locus and a four digit number (e.g. A0101, B0701, C0401 etc). MHC antigens are expressed on the cell surface in a co-dominant manner: i.e., products of both parental genes are found on the same cells.

HLA and connection to APCs

Class I antigens are expressed on all nucleated cells and platelets, but not on erythrocytes. Class II antigens are only expressed on B lymphocytes and a proportion of APCs: monocytes, macrophages and DCs. Small molecules (processed proteins) function as antigens and are presented in the cell membrane by MHC Class I and MHC Class II and both complexes are recognized by T cells which exhibit direct cytotoxic activity. CD4+ T cells further activate B cells causing humoral antigenic responses. When the MHCs are recognized by T cells as "foreign", cytolytic and humoral responses are induced. This is the mechanism underlying GVHD in stem cell transplantation, HVG in organ transplantation, and some autoimmune diseases.

HLA and autoimmune diseases

A number of autoimmune diseases have been found to occur at a higher frequency in individuals with certain HLA haplotypes. Most prominent among these are ankylosing spondylitis (B27), celiac disease (DR3) and Reiter's syndrome (B27). Unlike many other autoimmune diseases, APECED does not show an association to a specific HLA haplotype (151). We wanted to investigate if familial CMC with hypothyroidism, APECED excluded, was associated with certain HLA haplotypes.

Inherited thyroid diseases:
Autoimmune thyroiditis versus congenital thyroid disease.

Autoimmune thyroiditis is the main cause of acquired hypothyroidism in childhood in non-endemic goitre regions. In APSII, autoimmune thyroid disease accompanies Addison disease, and type I diabetes mellitus may also occur. CMC is not a feature of APSII. Autoimmune thyroiditis is a well known feature of APECED (=APSI), however in this context, it is far less frequent than hypoparathyroidism and Addison disease. There is increasing evidence that when APECED disease is excluded in the group of CMC patients, autoimmune thyroiditis is a frequent associated feature. In these CMC families, the disease is occurs sporadically or in an autosomal dominant inheritance pattern. Inherited resistance to TSH is the cause of the rare genetic congenital thyroid diseases. These patients may represent with neonatal hypothyroidism, but not all are detected on neonatal TSH screening. If hypothyroidism is detected, patients do not have a favorable outcome despite early and adequate treatment. Resistance to TSH is an inherited disorder of variable hyposensitivity to TSH. The metabolic consequences can range from euthyroid hyperthyrotropinemia to severe congenital hypothyroidism with thyroid hypoplasia. Causative gene mutations have been reported in the genes: *TSHR, PAX8* and also in the *TITF1, GNAS*, *FOXE1* genes associated with syndromic forms of congenital thyroid disease.

Mannose binding lectin – part of innate immunity

Mannose binding lectin (MBL) and other collectins

The collectins (C-type lectins containing collagen-like domains) are a small subfamily of large glycoproteins which includes the surfactant proteins A and D, and MBL among others {Lu, 2002 1594 /id;Holmskov, 2000 240 /id;Hansen, 1998 1595 /id}. Humans have serum and liver isoforms of MBL. Differentiation arises from alternative splicing and post-translational modification, as both are products of a single functional gene *(MBL-2)* on chromosome 10 {Kurata, 1994 1596 /id;Kilpatrick, 2002 280 /id;Cedzynski, 2004 1597 /id}. Surfactant A(SP-A) and D (SP -D) are important not only for enhanced lung compliance and low alveolar surface tension, but also play an important part in the innate immunity of the lungs. SP-A and SP-D are hydrophilic, in contrast to SP-B and SP-C which are hydrophobic. After secretion into the alveolar space, SP-A is located in the corners of the tubular myelin lattice and represents a first line defence against alveolar pathogens. There are extensive sequence similarities in structural configuration and functions in innate immunity between MBL, SP-D and SP-A, and particularly between SP-A and MBL(152;153). Their genes are also closely located on chromosome 10q (*MBL2* at 10q11.2-q21 and *SP-A (SFTP1)* and *SP-D (SFTP4)* at 10q22.2-q23.1).

MBL and complement activation, MBL deficiency and pneumococcal disease

MBL activates complement through the lectin pathway (Figure 18). Low levels of MBL results in an infantile illness characterized by recurrent infections, persistent diarrhea and failure to thrive(153-156). Homozygosity for certain MBL variant alleles has previously been shown to correlate with an increased frequency of invasive pneumococcal disease (157;158). Previous *MBL* genotyping of 100 Norwegian blood-donor controls showed that 3 % were homozygous deleterious for *MBL* variants (159)

MBL and *Candida albicans*

MBL is an important component of innate resistance to candidiasis. MBL binds to *Candida albicans*, inhibits *C. albicans* growth directly and activates complement(160). Parenteral administration of MBL increase resistance to hematogenously disseminated candidiasis in mice studies, suggesting that MBL therapy may prevent disseminated candidiasis in high-risk patients(161). MBL binds *C. albicans* via its lectin domain, resulting in agglutination of organisms with outgrowth of hyphae(160). MBL does not facilitate opsonophagocytosis by monocyte-derived dendritic cells. MBL inhibits the growth of *C. albicans* independent of complement activation. Upon complement activation, further inhibition is observed. In conclusion, MBL plays an important role in the first-line defense against *C. albicans* without the need for opsonophagocytosis by dendritic cells.

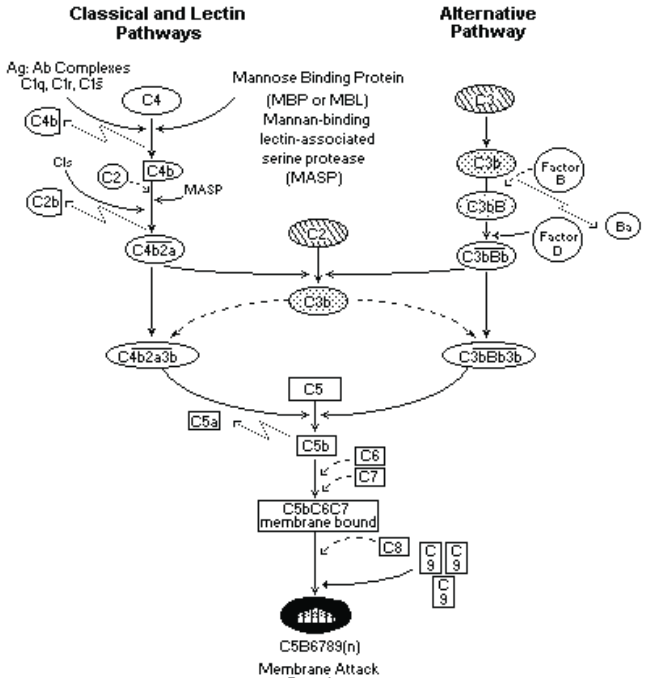


Figure 18 Complement pathway

There are two main pathways via which the effector functions of complement can be activated. The classical pathway is triggered by anitgen:antibody complexes, while the alternative pathway may be triggered by a number of substances such as bacterial lipopoly-saccharide (LPS) and cell wall components from various pathogens. The binding of serum lectins (such as mannose-binding protein) to pathogens can also initiate complement activation via the components of the classical pathway. Both pathways ultimately generate products that activate C3 and C5. The activation of C5 triggers the subsequent assembly of complement components C5b-C9 into the membrane attack complex, a hydrophobic "pore" that destroys the membrane integrity of the pathogen. In addition to its function in inducing cell lysis, the complement cascade produces fragments of complement components with specific biological functions, such as the chemotactic properties of C5a. A bar above the components indicates an enzymatically active complex of the components under the bar. Dashed arrows indicate contribution or release of a component. Solid arrows indicate an activation reaction (adapted from Janeway et al., 1997; Roitt et al., 1998).

***Streptococcoccus pneumonia* and immune defence**

The antibody response to pneumococcal capsular polysaccaride has been called a T cell independent process (TI type 2) with a predominant B-B cell interaction (162). Yet T cells and CD40 ligand contribute to regulation of this process (163;164). Wild-type infections or nasopharyngeal carriage normally induce pneumococcal antibodies, while vaccines (plain polysaccharide or conjugate) induce a more secondary response (165). Naturally occurring IgM antibodies to pneumococcus are polyreactive, while class-switched antibodies are serotype-specific (166). Oligoclonal pneumococcal antibodies of isotypes IgG2 and IgA predominate after vaccination and infection. Pneumococcal vaccines are capable of inducing antibodies within the IgG1 subclass (167). Specificity, avidity and protective efficacy depend on V(D)J rearrangements and somatic hypermutations. Certain V regions (V_H3 and $V_\kappa2$) dominate the repertoire, and specific V region genes, such as Vh3-48, are linked to high avidity and opsonic activity against a variety of pneumococcal serotypes (163;168;169). Several PIDs exhibit defective protection against *Streptococcus pneumonia*, including both immunodeficiencies caused by defects in adapted immunity (i.e. agammaglobulinemia or DNA repair disorders), and defects in innate immunity (i.e. IRAK4 deficiency or MBL deficiency). The use, utility and efficacy of pneumococcal vaccines and other vaccines in PIDs have been controversial. Vaccine responses are used in the diagnostic work-up of primary antibody deficiencies.

Aims of the study

Collect information on all PID patients in Norway

- to describe the incidence and prevalence of the various primary immunodeficiency diseases and their regional distribution in Norway, and
- to increase knowledge of the care and treatment of PID patients in order to
- facilitate better healthcare planning and care of patients.

Study of coping, quality of life and hope in a selected group of adults with PID

- To elucidate two main issues:
  a) How do adults with primary antibody deficiency manage their condition?
  b) What kinds of factors influence coping, quality of life and hope?

Studies in a selected group of PID, the patients with ataxia-telangiectasia

- To characterize the immunodeficiency in ataxia-telangiectasia:
  a) Find potential genotype-phenotype correlations in the immunodeficiency in A-T
  b) Test the response to conjugated pneumococcal vaccine in A-T
- To study alpha fetoprotein levels over time and assess for correlations with genotype, neurologic deterioration and liver enzyme serum levels.

Studies in other PID patients

- To study immunological abnormalities in patients with familial CMC with hypothyreosis, APECED excluded.
- To describe a patient originally considered to have A-T, but in whom genetic and immunological studies confirmed a chromosomal microdeletion of 14q and MBL deficiency

Material and Methods

Patients:

Patients with PIDs – epidemiology (Paper I)

Information concerning patients with PIDs were collected through their hospital physicians. Questionnaires were sent to all hospital departments taking care of PID patients. As of 1999, a total of 372 PID patients (303 living) were registered (Paper I). We were allowed to keep and update the Norwegian PID research registry until 2005. As of December 2004, a total of 566 PID patients (456 living) were registered. These data are presented in Results 2 (Table 5, Table 6 and Figure 1).

Patients with antibody deficiencies - selected for the coping, quality of life, and hope study (Paper II)

Studies of coping strategies, scales for measuring different aspects of quality of life and hope are designed for and need to be tested on adult persons with a more or less consolidated personality. The largest group of adult PID patients is found within the group of antibody deficiencies. The adult PID patients diagnosed with primary antibody deficiency and served by Rikshospitalet as of 1999 were selected for the coping, quality of life, and hope study. Based on data from the national PID registry (Paper I), we knew that a total of 122 patients ≥ 20 years of age had primary antibody deficiency, and that Rikshospitalet served 75% of these patients. After excluding one person with mental retardation, the cohort included 91 persons; 50 men and 41 women, aged 20–82 years, with various types of antibody deficiencies: Common variable immunodeficiency (n = 66), X-linked Bruton's agammaglobulinemia (n = 8), selective IgA deficiency (n = 16) and hyper IgM syndrome (n = 1). Twenty three patients had hepatitis, and of these 21 had verified HCV hepatitis. These 91 persons received the 30 page questionnaires by ordinary mail. Fifty five responded. Responders were representative of the original cohort of 91 persons with regard to specific PID diagnoses, gender and age.

PID patients selected for the interviews

The questionnaire study was supplemented with selected interviews of extreme cases. Cases were selected for interviews to detect possible patterns within two groups: patients with high QLI (quality of life) scores and patients with low QLI scores. The selected cases represented a strategic sample of patients with the lowest and highest QLI scores (n = 21). Originally, we wanted to interview all 21 extreme cases. **Ten patients consented** to participate in the interview study. Based on power calculations, 10 cases were regarded as sufficient to detect patterns within the different groups. One person, H.M.H. Sigstad performed the interviews. The different authors' contributions to this study are described in detail elsewhere (Acknowledgements).

Patients with Ataxia-Telangiectasia (Paper III-V)

A total of 14 patients with A-T have been studied prospectively studied during the immunological study, vaccine study and alpha fetoprotein study.

We were in contact with eight of the nine living Norwegian A-T patients registered per January 2000. These eight were first included in the immunological study. After initiation of the A-T immunological study, the last patient and two newly diagnosed A-T patients were also included. Similarly, after initiation of the pneumococcal vaccine study, an additional three newly diagnosed patients were included via their paediatricians. Information about the ongoing A-T studies had reached the new families through the A-T family meetings at Frambu Center for Rare Disorders and the Pediatric Rehabilitation Unit in Hedmark County. One patient had problems with repeated blood sampling and declined to participate in the vaccine study. His parents decided to withdraw him from the study. Two patients died after the immunological study was finished and before the vaccine study was initiated. After 2004 an additional three patients with A-T were diagnosed in Norway. As of 2006, we knew of a total of 32 persons diagnosed with A-T in Norway during the last 50 years, 14 living and 18 deceased, including the five patients reported by Smeby (61). Updated epidemiological and genetic data on A-T in Norway are presented in Result 2: Table 7, Table 8 and Figure 20

- and their parents (Paper III-V)

Twenty two parents were studied. The A-T patients' parents were included in the immunological study for two main reasons. Firstly, we wanted to check for possible immunological consequences of their carrier status, especially to see if the twelve parents who were carriers of the Norwegian founder mutation differed in immunological status from parents carrying other *ATM* mutations. Secondly, knowledge of the parents' immune status allowed us to assess the need for extra vaccines to prevent exposure of their immunodeficient child to certain infectious diseases. The results of the serum alpha fetoprotein measurements from these 22 parents are included in Paper V.

Ethical issues in the immunological studies of A-T patients

Oral and written information was given to patients and their parents. Written informed consent was obtained from the parents on behalf of each patient included in the study. The parents signed the vaccination consents on behalf of their children, and the older patients had to orally agree in the vaccination before participation. The vaccinee or a parent answered a questionnaire concerning adverse reactions after each vaccination. Information about pneumococcal vaccination status was documented in each person's medical record. The Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate approved the immunological study, and The Norwegian Medicines Agency approved the vaccination study protocol. Written informed consents were obtained for all collection and storage of biological material and *ATM* related genetic testing.

Control persons in the vaccine study (Paper IV)

Control persons for the vaccine study came from Rikshospitalet. We originally wanted to recruit healthy children, but this is difficult in a hospital setting. Persons with no to minor heart disease were selected from the Pediatric Outpatient Clinic at Rikshospitalet. Twenty-five individuals (13 males and 12 females) with no or minor heart disease served as sex and age matched controls. All controls had followed the national pediatric vaccination program. Exclusion criteria were: current infection, cancer/cancer treatment, corticosteroid treatment,

previous adverse reactions to other vaccines including diphtheria, other vaccinations six weeks prior to or after administration of the study vaccines.

Ethical issues control persons for vaccinations

The Norwegian Medicines Agency, the Regional Committee for Medical Research Ethics as well as the Norwegian Data Inspectorate, approved this study. Oral and written information was given to controls and their parents. Signed consent was obtained from each vaccinee or his/her parent.

Patients with Chronic mucocutaneous candidiasis (Paper VI)

Most of our patients with chronic mucocutaneous candidiasis CMC had APECED with verified mutations in the *AIRE* gene (170). Thirty eight patients have been registered with CMC in the PID registry (Update PID report, Results 2: Table 5), 23 had APECED (2 deceased), 4 had CMC linked to hypothyroidism and 9 had CMC without endocrinopathy. Recently, an additional 13 Norwegian APECED patients (3 deceased) have been reported (171). For several years, we have followed two families with several affected members with CMC associated with acquired, primary hypothyroidism, and where the disease does not segregate with the *AIRE* gene. Clinical and laboratory data on these two families are reported in Paper VI.

Ethical issues in the chronic mucocutaneous candidiasis study

The CMC study was approved by the Western Norway Regional Committee for Medical Research Ethics.

14 q deletion and MBL deficiency (Paper VII)

This patient was originally referred to us because ataxia-telangiectasia was suspected on the basis recurrent infections and neurological symptoms. He was investigated as part of our A-T study. He had a normal serum AFP level. No mutations in the ATM gene were found, and a constitutional chromosomal aberration was found. His proximal interstitial 14q microdeletion included the *TITF1* gene, a transcription factor for SP-A. The SP-A and SP-D collectins are structural homologues to MBL protein, and their coding regions are close to the *MBL2* locus. Since MBL deficiency was found in his serum, a family study was initiated to investigate if his MBL protein deficiency was caused by familial *MBL2* variant alleles or associated with the *TITF1* haploinsufficiency.

Methods

The epidemiological PID study

The simple PID questionnaire

In order to register all detected PID cases in Norway, a simple, two-page questionnaire was sent to all relevant hospital departments. Physicians were asked to classify each patient's disease as: antibody deficiency, T cell or combined B and T cell deficiency, phagocyte deficiency, complement deficiency, or other immunodeficiency. Since PID consists of a variety of different subtypes, subgroup categories were useful epidemiologically and allowed for simplification of the questionnaire. When necessary, additional information, such as laboratory results, was obtained at a later stage of the study. The questionnaire permitted reporting patients in whom PID was suspected, but not established. In April 1998 the questionnaires were mailed to 140 departments in 60 hospitals. All non-psychiatric hospitals in Norway were contacted. The patient organization, The Norwegian Immunodeficiency Association, distributed the questionnaire to its members. One reminder, with relevant ICD-9 numbers, was sent to non-responders. In addition, several departments were contacted by telephone to clarify information already given and to repeat the request for responses. The investigators arranged lectures, seminars and presentations for health personnel about PID and this particular study during the study period. All PID patients' medical records accessible at our hospital were reviewed, and the diagnoses were confirmed or reclassified.

The PID registry update report

After January 1999 we continued to receive reports of new PID patients from other hospitals and other departments within our hospital. Relevant medical information concerning these patients and all new PID patients referred to and registered at our paediatric department were consecutively included in the registry until January 2005 (Results 2).

The PID Research Registry Database

The collected data were registered in a non-network-connected database. In order to meet legal requirements, all patient administrative and personal data were coded when entered into the database. We were granted permission to keep a list with the database identification numbers linked to the patients' names and dates of birth. This list allowed us to update the database and avoid double registration. As initially recommended from the Norwegian Data Inspectorate, the decode list was stored separately from the PID research database.

Demographic statistics for calculating prevalences and incidences of PID

Children were defined as individuals below 16 years of age. Demographic statistics were obtained from Statistics Norway (31). The point prevalences in paper I were calculated using geographical population data from January 1999. Population data from January 2005 were used in the updated report. The regional prevalence results were based on the patients' home addresses. C1- inhibitor deficiencies and other complement deficiencies were reported to us with no more personal information than gender and year of birth, and these patients were therefore excluded from calculations of geographical prevalence. Incidences of some of the specific PID diagnoses, such as ataxia-telangiectasia, were calculated related to livebirths using national population data for the last four to five decades.

Coping, quality of life, and hope study in adults with PID

The comprehensive questionnaire

Five different scales were incorporated into one comprehensive 30-page questionnaire. Four standardized scales had previously been translated and tested in Norwegian populations. The standardized scales were: Ferrans and Powers Quality of Life Index (QLI), Short Form-36 (SF-36), Jalowiec Coping Scale (JCS), and Nowotny Hope Scale (NHS). An additional scale designed for this project (RPP Scale), focused on resources and pressures in the past. Factor analyses were used to assess the empirical support for each subscale in all instruments. Internal consistency was estimated using Cronbach's alpha coefficient.
The questionnaire together with an information letter was sent to each patient's home address. Definitions of the following concepts: coping, coping strategies, locus of control, resilience against stress, closeness, competence, and hope are defined in Paper II. In Paper II, the difference between global and health-related quality of life; QLI versus SF-36, is discussed.

The personal interviews

The questionnaire study was supplemented with selected interviews of ten extreme cases, five with low and five with high quality of life scores based on results from the questionnaire. The interviews were included as a supplement to the survey to elucidate preconditions for coping, good quality of life, and hopefulness. The interview study was designed to probe and to aid in the interpretation of some of the results from the questionnaire. Cases were selected for interviews to detect possible patterns within two groups: patients with high QLI scores and patients with low QLI scores. The selected cases represented a strategic sample of patients with the lowest and highest QLI scores. Ten patients consented to participate in the interview study, and the size of the cohort was regarded as sufficient to detect patterns within the different groups. The qualitative interviews were based on significant results related to QLI in the survey. The interviews were semi-structured with a pre-written interview guide, and lasted nearly two hours. The interviews took place at the patient's home and were performed by the same person.

Ethical issues in the coping, quality of life, and hope study

Participants were guaranteed anonymity and the right to withdraw from the study at any time. A letter to respondents provided information about the potentially sensitive items in the questionnaire. For each of the ten patient interviewed, the interviewer defined beforehand the content of the interview and explained her role. The coping, quality of life, and hope study was approved by the Norwegian Regional Committee for Medical Research Ethics and the Norwegian Social Science Data Services.

A-T studies - Longitudinal follow-up of a selected cohort

Blood sample collections – Repeated blood samples

Paper III, IV and V are based on results from **repeated blood samples** and clinical investigations. The blood samples were collected and consultations with the patients, parents and control persons were provided as an integrated part of the regular follow-up at the Pediatric Outpatient Clinic at Rikshospitalet.

The studies in paper III, IV,V and VII were conducted in collaboration with a variety of professionals from different hospital departments. The first author was responsible for the project planning, clinical investigations, vaccinations, sample collection and evaluation of the results of the laboratory tests. In this chapter (Materials and Methods) the principles of the methods used are outlined. Detailed descriptions are given in each paper.

Vaccination method

The seven-valent pneumococcal conjugated vaccine (PCV7, Prevenar® Wyeth Lederle) was given as 0.5 mL injection in the deltoid muscle. Prevenar contains polysaccharides from seven serotypes [serotype 4 (2 μg), 6B (4 μg), 9V (2 μg), 14 (2 μg), 18C (2 μg), 19F (2 μg) and 23F (2 μg)] which are conjugated to a carrier protein (CRM197 from diphtheria toxin, about 20μg). After 6-12 months, the patients received 0,5 mL of the 23-valent pneumococcal polysaccharide vaccine (PPV23, Pneumovax® Aventis Pasteur MSD) intramuscularly. Pneumovax contains polysaccharides from following 23 serotypes (25 μg of each): 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F. All vaccinations were performed at our hospital by one trained person. PCV7 and PPV23 were used in paper IV. Diphtheria and tetanus vaccines (0,5 mL containing 15Lf Diphtheria toxin and 3.8 Lf Tetanus toxin) in addition to the PCV7, were used in Paper VII. Prior to each vaccination and six weeks after, a blood sample was collected. The serum samples were stored at -20°C until antibody testing analysis, and pre- and postimmunization samples were assayed simultaneously.

Immunological tests

Immunoglobulins/antibodies

Quantitation of serum immunoglobulins, total IgG, IgA, IgM, IgE and IgG subclasses was performed by nephelometry (Dade Behring, Illinois, US). Lowest detection limits were 0.06 g/L for IgA and 3 kU/L for IgE. Age specific reference values were developed and are presented here in Materials and Methods and in the supplement of Paper III (172). IgD was measured by immunodiffusion (Behring) with reference values according to Haraldsson et al. (173).

Specific antibodies

An in vitro toxin neutralisation test was used for detection of diphtheria antibodies (174). The detection limit was 0.01 IU/mL, and the protective limit previously defined to 0.1 IU/mL (174). Tetanus antitoxin was measured with enzyme linked immunosorbent assay (ELISA), and the detection and protective limit were 0.1 IU/mL IgG (175). Antibodies to the capsular polysaccharide of *Hemophilus influenzae* type b (Hib) were measured with ELISA using an antigen composed of Hib oligosaccharides conjugated to human serum albumin (HbO-HA) The protective limit was defined to 1.0 microg/mL(176). Antibodies to viral antigens were measured using enzyme immunoassay (EIA) for anti-varicella-zoster virus (VZV) IgG, anti-herpes simplex virus (HSV) IgG, and anti-measles IgG. The microparticle enzyme immunoassay (MEIA) was used for detection of anti-rubella virus IgG and anti-cytomegalovirus (CMV) IgG. EIA was employed for detection of the anti-Epstein-Barr virus (EBV) nuclear antigen (EBNA) and anti-EBV virus capsid antigen (VCA) IgG.

IgG antibodies to *Streptococcus pneumoniae*

IgG antibodies to *Streptococcus pneumoniae* were tested against the 23-valent polysaccharide vaccine (PPV23) and measured with ELISA (177), levels given in arbitrary units (U/mL). We compared our results to historical controls of healthy, unvaccinated adults (178), and levels of pneumococcal antibodies below 2.5 U/mL were regarded as low and non-protective. IgG antibodies to individual pneumococcal serotypes 4, 6B, 14, 18C, 19F and 23F and also to a mix of PPV23, were measured with ELISA after CPS adsorption of sera (Statens Serum Institut, Denmark) (177;179). For each serotype an IgG level > 1 microg/mL were regarded as protective (178).

Autoantibodies

Antibodies against thyroperoxidase (TPO-Ab), thyroglobulin (TG-Ab) and thyroid-stimulating hormone receptor (TSHR-Ab) were assayed by a competitive lumino-immunoassay method with kits from B.R.A.H.M.S, Diagnostica GmbH, Berlin. Antibodies against the enzymes 21-hydroxylase (21OH), side-chain cleavage enzyme (SCC), 17α-hydroxylase (17OH), aromatic L -amino acid decarboxylase (AADC), and glutamic acid decarboxylase (GAD) were measured with a method based on the in vitro transcribed and translated protein as described by Ekwall et al.(180).

Investigation of complement and mannose-binding lectin

The complement components C3 and C4 and total haemolytic complement activation products (CH50 and C-alternative way) were measured as described by Nielsen et al. (181).

Mannose-binding lectin (MBL)

Serum concentrations of MBL were quantified using ELISA (Antibodyshop/Statens Serum Institut, Copenhagen, Denmark) with detection limit at 5 nanogram/mL. Low MBL levels were defined as <400 ng/mL and extremely low if <100 ng/mL. To further characterise the role of MBL, a functional ELISA measuring the lectin pathway activation capacity of exogenously added purified complement C4b-binding capacity was performed as described by Petersen et al (182;183).

Surfactant protein D (SP-D)

SP-D was measured by ELISA as described in (184). Briefly, microtitre wells were coated with F(ab´)2 prepared from rabbit anti-SP-D antibody, incubated with dilutions of test serum, later incubated with monoclonal antibody anti-human SP-D, and then peroxidase was added. Serum samples were tested simultaneously in 1:5 and 1:10 dilutions and the mean value calculated. Reference values for SP-D are 370-1300ng/mL according to (185). Three biallelic polymorphisms at the SP-D codons for the amino acids in position 11, 160, 270 in the mature SP-D were genotyped using an in house PCR-sequence specific priming method.

Alpha fetoprotein

Quantitation of alpha fetoprotein was performed in serum samples with AutoDELFIA™ hAFP (Wallac Oy, Turku, Finland). The AutoDELFIA™ hAFP assay is a solid phase, two-site fluorimmunometric assay with immobilized monoclonal antibodies and fluorescent urorium-labeled monoclonal antibodies technique. Reference value for AFP is < 14 kU/L with both techniques.

Flowcytometry for immunophenotyping/lymphocyte quantification

Flowcytometry is the generic term for techniques which involve analysis and/or separation of particles in a flowing stream by quantification of optical parameters. Flowcytometry allows analysis of various properties of cells or particles suspended in a fluid, which flows past a detector point, where the stream is illuminated by a focused laser beam. Cells are usually labelled using fluorescent probes/antibodies which bind to specific cell associated molecules. As the cells flow past the detector point and are illuminated, the probes fluoresce, the emitted light is detected and converted into proportional electronic signals. In this way measurements of various phenotypic, biochemical and molecular characteristics of individual cells are possible. In all our immunological studies, flowcytometric immunophenotyping of peripheral blood leukocytes was performed using the TruCount technique (Becton Dickinson, San Jose, California) with lysed heparinized blood. The monoclonal antibodies used were: anti-CD3, anti-CD4, anti-CD8, anti-CD19, anti-HLA-DR, anti-CD16, anti-CD56, anti-CD14, anti-TCR-αβ and anti-TCR-γδ. Age-adjusted ranges for the lymphocyte subpopulations were used.

Mitogen stimulation of lymphocytes

Mitogens are agents that are able to induce polyclonal cell division (mitosis) in a high percentage of T or B cells. There are T and B cell mitogens. A number of common mitogens are lectins, proteins which bind to glycoproteins on the surface of the lymphocytes and cause activation. Importantly, lectins do not act via conventional TcR-epitope or Ig-epitope interactions. The lectins concanavalin-A (ConA), and phytohemagglutinin (PHA) are T cell mitogens while pokeweed mitogen (PWM) activates both T and B cells. Another important mitogen which is NOT a lectin is LPS (lipopolysaccharide). This polysaccharide component of the outer membrane of gram negative bacteria is also known as endotoxin. LPS is a very potent mitogen for B cells. Mitogen stimulation in our immunological study of A-T patients was performed with ConA, PHA and PWM. Stimulation index was defined as counts per min (cpm) divided with the spontaneous proliferation.

REFERENCE VALUES for immunoglobulins and lymphocyte counts

As part of our study, age specific reference values were developed and defined for IgA, IgM and IgG with subclasses and also for some of the lymphocyte subclasses. Reference values for IgE were taken from Compendium in Laboratory Medicine, Denmark, and Fürst AS med-lab, Oslo, Norway (internet address: www.furst.no) and for IgD from (173). Age specific reference values in percentage for lymphocyte subsets were calculated from the absolute counts given in reference (186). The age specific reference values used in our study when measuring immunologlobulins and lymphocyte subsets are presented here and are also available at:

http://www.blackwellpublishing.com/products/journals/suppmat/CEI/CEI2492/cei2492tableS1.doc

http://www.blackwellpublishing.com/products/journals/suppmat/CEI/CEI2492/cei2492tableS2.doc

Table 3 Reference values Immunoglobulins

Age y	IgM [a] g/L	IgD [c] IU/ml	IgG3 [a] g/L	IgG1 [a] g/L	IgA [a] g/L	IgG2 [a] g/L	IgG4 [a] g/L	IgE [b] kU/L
2-4	.2-1.55	0-146	.14-1.26	3.2-9.4	.25-2.30	.52-3.00	<.03-1.27	3-70
4-8	.2-1.7	0-296	.13-1.42	3.7-10.8	.35-3.00	.72-4.10	<.03-1.87	3-150
8-12	.3-2.0	0-296	.13-1.49	4.0-11.5	.6-4.00	.85-4.80	<.03-2.1	3-180
12-16		0-169	.18-1.63	3.7-12.8		1.06-6.1	.04-2.30	3-195
>16	.5-2.5	.01-169	.18-1.10	3.7-11.4	.8-4.6	1.06-6.4	.04-1.40	3-120

Immunoglobulin(Ig) subclasses are listed in table columns according to the Ig heavy chains constant region's genetic reading frame

a) IgA, IgM, IgG, IgG subsets (2.5th to 97.5th percentiles) : Institute of Immunology (IMMI) Rikshospitalet, Norway, 2003
b) IgE (5th to 95th percentiles) : Compendium in Laboratory Medicine, Denmark, and Fürst AS med-lab, Oslo, Norway (internet address: www.furst.no)
c) IgD (5th to 95th percentiles) : (173)

Table 4 Reference values Lymphocyte subsets

Reference [a]	Number of persons	Age Y	CD3+ x109/L	CD4+ x109/L	CD8+ x109/L	CD4-/CD8- /CD3+ % of CD3+	CD4-/CD8- /TCR γδ+ % of CD3+	CD4-/CD8- /TCR αβ+ % of CD3+	HLA-DR+ % of CD3+ [b]	CD19+ x109/L	NK x10^9/L	SI-PHA [c]	SI-ConA [c]	SI-PWM [c]
Ref. 1		2	3.5 (1.4-8.0)	2.2 (.9-5.5)	1.2 (.4-2.3)				8.6 (2.9-20)	1.3 (.6-3.1)	.4 (.1-1.4)			
		2-5	2.3 (.9-4.5)	1.3 (.5-2.4)	.8 (.3-1.6)				8.7 (3.5-17.4)	.8 (.2-2.1)	.4 (.1-1.0)			
		5-10	1.9 (.7-4.2)	1.0 (.3-2.0)	.8 (.3-1.8)				10.5 (2.6-36.8)	.5 (.2-1.6)	.3 (.09-.9)			
		10-16	1.5 (.8-3.5)	.8 (.4-2.1)	.4 (.2-1.2)				4 (1.3-13.3)	.3 (.2-.6)	.3 (.07-1.2)			
	51 adults	>16	1.2 (.7-2.1)	.7 (.3-1.4)	.4 (.2-.9)				7.5 (2.5-16)	.2 (.1-.5)	.3 (.09-.6)			
Ref. 2	43 adults	>16	1.4 (.8-2.2)	.9 (.3-1.3)	.4 (.2-1.1)	3 (1-9.8)	2.3(.8-10.3)	1.1(.1-3.8)	10.1 (3.8-26.2)	.2 (.06-.4)	.3 (.08-.6)	≥ 25	≥ 5	≥ 5

a) Reference values are presented as median value and percentiles (5th to 95th percentiles) according to Institute of Immunology (IMMI) at Rikshospitalet, Norway and (186)
b) Age specific reference values in percentage are calculated from the absolute counts given in reference (186)
c) SI, Stimulation index - counts per min (cpm) divided with the spontaneous proliferation

Genetic tests

ATM genotyping

Several mutation analysis techniques have been used to locate the disease causing *ATM* germline mutation in A-T probands as the Norwegian AT study has been ongoing for more than a decade. Mutation analyses of DNA prepared from peripheral blood cells had been performed using several different techniques: protein truncating test (PTT), denaturing gradient gel electrophoresis (DGGE), heteroduplex analysis, denaturing high performance liquid chromatography (dHPLC) followed by sequencing in both directions using DyeDeoxy Terminator Cycle sequencing kits (Applied Biosystems, Inc., Foster City, CA) using an ABI 3100 DNA sequencer (Applied Biosystems, Inc) as previously described (187;188). dHPLC is a high-resolution method for separating large molecules and is often used as an automated method for the detection of DNA sequence variants. dHPLC-based mutation or single nucleotide polymorphism (SNP) screening relies on different DNA thermodynamic properties between perfectly matched base pairs in homoduplex molecules and mismatches in heteroduplex DNAs. dHPLC analysis is conducted on a WAVE machine and detects about 70% of *ATM* mutations.

AIRE genotyping

Sequencing of coding regions of the *AIRE* gene was done in one affected CMC patient from each family in Paper VI after publication. Reported in Paper VI are results of the microsatelite analyses excluding segregation with the *AIRE* gene locus.

MBL genotyping

Detection of mutations and promoter polymorphisms in the *MBL* gene was performed by polymerase chain reaction (PCR) according to (189). The following polymorphisms were studied: three point mutations in the promoter region (position -550 (H/L variants), -221 (X/Y variants), -70 (nt C or T)), one point mutation in the 5' untranslated (UT) region at position +4 (P/Q variants) and three point mutations located at codons 52, 54 and 57 in exon 1, at nucleotide positions 223, 230 and 239.

HLA haplotyping

Patient samples were typed for HLA-DQB1 using a reverse dot blot kit (Amplicor, Dynal, Oslo, Norway). The DQA1 alleles and HLA-DRB1-DQA1-DQB1 haplotypes were deduced based on known patterns of linkage disequilibrium in the Norwegian population.

Microsatellite marker analysis

Three highly polymorphic microsatellite markers (D21S268, D21S1224 and D21S1890) located near the *AIRE* gene were used as markers for the transmission of the *AIRE* locus in the two families. Fluorescence labelled PCR products were analysed on an ABI 310 Genetic analyser and the Genescan Analysis software (PE Applied Biosystems, USA).

Fluorescent *in situ* hybridization FISH

Fluorescent *in situ* hybridization (FISH) is based on chromosome region-specific, fluorescent-labelled DNA probes. Probes are cloned pieces of genomic DNA that are able to detect their complementary DNA sequences, and produce a fluorescent signal against background stained chromosomes. The technique requires prior knowledge of the type and location of expected aberrations and can only be used for the analysis of a limited number of chromosomal loci at one time. The efficacy of FISH is limited in some applications by low-resolution sensitivity. FISH is used to confirm findings on microarray CGH, MLPA etc. For the 14q deletion study,

cosmids covering *TITF1* and one covering *PAX9* (190) were biotinylated by nick translation. Labeling and FISH were performed as described (191).

Multiplex ligation-dependent probe amplification MLPA

The MLPA technique is used for the relative quantification of up to 40 different DNA sequences. Each MLPA probe consists of two oliginucleotides that are ligated by a thermostable ligase if they bind to the target sequence. Target sequences are small (50-70 nucleotides). The ligated probe is then amplified by polymerase chain reaction (PCR) rather than the target sequence as in conventional assays. Each MLPA probe is designed to have a specific size so that, when run on a gel, the product of each probe can be identified by its size. This technique can be used to identify exon deletions and duplications and, potentially, the copy number of any unique sequence. MLPA is used to confirm findings on microarray CGH, FISH etc. Neither FISH nor MLPA can determine sequence alterations and chromosomal break points with precision.

Microarray CGH

With the introduction of microarray-based comparative genomic hybridization (microarray CGH), it is possible to overcome the main limitation of conventional CGH, low resolution. Microarray CGH has more and more become the method of choice for studying genome-wide DNA copy number changes (191). With microarray CGH, the metaphase chromosomes are replaced by cloned DNA fragments (±100–200 kb) of which the exact chromosomal location is known. This allows the detection of aberrations in more detail and, moreover, makes it possible to map the changes directly onto the genomic sequence. Microarray-based CGH builds upon the conventional CGH procedure, by the use of differentially labelled test and reference DNAs. Both DNAs, then, are hybridized to cloned fragments, most often genomic DNA or cDNA, which are spotted on a glass slide (the array). The DNA copy number aberrations are subsequently measured by detecting intensity differences in the hybridization patterns of both DNAs. A high-density miniaturized array of oligonucleotides spotted onto a glass slide. Expression arrays hybridize mRNA to quantify gene expression. Genomic arrays hybridize DNA to identify cryptic deletions and duplications, and are useful for determining which genes are involved. CGH enables analysis of all chromosomes in a single experiment and, in contrast to conventional karyotyping, no dividing cells are required. A main disadvantage of conventional CGH is its inability to detect mosaicism, balanced chromosomal translocations, inversions, and whole- genome ploidy changes. For the detection of these types of abnormalities, other techniques, such as spectral karyotyping and (multiplex) FISH, can be used.

Statistics

The data leading to Paper I, III, IV and V was collected in Microsoft Access 97 databases; one database for the PID registry, one for the A-T patients and carriers, and one separate database for the control persons in the vaccine study. To meet legal requirement, the patient administrative data were coded when registered. SPSS programs for Windows (SPSS 9.0 or SPSS 11.0) were used for the statistical analyses performed in paper II-V. To compare two groups, t-tests were used, while one-way ANOVA models were used to compare more than two groups. Bonferroni corrections were performed to accommodate multiple tests. To study the linear relationship between two continuous variables, Pearson coefficients and linear regression components were computed. Associations between categorical variables were studied with Fisher's exact test. The programme SamplePower 2.0 was used to calculate statistical power of the data prior to and after the studies. Reliability analyses (Cronbach alpha) were performed for the factors in each subscale of the Hope/QLI/Coping questionnaire.

Results 1

Paper I

PID in Norway – epidemiology

The objective of this study was to perform a national epidemiological survey of PIDs. Questionnaires were sent to all hospital departments taking care of PID patients. As of 1999 a total of 372 PID patients were registered, 303 were alive, yielding a prevalence of 0.7 per 10.000 inhabitants in Norway. Distribution between the main immunodeficiency diagnoses was as follows:
(a) antibody deficiencies (n=189) 50.8%,
(b) combined deficiencies included other immunodeficiency syndromes (n=46) 12.4%,
(c) complement deficiencies (n=78) 21.0%,
(d) phagocytic disorders (n=25) 6.7%,
(e) immunodeficiency associated with other congenital diseases (n=34) 9.1%.
We were able to calculate more accurate incidences of the specific diagnoses, such as Wiskott Aldrich Syndrome, X-linked SCID, Bruton's X-linked agammaglobulinemia and ataxia-telangiectasia. When incidence and prevalence of each specific PID is compared, our results with some exceptions correspond to estimates from other countries. Compared to reports from other European countries, the proportion of combined deficiencies is larger compared to antibody deficiencies because we had fewer patients with selective IgA deficiency. We also had a large proportion of complement deficiencies because of the prevalence of hereditary angioedema.

Paper II

Coping, quality of life, and hope in adults with primary antibody deficiencies

Questionnaires were sent to all patients $\geq$ 20 years of age with primary antibody deficiencies served by Rikshospitalet. The questionnaires consisted of several standardized scales: Ferrans and Powers Quality of Life Index (QLI), Short Form-36 (SF-36), Jalowiec Coping Scale (JCS), Nowotny Hope Scale (NHS), and one scale we designed with questions about resources and pressures in the past. Fifty five of 91 patients (aged 23-76 years) replied. Among the 55, low quality of life scores were related to unemployment, infections in more than four organs, more than two additional diseases, or more than two specific occurrences of stress in the last 2-3 months. Previous experiences related to the immunodeficiency such as episodes of illness, absence from school and psychosocial consequences were important. Persons with selective IgA deficiency had significantly higher QLI scores than those with other antibody deficiencies. Other differencies between the various antibody deficiencies were not found. HCV infection had no influence on QLI or SF-36 scores. An optimistic coping style was most frequently used, and hope values were moderately high. The questionnaire study was supplemented with selected interviews of ten extreme cases, five with low and five with high quality of life scores. Based on these interviews, the patients could be divided into three groups: 1) low QLI scores, low hope values, and reduced coping, 2) low QLI scores, moderate hope values, and good coping, and 3) high QLI scores, moderate to strong hope values, and good coping. Coping was related to the patients' sense of closeness and competence.

In conclusion, low quality of life scores in adults with primary antibody deficiencies were linked to unemployment and disease-related strains. Closeness and competence were preconditions for coping, quality of life and hope.

Paper III

A-T and Immunology

During the epidemiological study it became evident that some patient subgroups including A-T had been less well characterized immunologically than others. All living A-T patients were localized via the national PID registry, included in the study and thoroughly characterized immunologically. In addition we looked for possible immunological correlations to *ATM* genotypes. Despite normal leukocyte counts and normal to subnormal levels of lymphocytes in peripheral blood, the A-T patients had low numbers of B cells (CD19+) and T cells, especially CD4+ cells. Among the 11 A-T patients, we found a variable degree of IgG2 and IgA deficiency, as well as low/undetectable IgE values. The five patients homozygous for the Norwegian *ATM* founder mutation had the lowest IgG2 levels. All 11 patients had very low levels of antibodies to *Streptococcus pneumoniae.* There was a significant positive linear relationship between pneumococcal antibodies and IgG2 levels. Previous vaccinations with T cell dependent diphtheria, tetanus and Hemophilus influenzae type b (Hib) vaccines had induced protective antibodies in most patients. There was a striking discrepancy between the pathological immunological findings in the laboratory and the sparse clinical symptoms of immunodeficiency in the patients. No age related progression of the immunodeficiency was found.
Patients' parents were included in the immunological study, because of evidence which suggests that *ATM* carriers may be radiosensitive and have a moderately increased risk of cancer. Twenty-two parents were included, half of them heterozygous for the *ATM* founder mutation. Some abnormalities in immunoglobulin levels and/or lymphocyte subpopulations were observed but there was no correlation to *ATM* genotype.

Paper IV

A-T and pneumococcal vaccine

This study was a follow-up of Paper III. Others have previously vaccinated A-T patients with the "old type" 23-valent pneumococcal polysaccharide vaccine (PPV23) without a significant pneumococcal antibody response. In Paper II we documented that A-T patients are capable of making protective antibodies to other vaccines, such as diphtheria and tetanus toxin. These vaccines are designed to trigger a more T-cell dependent immunological response, compared to the polysaccharide vaccines. The fact that A-T patients had antibodies against Hib, diphtheria and tetanus, suggested potential for effect of the "new type" seven-valent pneumococcal conjugated vaccine (PCV7). After protocol approval from the Norwegian Medicines Agency, the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate, all Norwegian A-T patients were invited to participate in the vaccine study. Nine A-T patients and 25 age and sex matched controls were vaccinated with PCV7 and PPV23, and three A-T patients were vaccinated with PCV7 only. No significant increases in pneumococcal antibody levels were observed among the A-T patients after the single PCV7. Subsequent PPV23 vaccination resulted in a significant increase in antibody levels to the PPV23 mix, as well as to some of the serotypes. The vaccinations induced a 1.5- to 7-fold

increase in antibodies to the six different serotypes tested. The increases in pneumococcal antibody titres were lower than those observed in the controls (9- to 34-fold increase) The results of the vaccination study are valuable in planning care of A-T patients. PCV7 can be used to trigger and PPV23 to booster immune response and possibly preventing severe pneumococcal disease. To prevent severe pneumococcal disease in A-T is important since older patients tend to suffer from chronic respiratory failure and to be susceptible to pneumonias.

No correlation between mannose binding lectin (MBL) levels and pneumococcal antibody levels was found. Six A-T patients had low levels of serum MBL. The four patients with the highest MBL levels were not prone to respiratory infections. Low MBL levels may represent a separate additive effect of the immunodeficiency in these patients.

Paper V

A-T and alpha fetoprotein

By closely following A-T patients with repeated blood samples for years, we have documented an increased level of alpha fetoprotein and a continuous increase in AFP serum levels with increasing age. This observation has not been published previously. The role of AFP in A-T remains to be elucidated and different theories of why AFP is increased in A-T are discussed in the paper.

Paper VI

Chronic mucocutaneous candidiasis and hypothyroidism

We describe the clinical and immunological features of two families with chronic mucocutaneous candidiasis (CMC) and primary hypothyroidism. Family A includes three siblings with both candidiasis and hypothyroidism and four individuals with hypothyroidism only. Family B includes four members with candidiasis, including one male child with hypothyroidism. All individuals with CMC had oral candidiasis and onychomycosis from infancy. Facial seborrhoeic dermatitis, generalized folliculitis and scaling blepharitis were the main manifestations. Hypothyroidism became evident during childhood. No thyroid antibodies were present in the affected siblings in family A, while the male with hypothyroidism in family B had antibodies against thyroid peroxidase at diagnosis. Immunological evaluation revealed intra-individual variations in serum immunoglobulin levels, lymphocyte subsets and proliferative responses, but no consistent abnormalities. An elevated serum MBL level was found in all patients. Vaccine responses were normal. The disease showed no HLA haplotype association. *AIRE* gene region microsatellite markers did not segregate with disease nor were autoantibodies typical for autoimmune polyendocrine syndrome type 1 detected. The link between hypothyroidism and chronic mucocutaneous candidiasis remains to be identified.

Paper VII

Proximal 14q Deletion and MBL deficiency

We report an eight year old boy with developmental delay, ataxia, choreoathetosis, muscular hypotonia, thyroid dysfunction and severe pulmonary problems characterised by recurrent episodes of respiratory distress and pneumonia. He had a heterozygous deletion of proximal chromosome 14q resulting in haploinsufficiency of *NKX2.1 (TITF1)* and *PAX9*. He was also homozygous for the mannose-binding lectin *(MBL)* haplotype B, a variant in codon 54 in the *MBL2* gene on chromosome 10q causing absence of functional serum MBL. The 14q deletion was confined to the index patient, while the MBL deficiency and corresponding *MBL2* genotypes were found in his mother and his younger sibling.
We conclude that the *TITF1* haploinsufficiency is causative for his neurological disease and respiratory distress, and his susceptibility to infections is intensified by the coexistence of MBL deficiency.

Results 2

Update report – PID epidemiology and treatment

PID epidemiology

We were allowed to keep and update the national Norwegian PID research registry until the end of 2004. As of January 2005, a total of 566 PID patients (456 living) were registered. With a population of 4.6 millions, the prevalence of PID was 1.0 per 10.000 inhabitants in Norway. These updated results are not published elsewhere. The number of patients according to specific PID diagnoses is presented in the table below, compared with the numbers from 1999, and with the specific diseases sorted by the same categories as in 1999. The distribution of PID and differences in prevalence between counties and regions are presented here in the Table 5 and Figure 19. 110 persons had another family member registered in the PID registry.

PID update report - Treatment

PID patients on treatment with subcutaneous (ScIg) and intravenous immunoglobulins (IVIg) are included in this update report, as are patients treated with hematopoietic stem cell transplantation.
As of January 2005, a total of 127 persons including 17 children were being treated with immunoglobulins. 113 were on ScIg, 24 of whom also received IVIg infusions periodically or parallel to ScIg. Fourteen individuals were treated with IVIg only.

Since Rikshospitalet is the national referral centre for stem cell transplantations in PID patients, the table of stem cell transplantation outcome in patients with PID is recent, from June 2007. The outcome of hematopoietic stem cell transplantation in patients with PIDs (Table 6 in Results 2) is presented with permission from Senior Consultant Anders Glomstein, Section of Pediatric Hematology, Department of Pediatrics, Rikshospitalet.

Table 5 Primary immunodeficiency diseases in Norway – Update report year 2005

Primary immunodeficiency diseases in Norway				Jan 2005	Jan 1999
Main PID Diagnoses	**Specific PID diagnoses**	Living	Deceased	Total	Total
Antibody deficiency	Bruton's X-linked agammaglobulinemia	25	6	31	15
	Common variable immunodeficiency (CVID)	103	32	135	117
	Hyper IgM syndrome type 1,2,3,5	8	5	13	11
	IgG subclass deficiency	15		15	10
	Selective IgA deficiency	48	2	50	36
	Other sorts of antibody deficiencies	4	1	5	
Total Antibody deficiencies		203	46	249	189
Phagocytic disorders	Severe congenital neutropenia	3	2	5	3
	Cyclic neutropenia	18	1	19	8
	Idiopatic neutropenia	10	1	11	7
	Interferon gamma receptor 1 deficiency	7	1	8	2
	Chronic granulomatous disease	6	1	7	4
	Leukocyte adhesion defects (LAD1)	1		1	1
Total Phagocytic disorders		45	6	51	25
Complement deficiencies	C2 deficiency	10		10	7
	C4 deficiency	1		1	1
	C5 deficiency	2		2	2
	C8beta deficiency	1		1	1
	C1 inhibitor deficiency	70		70	67
Total Complement deficiencies		84		84	78
T-cell defects, combined B- and T-cell deficiencies and other well-defined immunodeficiency syndromes	Severe combined Immunodeficiency (SCID) [a]	6	15	21	12
	Ataxia-Telangiectasia	12	17	29	17
	Del 22q11, DiGeorge anomaly with immunodeficiency	33	5	38	8
	Trisomy 22q11	1		1	
	IPEX* (192)	1		1	
	Wiskott Aldrich syndrome	8	3	11	8
	X-linked thrombocytopenia	1		1	
	Other T-cell defects	1		1	1
Total T-cell deficiencies and combined B- and T-cell deficiencies		62	40	102	46
Other immunodeficiencies	Autoimmune lymphoproliferative syndrome	7		7	
	Hyper IgE syndrome	2		2	1
	Chronic mucocutaneous candidiasis (CMC) [b]	36	2	38	23
	Ivemark syndrome with asplenia	5	6	11	3
	XL EDA-ID*/NEMO (193)		4	4	3
	NKX2.1 haploinsuffiency	1		1	
	Pearson syndrome		1	1	
	Schimke immunosseus dysplasia (194)	2	1	3	
	Shwachman Diamond Syndrome	4	0	4	
	Hoyerall–Hreidarsson syndrome	1	2	3	
	X-linked immunoproliferative syndrome		2	2	1
	Chromosomal defects combined with immunodeficiency	3		3	3
Total Other immunodeficiencies		61	18	79	34
Total PID Norway		456	110	566	372

a) SCID includes T- B+ NK- SCID, T- B- NK+ SCID, Omenn syndrome and other types of SCID such as reticular dysgenesis.
b) CMC includes patients with APECED*, CMC patients with hypothyreosis and also CMC patients without endocrinopathy.
*Abbreviations:
IPEX; **i**mmune dysregulation, **p**olyendocrinopathy, **e**nteropathy, **X**-linked caused by *FOXP3* gene mutations
XL EDA-ID; **X**-**l**inked **e**ctodermal **d**ysplasia **a**nhidrotic **i**mmuno**d**eficiency caused by *NEMO* gene mutations
APECED; **a**utoimmune **p**oly**e**ndocrinopathy, **c**andidiasis, **e**ctodermal **d**ystrophy caused by *AIRE* gene mutations

Distribution and prevalence of PIDs

Living patients with PID compared to the size of the population per 1. January 2005

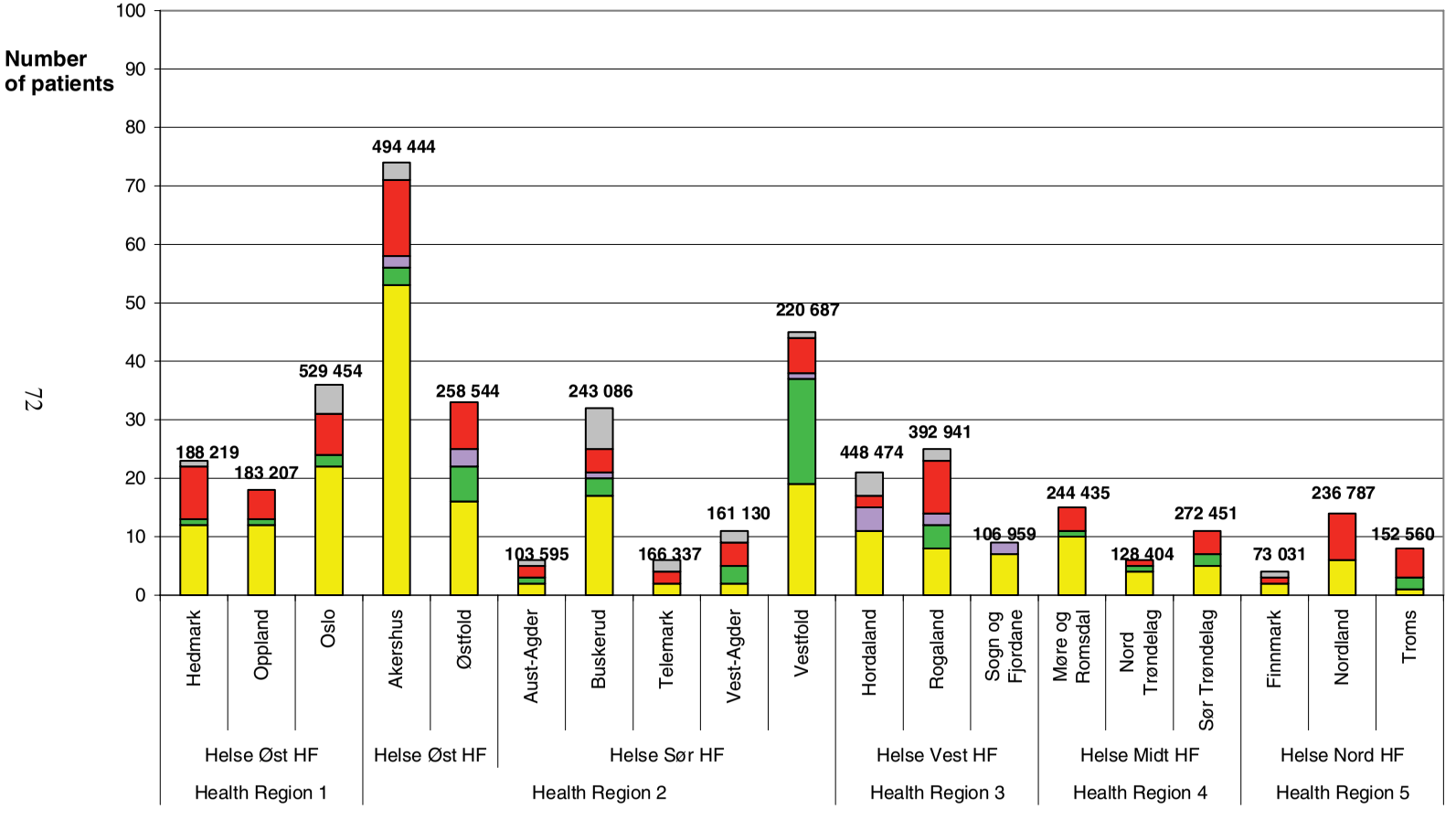


Figure 19 Distribution and prevalence of primary immunodeficiency diseases in Norway

Data based on where the patients lived when they got the PID diagnosis. Patients with C1 inhibitor deficiency are excluded from the data sets. The counties are sorted according to the former Health Regions 1-5. Since year 2001 the Health Regions 1-5 have changed into Helse Øst HF (East), Helse Sør HF (South), Helse Vest HF (West), Helse Midt HF (Middle), Helse Nord HF (North), respectively. Two of the counties, Akershus and Østfold that previously sorted to Health Region No. 2, are now sorting to Helse Øst HF (former Health Region No.1). The colours in the bars in the figure are referring to the same colours (and thereby groups of PID diagnoses) used in the Table 5 Primary immunodeficiency diseases in Norway – Update report year 2005: Yellow; antibody deficiencies, green; phagocytic disorders, violet; complement deficiencies, red; combined defects, and grey; other PIDs.

Table 6 Outcome of haematopoietic stem cell transplantations in patients with primary immunodeficiency diseases

PID diagnosis		Donor stem cells	Year of birth	Year of SCT	Year of death	Complications SCT	Preconditioning regimen	Where SCT
SCID	Reticular dysgenesis with deafness	Sibling, HLA identical	1995	1996 1997	Living	SCT x2, Successful	None	Norway
SCID	T-B+NK-	Parent, Haploidentical HCV+	1994	1995	1995	Pneumocystis jerovicii infection, Died of respiratory insufficiency	None	Norway
SCID	T-B+NK-	Parent, Haploidentical	1983	1983	Living	Chronic GVHD, ScIg treatment	NI	Abroad
SCID	T-B+NK-	umbilical cord non-identical + parental mesenchymal cells	2004	2005	2006	GVHD	Bu Cy ATG	Abroad
SCID	T-B+NK-	9/10 MUD, HLA C mismatch	2003	2004	2004	Aspergillus infection and TTP: Thrombotic Thrombocytopenic purpura	Bu Cy ATG	Abroad
SCID	T-B+NK-	MUD	2004	2006	Living	GVHD and ScIg treatment first year	Bu Cy ATG	Abroad
SCID	T-B-NK+	Parent, Haploidentical unfiltered	1989	1990	1990	Chimera, acute GVHD	None	Norway
SCID	T-B-NK+	Sibling HLA identical	2006	2007	2007	Died of pneumonia 2 months after SCT	None	Norway
SCID	T(+)B-NK+		2004	0	2005	Died during SCT preconditioning	Bu Cy ATG	Norway
SCID	T(+)B-NK+	Parent, Haploidentical	1996	1996, 1998, 1999	Living	SCT x3: the first two SCTs performed without preconditioning, the last SCT with modified Bu Cy. Successful	None x2, Modified Bu Cy x1	Norway
SCID	T(+)B-NK+	Parent, Haploidentical	1983	1984	1995	Chronic GVHD, lung fibrosis	None	Norway
SCID	T(+)B-NK+	umbilical cord, non-identical	2006	2007	2007	Death related to SCT preconditioning	Bu Cy ATG	Norway
Specific granula deficiency		9/10 MUD, HLA C mismatch	2005	2007	Living		Bu Cy ATG	Norway
Hyper IgM syndrome type 1 and cryptosporidiosis		Sibling, HLA identical	1985	2007	2007	Died one month after SCT of cryptosporidiosis + other infections		Norway
Leukocyte adhesion defect type1		Sibling, HLA identical	1987	2001	Living	Successful	Bu Cy	Norway
Severe congenital neutropenia (G-CSF treated) and leukemia		Sibling, HLA identical	1985	1998	2002	Successful despite chronic GVHD. Died of infection	Bu Cy	Norway
"Pearson syndrome"		MUD	1995	2004	2004	Died during SCT preconditioning	Bu Cy ATG	Norway
Wiskott Aldrich syndrome		Parent, Haploidentical	1986	1987	1988	GVHD, Death	Bu Cy	Norway
Wiskott Aldrich syndrome		MUD	1996	2001	Living	Successful, had GVHD first year	Bu Cy ATG	Norway
Wiskott Aldrich syndrome		MUD	1995	1996	Living	Successful	Bu Cy	Norway
Wiskott Aldrich syndrome		MUD	2002	2006	Living	Successful, had GVHD first years	Bu Cy ATG	Norway
Wiskott Aldrich syndrome		Sibling HLA identical	1995	1996	Living	Successful	Bu Cy	Norway
Wiskott Aldrich syndrome		Sibling HLA identical	1992	1993	Living	Successful	Bu Cy	Norway

Abbreviations: *SCT*, stem cell transplantation; *NI*, No information, *HCV*, hepatitis C virus; *GVHD*, graft versus host disease; *Bu*, busulphan; *Cy*, cyclophosphamide; *ATG*, anti-thymocyte globulin; *MUD*, matched unrelated donor; *G*-CSF, granulocyte-colony stimulating factor; *MTX*, methotrexate.

Ataxia-Telangiectasia in Norway, epidemiology and genetics

Disease incidence and A-T carrier frequency in Norway:

As of 2006, we knew of 32 patients born between 1951-2003 with A-T. The birth rate in Norway the last 50 years has been approximately 60.000 livebirths per year (Statistics Norway; http://www.ssb.no/). The incidence of A-T based on these figures is about 1:100.000 livebirths. The A-T carrier frequency is calculated to be 0.6 % in the general population. With 4.600.000 inhabitants per 2006, approximately 30.000 persons will be A-T carriers.

Nine of the 32 patients were born in Hedmark County which has a birth rate of 1800 per year (Statistics Norway; http://www.ssb.no/). In Hedmark County, the incidence of A-T is therefore 1:10.000 live births. The frequency of A-T mutant heterozygous alleles may be as high as 2 % in this area of Norway. The nine patients from Hedmark county were either homozygous or compound heterozygous for the Norwegian founder mutation, which originated in "Rendalen"valley in the northern part of Hedmark county. These data are presented in Results 2 (Table 7, Table 8, and Figure 20 Rendalen A-T Pedigree)

Eighteen of our 32 patients (born 1951-1985) are deceased; mean age of survival was 19,9 years (95% CI, 17.1-22.6) with three outliers: one patient died at 8, one at 9 and one at 23 years of age. Table 7 includes ATM mutations and cancer occurrence in 25 patients. In five families, two siblings were affected. Seven patients were homozygous and six compound heterozygous for the Norwegian *ATM* founder mutation.

Norwegian founder mutation from Rendalen in Hedmark County

Genealogical studies have identified a common ancestor for six of the Norwegian A-T families with the founder mutation. The common ancestor was born in 1495 in Rendalen, Hedmark County. The Figure 20 shows how the families are related. Further genealogical studies would most likely reveal common descent in more families given the allele frequency in Norwegian A-T probands.

Other mutations in *ATM*
and possible relationships between the Norwegian A-T families

Including the founder mutation a total of five mutations recur in the Norwegian families. These five mutations are responsible for 75% (30/40) of the parental *ATM* mutations. The mutation spectrum is presented in Table 8. The father in family NOAT 7 and mother in NOAT8 carry the same mutation and haplotype (187). They were born in the same community. The mother in NOAT7 carries the same mutation found in the mother of NOAT21, and this mutation was also found in both parents in family NOAT13 who were not aware of consanguinity prior to mutation analyses. The maternal and paternal grandfathers of NOAT13 carry the same mutation. These families originate from Southwest Norway. The father in NOAT3 and mother in NOAT20 carry the same mutation. The mother in NOAT9 and NOAT18 and father of NOAT15 carry the same mutation, and their families are probably originally from the same valley in Oppland County. In one family only one disease causing allele has been found (NOAT5). Linkage studies have concluded that the other mutation is *de novo* (187). Six of the mutations are also found outside of Norway, in Denmark, the US, the Netherlands, Great Britain, Germany, Poland and Brazil (187;195-201)

Predicted consequences of mutations

The mutations found in the Norwegian AT families (Table 8) have different consequences for the ATM protein: 27 (19 Rendalen + 8 other) of the 40 parental branches have frameshift mutations that lead to truncation of the protein, 5/40 have splice site mutations that lead to skipping of exons, 5/40 have nonsense mutations causing skipping of exons or inducing stop codons at the mutation site, 1/40 has a double substitution and 1/40 has a missense mutation.

Table 7 ATM mutations, gender, age and cancer in the Norwegian A-T patients year 2006

Identity	ATM Mutations[a] Paternal / Maternal	ATM Exons[d]	ATM[b]	Sex	Cancer	Age[c] di/re	Living 2006	Age y
NOAT1	c.3245_3247delATCinsTGAT(fs) / c.3245_3247delATCinsTGAT(fs)	24/24	R/R	M	ProT-ALL	7/8	N	22
NOAT2	c.3245_3247delATCinsTGAT(fs) / c.3245_3247delATCinsTGAT(fs)	24/24	R/R	F			N	26
NOAT6	c.3245_3247delATCinsTGAT(fs) / c.3245_3247delATCinsTGAT(fs)	24/24	R/R	F	B-lymphoma	14	N	21
NOAT10	c.3245_3247delATCinsTGAT(fs) / c.3245_3247delATCinsTGAT(fs)	24/24	R/R	M			Y	15
NOAT11	c.3245_3247delATCinsTGAT(fs) / c.3245_3247delATCinsTGAT(fs)	24/24	R/R	F	ProT-ALL	3	Y	13
NOAT14	c.3245_3247delATCinsTGAT(fs) / c.3245_3247delATCinsTGAT(fs)	24/24	R/R	M			Y	13
NOAT16	c.3245_3247delATCinsTGAT(fs) / c.3245_3247delATCinsTGAT(fs)	24/24	R/R	M			Y	11
NOAT5	c.3245_3247delATCinsTGAT(fs) / de novo	24/	R/a	F			N	17
NOAT8	c.3245_3247delATCinsTGAT(fs) / c.8264_8268delATAAG (ss)	24/58	R/a	F	ProT-ALL	6/9	N	9
NOAT17	c.6890A>C(ms)) / c.3245_3247delATCinsTGAT(fs)	49/24	R/a	M			Y	17
NOAT18	c.3245_3247delATCinsTGAT(fs) / c.4110_4110delG(ss)	24/30	R/a	M			Y	12
NOAT24A	c.3245_3247delATCinsTGAT(fs) / c.4588G>T(ns)	24/32	R/a	M			Y	6
NOAT24B	c.3245_3247delATCinsTGAT(fs) / c.4588G>T(ns)	24/32	R/a	F			Y	4
NOAT3	c.2880_2880delC(fs) / c.1931C>A(ns) .	21/15	a/a	F	B-lymphoma	13	N	23
NOAT4A	c.8978_8981delGAAAinsAT(fs) / c.7875_7876delTGinsGC (ds)	64/55	a/a	M			N	18
NOAT4B	c.8978_8981delGAAAinsAT(fs) / c.7875_7876delTGinsGC (ds)	64/55	a/a	F			N	33
NOAT7	c.8264_8268delATAAG (ss) / c.8432_8432delA(fs) .	58/60	a/a	F			N	21
NOAT9A	c.4110_4110delG(ss) / c.4632_4635delCTTA(ns)	30/33	a/a	F			N	21
NOAT9B	c.4110_4110delG(ss) / c.4632_4635delCTTA(ns)	30/33	a/a	M			N	22
NOAT13	c.8432_8432delA(fs) / c.8432_8432delA(fs)	60/60	a/a	M			Y	11
NOAT15	c.4110_4110delG(ss) / c.2938delA(fs) .	30/22	a/a	F			N	20
NOAT20A	c.5932G>T(ns) / c.2880_2880delC(fs)	42/21	a/a	F			Y	7
NOAT20B	c.5932G>T(ns) / c.2880_2880delC(fs)	42/21	a/a	F			Y	5
NOAT21A	c.6838C>T(ns) / c.8432_8432delA(fs)	49/60	a/a	F			Y	13
NOAT21B	c.6838C>T(ns) / c.8432_8432delA(fs)	49/60	a/a	M			Y	10
25 patients	20/46 are the c.3245_3247delATCinsTGAT(fs)		20R/30a	11M:14F				

a) Mutations in ATM gene are designed according to recommended nomenclature. Abbreviations: Ins, insertion; del, deletion; fs, frame shift; ds, double substitution; ms, missense; ns, nonsense and ss, splice site mutation.
b) R/R, homozygous for the Norwegian founder mutation; R/a, compound heterozygous for the founder mutation; a/a, other mutations in ATM, founder mutation excluded;
c) Abbreviations: di, age when the cancer was firstly diagnosed; re, age when the cancer relapse was detected. d) Blue and light blue indicate ATM mutations in early exons < 25 exon

Table 8 Parental mutations in *ATM* and predicted ATM protein alterations

Identity	cDNA	exon	PREDICTED EFFECT protein
NOAT3 mother	c.1931C>A	15	p.S644X
NOAT3 father	c.2880_2880delC c.2880delC	21	p.P961CfsX10
NOAT20 mother	c.2880_2880delC c.2880delC	21	p.P961CfsX10
NOAT15 mother	c.2938delT(fs)	22	p.Y980IfsX12
NOAT1 father	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT1 mother	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT2 father	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT2 mother	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT5 father	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT6 father	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT6 mother	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT8 father	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT10 father	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT10 mother	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT11 father	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT11 mother	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT14 father	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT14 mother	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT16 father	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT16 mother	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT17 mother	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT18 father	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT24 father	c.3245_3247delATCinsTGAT	24	p.H1082LfsX14
NOAT9 father	c.4110_4110delG c.4111delG	30	p.G1371GfsX16
NOAT15 father	c.4110_4110delG c.4111delG	30	p.G1371GfsX16
NOAT18 mother	c.4110_4110delG c.4111delG	30	p.G1371GfsX16
NOAT24 mother	c.4588G>T(ns)	32	p.E1530X
NOAT9 mother	c.4632_4635delCTTA(ns)	33	p.Y1544X
NOAT20 father	c.5932G>T(ns)	42	p.E1978X
NOAT21 father	c.6838C>T(ns)	49	p.Q2280X
NOAT17 father	c.6890A>C(ms)	49	p.Q2297P
NOAT4 mother	c.7875_7876delTGinsGC	55	p.DA2625EP
NOAT7 father	c.8264_8268delATAAG	58	p.Y2755CfsX12
NOAT8 mother	c.8264_8268delATAAG	58	p.Y2755CfsX12
NOAT7 mother	c.8432_8432delA c.8432delA	60	p.N2811TfsX46
NOAT13 father	c.8432_8432delA c.8432delA	60	p.N2811TfsX46
NOAT13 mother	c.8432_8432delA c.8432delA	60	p.N2811TfsX46
NOAT21 mother	c.8432_8432delA c.8432delA	60	p.N2811TfsX46
NOAT4 father	c.8978_8981delGAAAinsAT	64	p.R2993HfsX4
NOAT5 mother	Unknown - de novo?	-	

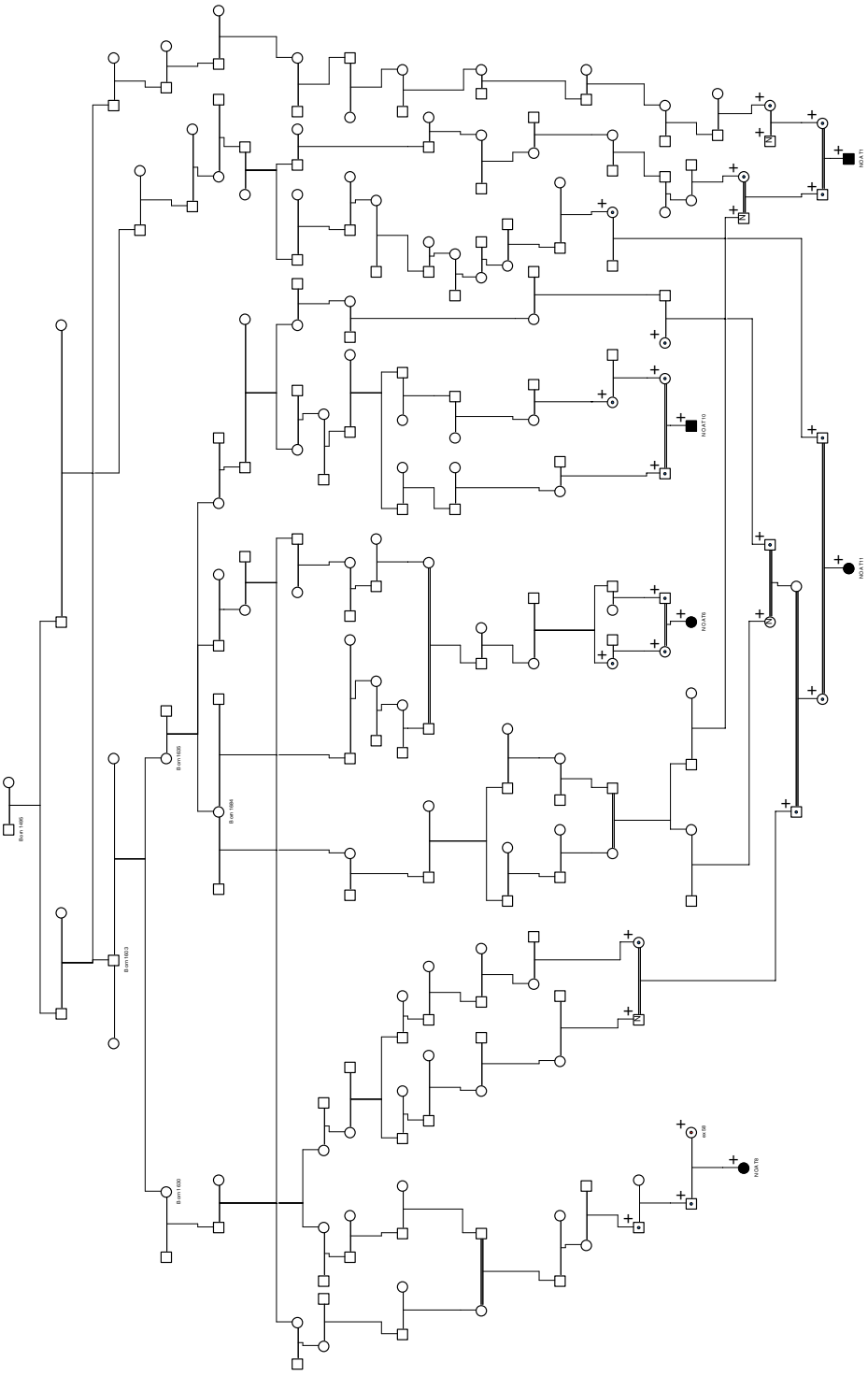


Figure 20 Rendalen A-T Pedigree

● ; Ataxia-telangiectasia

⦿ ; Carrier of the ATM founder mutation from Rendalen, Hedmark County, Norway

◎ Ex.58 ; Carrier of another ATM mutation

Ⓝ ; Not carrier of ATM mutation

◯⁺; ATM gene tested

By searching old Norwegian parish records and regional history books, a common ancestor have been identified for NOAT1, NOAT6, NOAT8, NOAT10, NOAT11 and NOAT14. Families NOAT16, NOAT17, NOAT18, NOAT24 know they have ancestors from the area around Rendalen, but the link to this pedigree has not yet been established. The big Rendalen pedigree is based on information given from the families including written information given by Anne Marie Andresen, the genealogical information are collected over years and have consecutively been supplied with new information, modulated and updated from the pedigrees drawn by Dr.scient Kirsten Laake and M.D. Andre Bregård
To avoid identification of the families, the unaffected siblings are not included in the pedigree

General Discussion

The presented papers (I-VII) all focus on collecting information concerning PID patients. We ascertained the number of PID patients (Paper I), and investigated how they were coping with their chronic disease and life in general (Paper II). The epidemiological study (Paper I) also revealed that there were some patients with uncharacterized immunodeficiency syndromes. In Paper III and IV we describe a cohort of patients with a specific PID, ataxia-telangiectasia. During the epidemiological study (Paper I) it came clear that the genotypes, but not the immunological phenotypes, had been defined in most A-T patients. We initiated the immunological study, searched for genotype-phenotype links, and were able to draw some interesting conclusions (Papers II-IV). When counting patients and sorting them into diagnostic categories and specific PID diagnoses, new and unclassified entities may appear. We found two families with CMC linked to hypothyroidism but without APECED (Paper V). Closer scrutiny of the A-T group revealed one patient with a new and different disorder (Paper VII).

Epidemiological studies in rare disorders and medical quality registries in Scandinavia

The great advantages of performing epidemiological studies in Scandinavia are our relatively small and surveyable populations, and our National Health Services with universal social healthcare (202). We also have a long tradition of national disease registries in Norway i.e. Cancer Registry of Norway http://www.kreftregisteret.no/, the Medical Birth Registry of Norway, and also medical quality registries for particular diseases such as the National Porphyria registry at the Norwegian Porphyria Centre (NAPOS) http://www.helse-bergen.no/avd/napos/. Using population-based registry data to calculate incidence and prevalence is contingent upon high detection rates. In the case of rare disorders official national-wide patient registries are necessary to ensure that all, or nearly all, patients are counted.

PID epidemiology - Not all PID registered

A main issue to discuss after Paper I is that not all Norwegian PID patients are likely to be recognised, diagnosed or registered. The geographical variation in prevalence of PID supports likely underdiagnosis. The true occurrence of PID may vary between regions (e.g., as for A-T) or medical professionals from some districts may not have reported all their PID patients, because of lack of awareness of the national PID registry. It is, however, very likely that the results reflect a difference in awareness of PID among medical professionals from different health regions and in different medical centres. PIDs are rare disorders and advanced immunological or specific molecular genetic investigations are often needed for accurate diagnosis. These tools are not available in all medical centres. Regional differences in primary, secondary and tertiary healthcare services including access to specialists may also have influenced the number of diagnosed and reported cases. Research activity and medical interests may influence the pattern of distribution of diagnoses causing overrepresentation of patients from certain medical centers. Half of the reported patients with primary neutropenia, for example, lived in the same county, and were referred or reported from the same hospital (Figure 19)(203). A similar example is the high frequency of hereditary angioedema in Northern Norway (Paper I). A special interest in PID research may contribute to the high prevalence of PID in the catchment of our hospital, Rikshospitalet, but does not explain all the geographical differences in PID incidence and prevalence. We have updated the PID research registry after the first publication, but this work was not done systematically. We only added

new PID referrals to the registry. An even more skewed PID distribution between regions was present in the latest update from January 2005 (Results 2 Figure 19). Some PIDs, such as APECED, are pleiotropic and require a multidisciplinary approach, Sometimes the immunodeficiency is not the clinically most important element of multiorgan disease, and reporting to the PID registry may very well be overlooked. The reported incidence of SCID based on recognized cases is 1/100.000 per live births, but is an underestimate due to death in infancy before diagnosis. SCID patients are missed and autopsy findings are nonspecific unless specific lymphocyte counts have been done prior to death. On the other hand, in some cases only autopsy could have had detected the PID. At least five factors cause underestimation of the true prevalence of PID: 1) lack of clinical recognition, 2) death before recognition, 3) variable access to cellular and molecular diagnostics, 4) lack of reporting to the PID registry, and 5) lack of standardized diagnostic criteria.

Classifications and definitions of PID are dynamic

One of the difficult challenges was to diagnose and classify PID patients correctly. Many patients registered as CVID may have had other PIDs, e.g., WAS, leaky BTK, XLP. Re-evaluation of each patient's complete medical record and laboratory results was necessary. After we started our epidemiological survey new PIDs have been defined as a result of molecular genetic research. X-linked Hyper IgM type 1 caused by CD40 ligand defect is a T cell defect, but this disease was previously classified as an antibody deficiency. Our former classification of female patients with agammaglobulinemia (combined with decreased or absent B cells) into the CVID group may therefore be incorrect. Some of these females may instead have one of the autosomal recessive μ heavy chain deficiencies, BLNK deficiency, Igalpha deficiency, λ(lambda)5 deficiency or other antibody deficiencies(204). The patients with hypogammaglobulinemia, low IgG and IgA and various levels of IgM have until now been classified into the CVID group, but may in reality have CD19 deficiency, ICOS deficiency, BAFF deficiency or defects in Ig subclass switching like AID deficiency, UNG deficiency, CD40 deficiency, the more comprehensive immunodeficiency CD40 ligand deficiency or another undefined immunodeficiency. Most of the agamma- and hypogammaglobulinemic patients are treated with immunoglobulin infusions, and one can argue that the new classifications into specific PIDs are of academic interest only. But for some of these PIDs knowledge about specific molecular genetic disease mechanisms may confer risk of additional diseases/complications and susceptibility to infection with specific microorganisms and have prognostic implications that should influence clinical management. For instance, it is important to know that patients with the CD40 ligand deficiency have a high risk of severe infection with cryptosporidiosis causing biliary canal stenosis and liver failure, or that lymphoproliferative and autoimmune signs are part of ALPS and WAS and that splenectomy may not resolve WAS patients' thrombocytopathia. The earlier classification of specific PIDs into the five groups of antibody deficiencies, combined deficiencies, complement disorders, phagocyte disorders or the groups of other PIDs/syndromes including PID was confusing. Recently classification has changed. For example, WAS classified as a predominantly antibody deficiency may cause neutropenia and T cell deficiency in addition to thrombocytopenia (205;206).

Medical records - from paper to electronic charts

Medical records were kept as paper charts during the study period. To collect and read thoroughly a large number of medical records was time-consuming and ineffective. Some patients with PIDs causing multisystem diseases, such as APECED, had multiple medical records within the same hospital; one medical record for each department. Today, at our

hospital and at most hospitals in our country, medical records are electronic and old documents are scanned to the chart. There is only one medical record for each patient. A given patient chart is accessible for more than one user at the same time. However laboratory results, results of radiological investigations and records for the same patient from multiple hospitals may not be easily accessible and searchable. When searching for new patients in the hospitals registry, the ICD-numbers have limited value as long as many of the specific PID diagnoses do not have their own ICD-10 numbers. For genetic diseases, OMIM numbers are of greater value because they are much more specific. It would be an advantage if these numbers were used in patient reporting hospital data systems.

Change in organisation of health care system in Norway – implications for management of PID patients and disease awareness

After we began to register PID patients, the five health regions in Norway were reorganised into five new regional health trusts (207), and more recently fused into four regional health trusts. The proportion of patients receiving medical treatment across regional health trusts was only 0.9% in 2003 (208). Since we have indirectly shown that awareness of PID differs among medical professionals, this reorganization of specialized healthcare services and hospitals may have consequences for the diagnosis and follow-up of new PID patients.

Our epidemiological data has been useful for others

Despite the limitations of our epidemiological survey and report update we collected a fairly large number of PID patients compared to studies from other countries. This may be consistent with variance in rates of detected/reported PID patients, rather than true differences in frequency of PID between the countries. Our population based calculations of frequencies of PID have already been valuable in planning healthcare services for PID patients in our country as well as in other countries, in documenting the need for PID awareness among healthcare professionals, and has also been useful comparing PID frequencies in different populations(209;209;210). Our results have been used by the US' Department of Health and Human Services' Centers for Disease Control and Prevention (CDC) in their recommendations and report "Applying Public Health Strategies to Primary Immunodeficiency Diseases" January 2004:
http://www.cdc.gov/genomics/hugenet/reviews/tables/SCID_Tables.htm

Other national PID registries and the ESID registry

In paper I our data were compared with the data published from other countries and the data from the old ESID (European Society for Immunodeficiencies) registry. After our publication in 2000 there have been some reports or update reports from other countries: Spain in 2001(211), Ireland in 2005 (212), Israel in 2002 (213), Iran in 2002 and 2006(214;215), Australia/New Zealand in 2007(216), and a second report from the Latin American PID registry in 2007(217). A new report calculating the population prevalence in US was published in 2007. This study was based on telephone calls to 10.000 random households, trying to find the true prevalence of PID(218). The internet based registry from Australia and New Zealand (2007) showed a geographic variation in PID registration from 1 to 12.5 per 100.000 living individuals (216). We had a mean registration of 7 PID patients per 100.000 (Paper I), increasing to 10 per 100.000 in our update report (Table 5). We also found substantial variation in PID registration between the different regions in our country (Paper I and Update report: Figure 19). The true frequency of certain diseases may vary between countries. Of a total of 1209 reported PID patients, only ten had A-T in Australia and New

Zealand (216). In the PID report from Iran (2002) a higher proportion of A-T patients were reported; (48/440). The report of PID from Australia and New Zealand was based on a single time point registration, while PID registries from other countries have included deceased patients. In comparison, our first PID epidemiology report (Paper I) contained 17 A-T patients including 10 deceased individuals. During our clinical immunological A-T studies newly diagnosed patients were referred to us (Papers III-IV), and we also received information about previously deceased A-T patients. This information was included until end of year 2004 in the national PID registry (Table 5). Recently, even more A-T patients have been diagnosed in Norway and our summary from year 2006 (Results 2) contained 32 known A-T patients (14 living, 18 deceased). Our first PID registry report (Paper I) was based on single time point registration combined with collection of retrospective data. This allowed us to register deceased patients, such as patients with fatal SCID. The epidemiological results emerging from our A-T studies illustrates the advantage of longitudinal collection of data, both retrospectively and prospectively, compared to single time point registration.

The A-T report from Iran 2007 (219), corresponds in many ways to what we have done based on our national PID registry. Characterizing and following cohorts of patients increases our knowledge of the specific disease. National multicenter registries, such as the CVID cohort study from Italy (220), can be used for follow-up of cohorts of patients with the same PID diagnosis. For assessing outcome of treatment, e.g., bone marrow transplantation, national and international collected medical data are important for formulating treatment recommendations for specific PIDs (23).

It is necessary to emphazise that prevalence and incidence of *specific* PID diagnoses, and not groups of PID such as "antibody deficiencies" or "phagocyte defects", should be used in making comparisons between countries. For example CMC patients in some national PID reports may have been classified as "T cell deficiencies" or "other immunodeficiencies", while new research shows that CMC is a phagocyte disorder. In contrast to the old ESID registry, specific PID diagnoses are available the in new internet based ESID registry (221-223). As we do in Paper I, the report from Australia states that the access to cellular/immunological and molecular diagnostic tests seem to lead to more patients being recognized and correctly categorized. Mutation registries, such as Bruton's X-linked agammaglobulinemia registry from Argentina 2003(224), represent a basis for future PID cohort studies.

Mutation databases for specific PID are already established (225) with a connection to the internet based European PID registry(221). The new ESID database has been designed for supporting continuous long-term documentation of patients, in order to improve diagnosis, classification, prognosis and therapy of PIDs. The ESID Online Database is a web-based system aimed at data storage, data entry, reporting and import of pre-existing data sources. It fulfills requirements of high-standard security and complies with present EU data protection laws and the demands of a modern research platform. The IDRbase (PID mutation databases) integrate clinical, biochemical, genetic, genomic, proteomic, structural, and computational data of primary immunodeficiencies.

International collaboration: National PID registry ⇆ ESID registry

Strictly speaking, few data from registries or large case-series have been published in the past 5 years in international journals and there should be a greater focus on international collaboration and pooling of data (212;213;215;226). The present and future *updated* reports

of all PID patients in one country will be of national interest, but of more minor international significance. Some of the recent updated national registry reports are published in national languages, not in *lingua franca* (211;213). It seems no longer relevant to publish separate updated reports of all PIDs from each country in Europe. However, it is still very important to collect information about all PID cases in each country, collaborate with the ESID registry and with other international databases for specific PIDs and publish national updated reports collectively, as part of an international clinical scientific effort.

Focus on particular groups within the PID registry

Using the PID registry, we were able to focus on particular groups and study more closely areas of special research interest e.g., psychosocial factors such as coping, quality of life and hope. Studies of coping strategies, scales for measuring different aspects of quality of life and hope are designed for and need to be tested on adults with a more or less consolidated personality. The largest group of adult PID patients is found within the group of antibody deficiencies. Even the selected group consisted of patients with different PIDs and represented a rather heterogeneous group of disorders. However, some common denominators were present. Including subjects with other immunodeficiencies would have increased the sample size at the cost of homogeneity.

Coping, quality of life, and hope study in adults with PID

The main purposes of the study reported in Paper II were to examine how adults with primary antibody deficiency manage their condition and to identify factors which are conducive to coping, good quality of life, and hopefulness. We found that low quality of life scores in adults with primary antibody deficiencies were linked to unemployment and disease-related strains. Previous experiences related to the immunodeficiency such as episodes of illness, absence from school, psychosocial consequences were important. Individuals with selective IgA deficiency had significantly higher QLI scores than those with other antibody deficiencies. Those with IgA deficiency had higher QLI scores in the socioeconomic and not surprisingly as expected in the health function domain. Other differences between the various antibody deficiencies were not found. During the years 1987-1993 the immunoglobulin preparation for intravenous administration (IVIg) was contaminated with *hepatitis C virus.* Several of the patients with antibody deficiency who had been treated with IVIg during that period were infected with HCV with subsequent significant morbidity. We found that HCV infection had no influence on the QLI or SF-36 scores.

Closeness and competence

The concept “competence” encompasses an individual’s skills and experience of usefulness. “Closeness” is defined on three levels: 1) dyadic relationship with one competent adult, 2) family, and 3) social network. Closeness and competence contribute to self confidence and thereby to resilience against stress. We found that closeness and competence were preconditions for coping, quality of life and hope. Closeness and competence emerged as areas where psychosocial interventions may contribute to better quality of life in adults with antibody deficiencies. The results from the adult PID study confirm Lazarus and Folkman’s universal theory of coping and resilience against stress. Our results should influence the organisation of medical care and follow-up for this patient group.

Why 60% represented a satisfying response rate

The questionnaire used in the coping, quality of life, and hope study was comprehensive and over 30 pages long. It was time-consuming and potentially challenging to answer. In order to perform statistical analyses and formulate conclusions of general validity in the study of "soft" factors such as coping, hope and quality of life, standardized comprehensive questionnaires and a high response rate are essential. A 60% response rate may be considered low, but is average for comprehensive quality of life questionnaires (227;228) That more than 60% completed and returned the questionnaire, may indicate that our adult PID patients, on the whole, were motivated. The distribution of gender, age, types of antibody deficiency and presence of HCV was similar in non-responders and responders. The response rate was judged sufficient for the statistical analyses. Including subjects with other immunodeficiencies would have increased sample size at the cost of homogeneity.

Triangulation – combining quantitative and qualitative studies

Triangulation, combining quantitative and qualitative methods based on different theories of coping, hope, quality of life, was a fruitful strategy. Triangulation of different methods strengthens the validity of research findings (Patton) (229). Our interview study supported the theoretic basis and constructs in the questionnaire, thereby strengthening the study as a whole. The interviews probed the individual's and general results from the questionnaire. However, the interview study also raised some new questions and hypotheses, which were not covered by the questionnaire. The interviews contributed to more nuanced knowledge and complemented the questionnaire, increasing the relevance of the project as a whole. The combined findings strengthened the validity and utility of our results.

Researchers have long debated the relative value of qualitative and quantitative inquiry. Phenomenological inquiry, or qualitative research, uses a naturalistic approach that seeks to understand phenomena in context-specific settings. Quantitative research uses experimental methods and quantitative measures to test hypothetical generalizations. Where quantitative researchers seek causal determination, prediction, and generalization of findings, qualitative researchers seek instead illumination, understanding, and extrapolation to similar situations. Qualitative analysis results in a different type of knowledge than does quantitative inquiry. Some researchers claim that qualitative and quantitative research can be effectively combined in the same research project, giving insights that neither type of analysis can provide alone (229;230). Also in medical science qualitative studies may be relevant (231).

The triangulation of methods, combining quantitative and qualitative studies and finding coherence with theories of human behaviour and attitude, is elegant and has been used in other recent quality of life studies in various patient groups(230). For example Lukkarainen studied health-related quality of life in people with coronary artery disease with this approach. Significantly, the qualitative approach provided an explanation for the poor psychosocial health-related quality of life scores obtained by the quantitative approach (232). Interviews and the application of phenomenological psychology helped us to gain insight into the informants' situational experiences of health-related quality of life and life course. This information was not elicited by questionnaire. Merkouris studying patient satisfaction found that the combination of qualitative and quantitative methodology appeared to contribute to the completeness of description and understanding of phenomenon (233). Anderson and Ferrans, in 1997, reported that indepth interviews provided a more complete understanding of the quality of life in their patient group and clarified the low ratings that were found on the quality of life index(234).

Choice of Instruments

Quality of life and patient reported outcome studies depend a great deal on the choice of appropriate instruments. Tools must be selected according to the domains they measure and the populations and pathologies for which they are designed. Practical issues, such as the availability of different translations, copyrights, and access to instruments are additional major criteria in the choice of instruments. In our project following instruments were used: Ferrans and Powers Quality of Life Index (QLI), Short Form-36 (SF-36), Jalowiec Coping Scale (JCS), Nowotny Hope Scale (NHS). These instruments had been used in similar studies of other patient groups and had been translated into Norwegian and previously validated in our country (235;236). In addition, past events in the patients' life needed to be documented. The first author (H.M.H.Sigstad) devised a new scale to measure resources and pressure in the past. Similarly to the JCS, this RPP Scale was founded on and Lazarus and Folkman's theory of stress and coping(237).

Coping Scale

Jalowiec Coping Scale (JCS) were used to assess coping (238). JCS is based on Lazarus and Folkman's theory of stress and coping. Other coping scales include, for example, the Coping Strategies Questionnaire (CSQ) that has recently been translated, cross-culturally adapted from an American to a German version. The reliability and validity of the translated version has been tested(239), but this coping scale is not yet ready to be used in the Norwegian population. Multicenter, multinational studies of psychological and social consequences of disease require questionnaires in the patients' own languages and the scales must be cross-culturally adapted, tested and validated on sample cohorts.

Hope Scales

As with social network and support, being able to hope may be helpful in the adaptive process and promote good quality of life. Hope has not been investigated in the other quality of life studies of PID patients. Different instruments can be used to study hope. Scales have been developed within various healthcare disciplines. Researchers with nursing background, in particular, have made important contributions. Examples of instruments used to study hope in patient groups are: Stoner Hope Scale (240), Miller Hope Scale (241), Nowotny Hope Scale (236), Herth's Hope Scale (HHS) and Herth's Hope Index which is a short version of HHS, recently translated and tested in a Norwegian population (242). The various hope scales have been developed based on different definitions of hope. For instance, Herth's Hope Scale and Index are based on Daufalt and Martocchios theories and understanding of hope. We chose Nowotny Hope Scale. Nowotny identified hope as an important factor in the quality of life of individuals with cancer and found that hope was present in all aspects of life and could vary over time, as difficult situations evolved. Nowotny defined hope as a multidimensional dynamic attribute of the individual which includes future orientation, active involvement, trust that what is hoped for is possible, belief that other people or a higher being are involved, and importance of outcome for the individual. Hope may act as a buffer against decreased quality of life and can be regarded as an expression of personal resources. In spite of having ''the same diagnosis,'' disease progression and quality of life vary between individuals and settings. We estimated that hope was responsible for 1/5 of the total variance of QLI in our adult patients with antibody deficiencies. A low hope score was found in patients with extensive disease. The subscales of the NHS provide a framework for the development and implementation of strategies to maintain, reinforce, and facilitate hope. Sources of hope, as well as divergence in the hopes of health care providers and patients with chronic diseases, could also be discussed.

Quality of life scales: QLI or SF-36

The validity and reliability of the instrument we used to measure global quality of life, the Ferrans and Powers Quality of Life Index, has recently been reassessed (243;244). SF-36 which measures health-related quality of life is well-known and widely used in health care assessments. It is available and has been tested in almost all languages and populations. The SF-36 Scale has recently been implemented in the ESID internet registry as well, and is well-suited for longitudinal follow-up reports of individual PID patients.

SF-36 versus measuring global QLI

As a rule, in quality of life studies of patient groups only the SF-36 scale is used. This also applies to older and recent studies of patients with PIDs (30;245;246). Health care professionals including nurses and physicians are primarily focused on the health-related quality of life, and can forget that life is more than concerns about disease and treatment. Other factors may contribute substantially to coping and good quality of life. These factors are easily missed with a focus limited to SF-36 scale scores. Some non-medical factors have implications for how patients and families handle their lives, deal with disease and treatment, and interact within the family. Therefore, to focus on the global factors related to quality of life is important for all types of patient groups. Patients with symptomatic selective IgA deficiency are usually healthier than other patients with antibody deficiencies and, as expected, they achieved a higher global QLI score (247-251). But very interestingly, the QLI differences were not found in the health/function domain, but in the socioeconomic, family and psychological/spiritual domains. If only SF-36 had been used, this finding would not have been evident. We need to be cognizant of factors than strictly medical issues when planning interventions which can have a positive impact on how patients and families cope with disease in the long-term. In addition to developing effective medical treatment strategies, it is important to support independence from an early age, focus on school achievements and ability to work, prevent social isolation and personal financial problems.

Health-related quality of life and treatment methods

The SF-36 scale measured health-related quality of life. Our study also focused on global quality of life. Answers about the immunoglobulin treatment regimens were not fully informative. We observed a difference in quality of life between those who treated themselves (ScIg) compared to those who were treated by others (IVIg), but the difference was not statistically significant. Patients who treated themselves (ScIg) had a significantly higher score in social functioning as assessed by SF-36 compared to the others. Gardulf found significantly increased health-related functioning, and improved self-rated health among patients with antibody deficiencies after initiation of ScIg infusions. This finding has been confirmed by others (30;31;245;252;253). In contrast to most other recent quality of life studies of patients with antibody deficiencies our study was not designed to detect differences before and after introduction of ScIg. In Norwegian patients, ScIg is the primary and initial immunoglobulin treatment for adults and children with hypogammaglobulinemia. Patients are put on IVIg only if they need high doses of immunoglobulins to maintain adequate serum IgG levels, or intermittently for other practical reasons, such as travelling. Studying implications on quality of life of a change from IVIg to ScIg is therefore less relevant.

In contrast to hypogammaglobulinemia, selective IgA deficiency is usually not treated with immunoglobulins because of the potential for allergic reactions. Individuals with selective IgA deficiency do not have fewer intercurrent infections during adolescence or less of a health burden from autoimmune disease, but are perhaps less absent from school due to

hospitalisations for routine investigations and IVIg treatment. These factors may reduce the tendency to define oneself as sick. We found that individuals with selective IgA deficiency had significantly higher QLI scores in the socioeconomic but not the health-function domain compared to individuals with other antibody deficiencies including hypogammaglobulinemia. Selective IgA deficiency may have less psychosocial consequences and fewer implications for education, ability to work, employment opportunities and income. Other differences between the various antibody deficiencies were not found in our study, and surprisingly HCV infection had no influence on the QLI or SF-36 scores.

Further discussion of results of the coping, quality of life, and hope study – implications for follow-up

Avoid unemployment

The sum of medical problems, such as infections and additional diseases, were important for scholastic achievement. Based on the interviews a state of unemployment and, quite unexpectedly, a close bond to mother were noted as two important negative factors for coping. Our medical interventions should reduce the patient's burden of illness, and support his / her chances of employment. Finding that work is an important independent external determinant of quality of life, is in agreement with results from recent studies of other chronic diseases(228). Adolescent patients must be encouraged to continue their educations in order to increase their flexibility in the employment market. Practically speaking, in caring for patients with antibody deficiencies, appointments should be planned to minimize absence from work and school. Self-administered subcutaneous treatment with immunoglobulin administered at home reduces travelling time to hospital for intravenous treatment. Appropriate pre-filled prescriptions for antibiotics and avoidance of unnecessary consultations may reduce sick leave. Medical interventions aim to reduce health related strains, but may not necessarily influence the patient's perception of disease. A focus on coping, taking into account factors that have been defined as relevant, is an extra tool when planning health care strategies for the group and for each individual. Non-medical interventions can also have implications for the patients' global QLI (30;31;222;253-255). Treatment and interventions may have consequences in other areas than anticipated. Health care providers can play an important role in enhancing the quality of life for patients with chronic illness through developing appropriate, individualized, coordinated and compassionate medical management.

Support independence in adolescence

Interpretation of the results from the interviews must be interpreted with some caution, since individuals were selected for interview based on QLI scores. The two elusive factors "closeness" and "competence" were found to be important for coping in PID patients. This was thought provoking, but consistent with Lazarus and Folkmans' theories (237). Of particular note was that a complicated relationship to an intervening mother was related to low QLI scores. This raises a more universal issue for individuals with congenital and/or chronic disorders. Parents of children with chronic disorders may feel primarily responsible for the medical care of their children for many years. Particularly in children with rare disorders, parents may experience that healthcare providers may have less knowledge of the disease than they themselves have. Helping parents to withdraw from having total responsibility for an adolescent or adult child's health, is an important management issue. Supporting independence already in childhood may be achieved by arranging training courses i.e. for introducing and promoting self-injection of ScIg at an early age. Attention to the transition

through adolescence, family dynamics and other psychological issues is required for successful management. Transfer of care to adult services should be planned carefully within the context of a multidisciplinary team.

Optimistic coping style – also found in other studies

An optimistic coping style was the most frequently used strategy found in our cohort of antibody deficient patients. In studies of other groups of patients, a majority have been found to employ an optimistic coping style (256-259).

Ethical issues relating to the questionnaire and interviews

Ethical issues can arise when patients are asked to complete questionnaires about coping, quality of life and hope. Answering such a questionnaire may lead to emotional distress and a feeling of being intruded upon. However, reflecting around these issues may also lead to increased insight, personal growth and better adjustment. The introductory letter, which accompanied our questionnaire, warned patients of the potentially sensitive nature of some items in the questionnaire. The interviews took place in the patient's home with his / her consent. This may have felt intrusive, but on the other hand, may have been perceived as considerate and positive. The interviewer strived to be professional, empathetic and respectful and if necessary, provided information about follow-up support available through the National Health Service.

Studies of coping, quality of life, and hope in other PID patients

Quality of life studies are needed in patients with PID diagnoses other than antibody deficiences. In particular, there is a major need for psychosocial and behavioural studies in children with PIDs. There are additional ethical issues which arise with psychosocial testing and intervention studies in children. The childrens' welfare should be given priority.

Clinical studies based on the PID registry

Clinical studies are time-consuming and will eventually occupy a part of the health centre capacity. However, the dividends from systematically following groups of patients with rare disorders have substantial value for patients and professionals. Health care professionals increase their knowledge of medical aspects of the disease, and patients are met by professionals with relevant clinical experience. Research results can be translated into practice directly and can be used for rational planning of health care and social services. Practical strategies for follow-up care, increased awareness and competency among health care professionals and results of the clinical studies could all have carry-over benefit for other patient groups. With the A-T studies (Papers II-V) we have shown that national registries can be used for clinical research and follow-up of cohorts of patients with the same PID.

A characteristic immunological pattern in A-T patients

A very characteristic immunological pattern is recognizable in A-T patients, and reflects underlying disturbances in T cell and B cell development including immunoglobulin formation. The A-T patients' levels of immunoglobulin isotypes situated at the beginning of the constant region's reading frame are increased and then successively reduced downstream the genetic reading frame. This is compatible with the role of ATM protein in the end joining repair machinery in CSR. Patients usually have low IgE, IgA and/or IgG2 levels, and oligoclonal-/monoclonal increase in IgM and/or IgG can be present in A-T patients without signs of hematological malignancy (210;260). Quantitation of lymphocyte subsets is required to detect the specific pattern of cellular immunodeficiency. Routine leukocyte counts will

rarely, if ever, detect these lymphocyte subclass defects. The low T cell number in A-T patients has been observed by others (81;261), but we also call attention to the low B cell numbers (Paper III), a phenomenon which had not been reported previously. Lymphocyte flowcytometry can be performed as a diagnostic tool when A-T diagnosis is suspected in a child and will usually reveal both low B cells and low T cell numbers, with normal NK cell number. Not all patients are lymphopenic. Note that age-specific reference values should be used. Consistent differences in immunological status between patients can be found. The overall pattern is the same, but the severity of the immunodeficiency varies. The measurements in each patient showed variation over time, but interestingly, there was no significant progression of immunological abnormalities with age. We performed a longitudinal study, but only followed each patient for two to three years in the first study. If we include the subsequent years during which we conducted the pneumococcal study and measured alpha fetoprotein, we have followed seven patients for six to seven years each and not seen major progression of the immunological aberrations, however, an age effect over decades cannot be completely excluded. However, in a cross-sectional analysis of 100 A-T patients Lederman et al showed that the immune defect is not age-related (262).

Laboratory immunological evaluation

The defects in immune response are more quantitative than qualitative in A-T (42;263) and there is a discrepancy between patients' modest clinical symptoms and the abundance of immunological laboratory abnormalities. The effectiveness, not only the numbers of B cells and T cells is important(262). We investigated the immunological status in our A-T patients using repeated blood sampling for quantitation of lymphocyte subsets and immunoglobulin levels. We did not determine the specific degree of deficiency in V(D)J recombination, isotype switch (CSR) or somatic hypermutation (SHM). However, the low T cell and B cell numbers as well as the signs of reduced ability to make certain immunoglobulin classes such as IgE and IgA, point indirectly to a deficiency in certain processes in the development of secondary immunocompetence. Investigation of presumed restricted V-region usage and oligoclonality of T cells and B cells requires methods not used in our studies (264;265). Others have shown that ATM is involved in V(D)J recombination and CSR, but not in SHM (120;266). A theoretical explanation for the extremely low B cell numbers in A-T is that B cells undergoes both V(D)J recombination and isotype switch, both of which are processes involving ATM. T cell precursors only undergo V(D)J recombination involving ATM, not CSR. This may be the reason why we found low T cell numbers and even lower B cell numbers (Paper III). A limitation of V(D)J repertoire of T cells and also B cells is to be expected in A-T and has been described by others (89;264;267-270). Recombination is an important step during development of B- and T-lymphocytes. Failure to perform appropriate recombination may lead to early apoptosis resulting in a low number of mature cells (89;94). The resulting few circulating B cells and T cells may be effective enough to prevent severe invasive infections in A-T in the absence of impaired respiratory function.

Clinical immunodeficiency

The A-T patients' immunodeficiency was of less clinical significance than anticipated based on the laboratory abnormalities. In a nutshell, we found a severe immunodeficiency in the laboratory with mild clinical immunodeficiency in children. Despite low T cell numbers, opportunistic infection with *Pneumocystis jerovici* was not a problem. Common warts, molluscum contagiosum, paronychia/onychomycosis, seborrhoeic eczema were present. These are considered clinical signs of minor T cell deficiency and frequently described in A-T patients (84). Some correlation between laboratory and clinical findings was observed,

however: Low IgA values were for example found in patients with diarrhoea, and decreased IgG2 levels were noted in patients with recurrent respiratory tract infections. This reproducible discrepancy between laboratory and clinical immunological findings in A-T patients had been recognized previously (42;42;263;263), but had been overlooked in some recent literature. Lederman et al also recently described this peculiar discrepancy(262). Our laboratory investigations represent fragmented snapshots of ongoing immunological processes and underscore that laboratory results should be interpreted in a clinical context.

Immunoglobulin supplementation in A-T

None of the A-T patients in our studies were treated with immunoglobulin substitution. Internationally IVIg is recommended in A-T patients, while we consider home-based subcutaneous therapy the better alternative when Ig substitution is indicated. In Norway we generally reserve supplemental gammaglobulin for PID patients with verified symptomatic hypogammaglobulinemia. None of our A-T patients had hypogammaglobulinemia defined as low serum IgG1 levels (Paper III). We have followed a small number of A-T patients, and may have a restrictive policy compared to others with larger cohorts who estimate that 10% of A-T patients require immunoglobulin supplementation (262)

Frequency and severity of infections – interpretation difficulties

Recording and categorizing infections was challenging, especially in retrospect. Even when we tried to use annual summaries, it was difficult to get a clear picture of frequency and severity of infections. Microbiological evaluations were often sparse. Chest x-rays had not been done in all cases of clinical pneumonia because of radiosensitivity. One reliable measure of infections was information about the prescription and effect of antibiotics. Another objective measure was hospitalisation for infection. Also in the Iranian study of 104 A-T patients as many as 25% had no more infections than predicted for the population in general. 63% had somewhat more infections than average, but within this group only one admission to hospital was registered. Only 12% of 104 suffered from suppurative lung disease or severe infections (219). Moin et al found a positive correlation between immunological symptoms and immunoglobulin deficiencies in A-T patients. Because one of our main conclusions is that immunodeficiency is generally not a major clinical issue, we are able to reduce parents' anxiety concerning the risk of severe and life threatening infectious diseases in children. However, increasing risk of lung infections with age is an issue. If increased vulnerability to pulmonary infection is not mainly caused by a progressive immunological defect, it may be the result of progressive lung failure secondary to microaspiration from dysfunctional swallowing or other reasons such as interstitial changes due to ATM protein deficiency in the lung cells. These research questions remain to be addressed (39;74;262).

Vaccination in A-T

PCV7 vaccine induced, and PPV23 vaccine boostered, pneumococcal antibodies in our A-T patients (Paper IV). Six of nine achieved protective antibodies to the PPV23 mix. The PCV7 containing diphtheria CRM_{197} induced an increase in diphtheria antibody levels. Pre-existing protective antibody level to diphtheria and tetanus seemed to be predictive of capability to make protective pneumococcal antibodies, which was both interesting in itself and illustrative of the variable nature of immunodeficiency. The results of the vaccine study were important for the A-T group, because they show that vaccination may partially circumvent immunodeficiency. The clinical immunological study revealed that many children had

received the MMR vaccine prior to being diagnosed with A-T and that no adverse reactions to the living vaccine had occurred (Paper III). We have gone from advising against vaccines, to advising that children follow the standard vaccination program, excluding only vaccination against BCG (*Mycobacterium bovis* Bacille Calmette-Guerin). In addition to the vaccines in the national vaccination programme, we recommend vaccination against chickenpox and annual vaccination against influenza. Based on our study, as well as the Turkish and the German study of antibody responses to pneumococcal vaccines, we now recommend two successive PCV7 vaccinations plus a booster vaccination with PPV23.(85;271;272). Revaccination with PPV23 is recommended every five to ten years in splenectomized individuals. This should also be recommended in A-T patients in an effort to prevent pulmonary pneumococcal disease in the longterm. After we completed our study, the PCV7 vaccination became a part of the national pediatric vaccination program. All children born in Norway after 1st January 2006 presently receive PCV7 vaccinations at 3, 5 and 12 months of age. Introduction of PCV7 vaccine in the general population can cause a shift in pneumococcal serotypes causing severe pneumococcal disease. Our recommendation for pneumococcal vaccination in A-T may therefore need to be modified with time.

Reference values and protective antibody levels

The importance of using age-adjusted reference values for serum immunoglobulins and lymphocyte populations needs to be stressed (Paper III). Immunoglobulin levels and lymphocyte counts vary with age in early childhood. The problems arising from undefined protective levels for specific antibodies to microbial antigens are discussed in Paper IV for pneumococcal antibodies, and illustrate a more general problem. For many of the antibodies detected, protective levels are not clearly defined. Undetected differences in antibody specificity may exist, or protective levels may only have been defined based on animal studies.

Antibody responses, not clinical outcomes, have been studied

To test the response to conjugated vaccines in A-T, a study of the incidence of pneumococcal infections or pneumococcal carrier status is necessary. Only antibody response, not clinical effect, has been studied to date. Determination of protective antibody levels is associated with uncertainty because antibody specificity may vary at the same antibody level. Why A-T patients have difficulty specifically with making protective antibodies against polysaccharide antigens and pneumococcal polysaccharide, in particular, remains an unanswered question. Our patients with A-T had extremely low levels of pneumococcal antibodies (Paper III and Paper IV). The difficulties in ligation of DNA strands after excision of larger loops in CSR (Figure 5) are demonstrated by low levels of IgE, IgG2 and IgA compared to more normal IgG1 levels. The same mechanism may also be responsible for the difficulties in making B-cells with certain V-regions during V(D)J recombination. Certain V genes present particular conformations suitable for certain types of antigen. Successful clones in the memory response to pneumococcal polysaccharide use V_H with high numbers, requiring sufficient DNA ligation after excision of large loops. Assessment of TCR repertoire and use of specific V regions (Figure 5, Figure 7) could have been investigated, before and after vaccination. Nasopharyngeal carrier status for pneumococcus could have been ascertained (273).

BCG vaccination in A-T

BCG vaccination in A-T patients is still somewhat controversial. In countries where BCG vaccination is administered routinely shortly after birth, active BCG infection has not been

observed in individuals subsequently diagnosed with A-T (Prof. Ozden Sanal, Turkey, personal communication) (274). However, there is a recent publication showing impaired interferon-gamma production in response to BCG in T cells and NK cells in patients with A-T (275). Granulomatous lesions have been reported in A-T patients (276;277). Some of these reports are from countries were BCG vaccination is routinely given in early childhood (278;279). Even in the absence of mycobacteria in granulomatous lesions or absence of evidence of disseminated BCG infection, the safety of BCG vaccination in A-T remains uncertain.

Vaccination in other PIDs

Vaccines represent an extra therapeutic tool in the treatment of patients with primary immunodeficiencies other than A-T and can reduce effects of specific immunological defects. For instance, in patients with cyclic neutropenia vaccines are safe and may effectively prevent certain diseases. Whether or not to vaccinate patients with hypogammaglobulinemia, including CVID, is under debate. Effects are expected to be modest, but given that vaccines are safe in this population, they are not medically contraindicated.

The thymus is underdeveloped

The term “thymus atrophy in A-T” is inappropriate, because lack of thymus tissue is not a primary cause of immunodeficiency in this disease. The thymus appears underdeveloped on MRI and ultrasound scanning because of a paucity of T cells. Measurements of TRECs (T cell recombination excision circles) demonstrate low thymic output (264). However, there is no primary thymic aplasia or hypoplasia as in DiGeorge/deletion 22q11 syndrome. Thymus aplasia or hypoplasia is not a distinct diagnostic criterion for A-T.

Using immunological laboratory findings to support the diagnosis of A-T

Characterization of the immunodeficiency in A-T should be done as part of the diagnostic work-up because a characteristic pattern of abnormality is supportive of the diagnosis prior to molecular genetic confirmation. Although the degree of immunodeficiency on immunological testing is variable, the overall immundeficiency pattern is very characteristic. These immunological findings have consequences for management. Only a minority of the A-T patients appear to need immunoglobulin treatment.

Revising the ESID diagnostic criteria for A-T

The ESID diagnostic criteria for A-T (Table 1) should be revised to not only include IgA deficiency, but also the other typical immunological findings. Only half out patients had IgA deficiency. IgG2 deficiency and IgE deficiency were much more common. Low numbers of B cells and CD4+ T cells were an even more consistent finding.

A-T and phenotype - genotype correlations

We have a large cohort of A-T patients, relative to population size, which we have followed closely for several years. We have noted some phenotype-genotype correlations in IgG2 serum levels, and in the debut age of telangiectasias (Paper III)(63). The patients homozygous for early truncating mutations (two null mutations) had relatively lower IgG2 and earlier debut of telangiectasias. We also found a correlation between serum IgG2 levels and

pneumococcal antibody levels (Paper II). A correlation between IgG2 levels and early truncation mutations in *ATM* has been noted in a subsequent and on-going pneumococcal vaccination study from the UK (http://www.atsociety.org.uk/newsletter12.06.pdf., preliminary results presented as a poster at the 12th ESID Biennial Meeting, Budapest Oct 2006). However, a correlation between IgG2 levels and pneumococcal antibody levels was not shown in a recent Brazilian study of ten A-T patients (274). The A-T mutation spectrum in our country is heavily skewed towards truncating mutations.

Ideally, genotype-phenotype comparisons for immunological phenotype, neurological phenotype, eye findings including debut of telangiectasias and AFP levels should be performed in individuals with milder disease (63). In the UK several individuals with atypical/mild A-T have been diagnosed. They have a less severe neurological course than classic A-T patients. Ataxia is milder and starts later in life while other neurological features, such as extrapyramidal signs are more apparent. These British patients with a milder phenotype have missense mutations in *ATM* predicted to produce altered ATM protein with residual function. Comparison of immunodeficiency between typical and atypical A-T patients with measurement of ATM protein levels and ATM protein functional activity, could determine whether complete loss of ATM protein or ATM kinase activity, causes more severe immunodeficiency than partial loss. In 2004, Trimis et al. reported an interesting, unusual A-T case; a 6 year old girl without any neurological symptoms. She was homozygous for a mutation in one of the terminal exons in *ATM*, had classical immunodeficiency (280) and only moderately increased AFP. This case highlights that a possible broader disease spectrum than previously recognized may be associated with *ATM* mutations.

Our study was the first to find a correlation between the degree of immunodeficiency and mutation type (Paper III). A correlation between cancer incidence and mutation type may also exist. The immunodeficiency and hematological cancers in A-T represent the bi-faceted result of defective ATM functional activity in T cell and B cell maturation. Of the five Norwegian patients who had developed leukemia/lymphomas, three were homozygous for the founder mutation and one was compound heterozygous for null mutations in proximal exons (Results 2, Table 7).

Non-allelic polymorphisms may contribute to variation in phenotype

Sequence alterations/variations in genes other than *ATM* may explain some of the phenotypic differences. MBL deficiency may have a separate additive effect on the immunodeficiency and further increase the risk of infections (Paper IV). In contrast to the risk of hematological cancers, leukaemia and lymphomas, the increased risk of other solid tumours in A-T patients and carriers are unexplained. Increased cancer risk may be related to reduced TP53 function. Li Fraumeni syndrome patients have germ line *TP53* haploinsufficiency and a greatly increased risk of cancer (solid tumours, sarcomas). A common polymorphism in the *MDM2* gene is linked to earlier cancer debut in Li Fraumeni patients. MDM2 inhibits TP53. This specific SNP309MDM2 polymorphism causes gain-of-function of MDM2. ATM kinase inhibits MDM2 and stimulates TP53 as part of the cells' cancer protection machinery (Figure 8). High levels of MDM2 could theoretically exaggerate the effect of ATM deficiency and increase cancer risk. Studies of cancer incidence, especially for solid tumours, in A-T patients and A-T mutation carriers correlated to co-existence/prevalence of SNP309MDM2 have not yet been carried out.

Alpha fetoprotein in A-T

The role of alpha fetoprotein in A-T is still not clear. Serum AFP is elevated, and increases with age (Paper V). It might be useful as surrogate marker in clinical trials. We will continue to monitor serum AFP in our cohort. The AFP study originated from long term systematic study of A-T patients. AFP measurement is reliable and reproducible. Our simple observation provides important knowledge about the disease. However, in order to assess the value of AFP as a biomarker, we would have needed to have done standardized neurological assessments in parallel with serum AFP measurements. Interestingly, AFP is also increased in AOA2 and vitamin E deficiency, but not in Mre11 and NBS. The process leading to increased AFP expression in A-T is different from the process leading to immunodeficiency, in light of the common the immunodeficiency pattern in Mre11, NBS and A-T. Theoretically, liver cells may be affected by ATM deficiency, and reduced TP53 activity may be the linked to increased AFP expression. Common for ATM deficiency, AOA2 and vitamin E deficiency is increased ROS (reactive oxygen species) and reduced cellular antioxidative capacity. If and how ROS in hepatocytes is related to AFP expression in A-T is not clear.

The genes which code for AFP, albumin, vitamin D binding protein and alpha-albumin are located close to each other at chromosome 4q11-q13 and share considerable sequence homology (281)(MIM+104150). These four three-domain gene family proteins are all synthesised in liver and secreted in serum. Their half-life in serum is 5 days.
Human AFP was previously thought to be fetal albumin. Similarly to albumin, AFP binds and transports a multitude of ligands, including bilirubin, retinoids, steroids, heavy metals, dyes, flavonoids, phytoestrogens, dioxins, and various organic drugs and fatty acids. In contrast to albumin, AFP reversibly binds with high affinity and transports polyunsaturated fatty acids, mainly docosahexaenoic acid (282-285). AFP is first synthesised by the embryonic yolk sac cells, and later by the fetal liver. High AFP levels persist until birth when they decrease rapidly, and reach levels typical for adults (< 14 kU/L) by the end of the first year (286). After birth and in adult life, extremely high levels of AFP are linked to malignant liver and germ cell tumours. Lectin binding studies of serum AFP from patients with A-T reveal a profile characteristic for AFP of hepatic origin (287) which is similar to that seen in benign chronic liver disease and neonatal hepatitis. Increased serum AFP levels are not indicative of severe liver disease. Autopsy of A-T patients usually show no or sparse liver damage (287;288). However, cirrhosis has been reported in a few A-T patients, as has hepatocellular carcinoma and hepatic veno-occlusive disease (289-291). Mild elevation of serum liver enzymes was reported in nine of 20 A-T patients in one series (Waldmann 1972) (292). Other fetal proteins are not increased in A-T (293). Serum AFP level is lower in patients with A-T than with yolk sac derived tumours and liver cancers, and similar to the levels observed in hepatitis.

ATM deficiency, AFP and signs of liver damage. TP53 inhibits AFP expression

ATM deficiency may induce an inflammatory and regenerative process in the liver cells analogous to the changes that can be seen in hepatitis and other benign liver disease. Increased oxidative stress has been reported in *Atm*-/- mice and is linked to growth retardation (Ziv et al). (294). AFP expression can be induced in experimental animal model systems by partial hepatectomy and by administration of hepatotoxic agents (295). Increased expression of AFP mRNA correlates with liver regeneration (296). Study of Atm function in a liver regeneration model system showed that Atm is required for survival of hepatocytes, that liver regeneration is impaired in *Atm-/-*mice, and that AFP expression during liver regeneration can be observed both in wild-type and *Atm-/-*mice (Shu Lu et al)(297). Increased AFP expression in A-T patients may be caused by a constant hepatic regenerative response to genotoxic

insults/oxidative stress. AFP has also been identified as a novel downstream target of NF-kappaB. Repression of IKK-2 activity in hepatocellular carcinomas promotes down-regulation of AFP gene expression, linking AFP directly to apoptotic pathways (298)

The tumour suppressor TP53 acts downstream of the ATM protein kinase in cell cycle DNA damage checkpoint control. In response to DSBs, ATM phosphorylates TP53 and induces processes leading to cell cycle arrest at the G1-S transition (44). Recently, it has been documented that TP53 and its family members act as repressors of AFP gene expression during liver cell development (299-301). Increased AFP expression may be related via TP53 to ATM deficiency.

Large cohort of A-T patients for population size

In the primary immunodeficiency registries in Iran and Turkey (219), A-T is more prevalent than in most countries. However, consanguinity was found in the majority of Iranian and Turkish patients, and consanguineous marriages are common in both countries. In contrast, the majority of the parents of our A-T patients with homozygous ATM mutations were not aware of consanguinity. Hence, not consanguinity, but a high carrier frequency is responsible for the rather large cohort of A-T patients compared to our population size. People in Norway have traditionally lived most of their lives in their birth counties. Our universal national healthcare system is well developed. It is safe to assume that all diagnosed A-T patients in Norway are included in our report. This gives us the opportunity and the obligation to undertake longitudinal studies on all aspects of disease progression. The Norwegian A-T cohort is well suited for participation in future clinical trials. In contrast to other groups of PID, our cohort is well characterized molecular genetically. Participation in clinical trials for specific *ATM* mutations (i.e. read-through aminoglycoside trials) are feasible (302).

A-T Clinical Care Centers

The centralisation of some medical care and follow-up of patients with a rare disorder such as A-T is highly desirable. It allows a few people to collect information and to develop knowledge about the disease and to build a team with expertise. The expert team may then be able to give evidence-based as well as experience-based advice to medical and multidisciplinary teams around each patient. In the US and Israel A-T clinical care centers have been established. The population in our country is scattered, and it is not practical for a single central clinical center to cover all medical aspects, serve acute medical needs or to provide frequent follow-up of each patient. To have the opportunity to see each patient infrequently but regularly, and to be able to follow patients with rare disorders over time, would confer great advantages for the development of multi-specialist teams and good patient care. Taken into account the great distances in our country, a Norwegian A-T Clinical Care **Network** with extensive collaboration across health care regions, is probably the best approach for us.

Longitudinal follow-up is needed for rare disorders.

Longitudinal follow-up of our small patient group has already led to increased knowledge of the disease. Some previous misconceptions have been cleared up. For example, the typical immunological findings in A-T patients no longer result in emergency telephone calls to parents and colleagues. We now advise completing the routine pediatric vaccination program instead of avoidance of vaccines

Prior to the death of one of our oldest patients because of unexpected cerebral hemorrhage Paper III), we did not know that older A-T patients may have microangiopathy of the brain. Only after our patient died, did we became aware of the few reports of similar autopsy findings in patients with this rarely described complication. If the eyes are a mirror of the cerebrum, the findings from the ocular studies of our A-T patients are interesting. The conjunctival biopsies revealed increased blood vessel tortuosity (telangiectasias) and also increased number of cross-sections of neurons. The difference between light-exposed areas (rima) and less light-exposed areas (fornix) points to an UV-effect. Telangiectasias regularly found on sun-exposed areas in the skin such as hands and ears also support the theory of an UV-effect on blood vessel tortuosity in A-T. Since the brain is not UV-exposed, there must be additional factors as well which promote increased vessel tortuosity. On post-mortem, brains from radiation treated patients resemble brains from older A-T patients.

Awareness of the risk of interstitial lung disease in A-T has increased. The immunodeficiency alone does not explain the lung problems which increase with age. In the future, we need to follow A-T patients' lung function regularly. Prolonged or recurrent pulmonary infections need to be documented and treated aggressively to prevent further lung damage. There is also a need to standardize neurological assessment for use in future drug intervention studies. Immunological status should be assessed intermittently in order to check for development of hematological malignancies or hyperimmunoglobulinopathy. We observed individual patients over a period of years, without detecting worsening of immunological laboratory findings. It would have been interesting to see if immunological findings may change over *decades* and if any changes have predictive value relative to cancer risk or other longterm outcomes. In general, the advantages of longitudinal studies in small cohorts of patients with rare disorders should not be underestimated. Repeated observations at the individual level have more power than cross-sectional observational studies.

Children and adolescents with a neurodegenerative disease and participation in clinical research studies – Ethical issues

Frequent blood sampling or other unpleasant investigations for research purposes only, may be an extra stress factor for children and adolescents with a neurodegenerative disease. The cumulative blood sample collection of 50-100 ml represented proportionally large volumes for the smallest children. One family did not want to participate in the AFP study because of frequent blood sampling. Consultations were coordinated with other planned medical examinations in order to minimize disruptions in daily life. Parents consented for participation on behalf of young children. Adolescents and adults gave written informed consent. Since it was physically impossible to obtain proper written consent from the oldest A-T patients, oral consent was accepted. Parents and patients older than 10 years knew that withdrawal from the studies was possible at any time, without consequence for their routine medical management. On one hand, participation in research studies may be an extra burden, because of the focus on disease rather than health. On the other hand, in rare diseases without curative treatment the families and patients are often extremely motivated to participate in clinical studies, representing hope for future therapy and improved quality of care (303;304). There is also a remarkable willingness to contribute to research which can help other families.

Study humans in human disease, in addition performing animal studies

Further basic and clinical research are a means of building knowledge that can be used for development of therapy. Clinical studies often represent substantial intervention in a patient's

life, but it is important to study humans in human diseases. Scientific knowledge from mouse models has contributed, and will continue to contribute, substantial knowledge to every medical field, especially in the areas of immunodeficiency, DNA repair and neurodegenerative disease. Mouse models are extremely practical. However, there are numerous examples of differences between humans and knock-out mice as well as mice knocked-in for human mutations (305). In the future, the optimal strategy would be to study effects in mice and humans in parallel. More collaboration between clinical and laboratory researchers is needed.

Caring for A-T carriers and other relatives of PID patients

Special attention should be given to the susceptibility of adult members of families with A-T to malignant neoplasms and to the importance of regular examinations for cancer detection. Relatives of patients with other kinds of PID may also need to be offered genetic counselling. Investigation of other family members for the same disease may be an issue. In our CMC patients (Paper VI), the younger family members were diagnosed and treated more promptly after debut of symptoms than their older relatives. In our recent publications of autosomal dominant IFNGR1 mutation the youngest family member received prompt antimycobacterial treatment only because of awareness of PID in the family (Glosli et al, See other publications). In Paper VII the results of MBL testing had implications for understanding the susceptibility for infections also in other family members. Genetic counselling can be important for parents planning new pregnancies, for families with multiple consanguineous couples and for female relative of individuals with X-linked PIDs.

MBL deficiency, IgA deficiency and cancer risk in A-T, CVID and other PIDs

We have studied MBL levels in A-T patients. MBL deficiency contributes to their clinical immunodeficiency by causing an increased risk for certain infections, such as mycoplasma pneumonia. In patients with other PIDs, for example CVID, coexistence of MBL deficiency may increase pre-existing susceptibility to infections, and explain some phenotypic variation (306). The IgA deficiency in A-T seems to be related to defective DNA repair processes in Ig class switch recombination, while the cause of selective IgA deficiency in CVID families can not be explained by identical defects. An increased risk of cancer is observed in A-T patients, and, to a lesser extent, in A-T carriers. In patients with other PIDs an increased risk of cancer has been observed, e.g., leukemia in severe congenital neutropenia, lymphomas in WAS and in CD40 ligand deficiency. There have been reports of an increased risk of cancer in CVID patients, but this is controversial. The heterogeneous CVID group may consist of a wide variety of different specific PIDs.

Immunological study of patients with familial CMC with hypothyroidism -but without APECED

Patients with chronic mucocutaneous candidiasis (CMC) are selectively unable to clear the yeast *Candida*, which results in persistent debilitating infections affecting the skin, nails, and mucous membranes. The underlying defect is unknown. Studies in *Aire-/-* knockout mice highlighted the importance of type 1 cytokines in protection against *Candida*, and previous work suggested that CMC patients may exhibit altered cytokine production in response to *Candida.* Protection against *C. albicans* infection is generally considered to be T cell mediated. Therefore, it is surprising that CMC patients suffer from chronic mucocutaneous candidiasis in spite of an exaggerated T cell mediated destruction of endocrine organs. A

dysregulation of T cell responses, both Th1 and Th2, in APECED and other CMC disorders has been suggested. Th2 responses are related to IgE and IgG4 production. Low IgE and IgG4 levels, as we found in our CMC patients (Paper VI), points to a lowered Th2 response. The low NK cell numbers found in some of our patients together with the low serum IgG4 and IgE levels (Paper VI) may also fit into this pattern of low Th2 response, since also NK cells have been shown to be involved in Th2 responses. On the other hand, clearance of *Candida* is dependent on an appropriate Th1 response. Specifically, it has been shown that Th1 type response, characterized by antigen stimulated lymphocyte production of cytokine IL-12 and interferon gamma and delayed-type hypersensitivity reactions, are associated with protection against *Candida* in animal studies(150), but the role of the Th1 response is less well understood in humans. Cytokine IL-12 is produced by myeloderived dendritic cells and macrophages. IL-12 plays a key role in regulating the type of adaptive immune response produced, whether Th2 or Th1 type responses are induced. In general, Th2 responses are linked to induction of humoral immunological responses and Th1 responses to cellular responses. The clinical autoimmunity accompanied by various autoantibodies present in APECED patients, suggests an overactive and dysregulated Th2 response. APECED may result from biased Th2 responses to self-antigens and defective protective Th1 responses against *C. albicans.* In four of seven of our CMC patients we found low levels of CD8 T cells (Paper VI) which may be consistent with a low Th1 response. Others have tested cytokine production in CMC patients in response to *Candida* and found impaired production of IL-12 in parallel with increased levels of IL-6 and IL-10. Their results suggest that patients with CMC have impaired production of Th1 inducing cytokines and an inability to mount protective cell-mediated responses and a failure to clear *Candida* (307). Decreased interferon gamma production has been found in whole blood from CMC patients (APECED excluded) after *in vivo* stimulation with *C. albicans* (308). Taken together, these data suggest that although cell-mediated Th1 immunity is essential for protection against fungal infections, the link between type 1 inducing cytokines, induction of Th1 responses, and fungal clearance is more complex than initially appreciated.

If T cell deficiency, why not warts and molluscs? Lessons from APECED

Usually in patients with minor signs of T cell deficiency (i.e. as in A-T), candidial onycomycosis and seborrhoeic dermatitis are accompanied by warts and molluscum contagiosum. The altered T cell responses could be explained by a defect in the antigen presenting cells (APC) including the dendritic cells. Faulty dendritic cell function may lead to autoimmune disease. The trigger for inappropriate immune reactions may be *Candida*, as proposed for Hyper IgE syndrome (309). Chronic candidiasis may on the other hand only be another sign of the specific immunological defect. These diseases (APECED + other CMC disorders) may serve as models for understanding the pathogenesis of organ-specific autoimmunity in general. The question of what skews cytokine responses remains unanswered, while the candidate cells that harbour the defect could be dendritic cells or macrophages by virtue of their cytokine-producing function and role in orchestrating Th1 and Th2 responses. These findings have bearing on understanding CMC itself, as well as on our understanding of the mechanisms involved in protective immunity to fungi in general, a subject which is still poorly understood. Lessons from APECED may help us further since the genetic defect in the other CMC disorders, including our CMC families, is not yet known. Importantly, the *AIRE* gene is expressed in the peripheral monocyte/dendritic cell lineage (310). Brannstrom et al found both a defect internalization of *Candida* and kinase activation in monocytes derived from APECED patients. The total number of monocytes in mice with *Aire -/-* is increased roughly two-fold. (311). A recent paper by Peltonen et al (2007)

mentioned monocytosis in CMC in humans, but to our knowledge there is no published data on the subject.

Compensatory responses but no distinct immunological defect found in our CMC study

We were not able to find an underlying immunological defect in our CMC patients (Paper VI). Perhaps we failed to detect or oversaw laboratory signs of an immunological defect. Monocyte numbers were somewhat high in family B, but still within the normal reference range and are not commented upon in our report. The disease showed no specific HLA haplotype association. Even the autoantibody evaluations that are valuable and disease-related in APECED were not conclusive in our study. Usually in AIRE deficiency autoantibodies are present, however, we found no autoantibodies in our CMC affected family A. TPO-Antibodies and TG-Antibodies were found in the patients with newly diagnosed hypothyreosis in family B. APECED patients tend to have anti-thyroid antibodies which are detectable several years after the onset of overt hypothyroidism. It is also possible that our two families represent two distinct CMC disorders, autosomal dominant inheritance was evident in family B, but a recessive inheritance pattern can not be excluded in family A. Candida antigen responses were not investigated in our project, but three of the patients had been tested previously and two had shown anergy on skin-test reactivity against Candida antigen (Paper VI). Increased MBL levels and complement activation (Paper VI) may be part of a compensatory response to the immunological defects. MBL plays an important role in the first-line defense against *C. albicans* without the need for opsonophagocytosis by dendritic cells. MBL inhibits the growth of *C. albicans* independent of complement activation. With complement activation, further inhibition is observed (160).

If monocyte/APC/dendritic defect, why the absence of susceptibility to mycobacteria?

Mouse Aire is expressed both in thymic medullary epithelial cells and in peripheral antigen-presenting cells, suggesting a role in both central and peripheral tolerance. Very interestingly, T cells from Aire deficient mice show a competent *in vitro* response against *C. albicans.* APC-mediated T cell activation is not decreased, but increased both in mice and humans deficient in this autoimmune regulator. Maturation time for monocytes in bone marrow is decreased in Aire deficient mice. Monocytes from APECED patients have defective internalization and intracellular signalling after exposure to *Candida* antigen. Evolving evidence points to one or more immunological defects within the APCs. However, in contrast to other inherited disorders of macrophage function, there is no obvious susceptibility to mycobacterial infection in CMC patients (144). None of our CMC patients had had mycobacterial infections.

Inherited thyroid diseases: Autoimmune thyroiditis versus congenital thyroid disease and response to treatment.

In contrast to the autoimmune thyroiditis in CMC patients, the thyroid problem in TITF1 deficiency is congenital. Patients with CMC and hypothyreosis, respond to treatment with thyroxin, while patients with TITF1 deficiency do not. The mild elevation of TSH with normal thyroid hormone secretion points to a functional thyroid defect in our patient with 14q deletion (Paper VII). There is a reduced activation of TITF1 targets, such as the thyroglobulin promoter in the thyroid follicular cells (312). Other TITF1 targets are TSH receptor and thyroid peroxidase gene (313;314). Thyroid dysgenesis has been found in other patients with *TITF1(NKX2.1)* haploinsufficiency (315), but ultrasound of the thyroid gland in our patient showed a thyroid of normal size and shape. As expected, he did not respond to thyroxin.

Recurrent pulmonary problems, related to MBL deficiency and IgG2 deficiency?

We suggest that the MBL deficiency and probably also the IgG2 deficiency explain why our patient described in Paper VII had more severe and recurrent pulmonary problems than previously reported for *TITF1* haploinsufficiency. The mother and younger brother who also had MBL deficiency, had history of respiratory infections, although the infections were fewer and less severe than in the index patient. The 14q deletion involved *PAX9* and probably also haploinsufficiency for other genes that may have contributed to his cleft lip-palate, clubfeet (as case 777 in (316)) and feeding difficulties with gastrooesophageal reflux.

Contiguous gene syndromes and the contribution of single genes

It has become increasingly evident that the clinical consequences of chromosomal aberrations are linked to the excess or reduction of involved genes. The phenotype in known chromosomal syndromes is dependent on which genes are involved. However, phenotypic variation is also observed between patients with identical single gene mutations. Further studies of patients with single mutations in genes within the region may further elucidate genotype-phenotype correlations of proximal 14q deletions. Polymorphisms located outside this chromosomal region may also influence the clinical phenotype.

Conclusions

We have shown many advantages of collecting information on PID patient cohorts. We have been able to document prevalence and incidences of the various types of PIDs in our country (Paper I), which in turn has facilitated estimation of the need for different treatment modalities. Baseline statistics are important for securing subgroups of PID patients nation-wide drug-availability, e.g. immunoglobulins. Using the national registry we have been able to focus on particular PID groups and to study more closely areas of special interest, e.g., coping, quality of life and hope in adult patients with antibody deficiency and genotype-phenotype correlations in A-T cohort. We have shown that national registries can be used for clinical research and follow-up of cohorts of patients with specific PID diagnosis. Previously unrecognized disease manifestations may also be detected when data are systematically registered, e.g., increasing serum AFP levels with increasing age (Paper V). The epidemiological results emerging from our A-T studies illustrate the advantage of longitudinal data-collection, both retrospectively and prospectively, compared to single time point registration. Characterizing and following cohorts of patients with rare disorders increase our knowledge of specific diseases, and may uncover atypical patients with previously undescribed diseases entities, e.g., CMC patients in Paper VI and the patient with a microdeletion 14q and MBL deficiency in Paper VII.

The prevalence of all types of PID taken together was 7 per 100.000 living population in 1999 (Paper I), and 10 per 100.000 in our update report from 2005 (Table 5). Even in light of the geographic variation in PID registration within the country, the total prevalence estimate is high compared to other countries. Compared to other European countries, the proportion of combined deficiencies is decreased because we have fewer patients with selective IgA deficiency, and the proportion of complement deficiencies is increased because we have many patients with hereditary angioedema. Compared to international estimates we have a large proportion of ataxia-telangiectasia patients. Within Norway there is geographic variation in the incidence of A-T from 1 to 10 per 100.000 livebirths, due to a high heterozygote carrier frequency in certain areas of the country.

Using the PID registry we were able to focus on particular groups and research questions. Our investigation of psychosocial factors in the adult cohort of patients with antibody deficiency showed that unemployment and disease-related strains were linked to low quality of life scores. Previous experiences related to the immunodeficiency such as episodes of illness, absence from school, psychosocial consequences were of importance, but the patients with hepatitis C virus infection had not lower scores on SF-36 or QLI scales than the others. Persons with selective IgA deficiency had significantly higher QLI scores than those with other types of antibody deficiencies. They had higher QLI scores in the socioeconomic and not in the expected health function domain. Other differences between the various antibody deficiencies were not found. Closeness and competence were preconditions for good coping, quality of life and hope. In addition to representing approaches for future interventions, the results from the adult PID study confirm Lazarus and Folkman's universal theory of coping and resilience against stress.

The immunological phenotype in A-T patients was characterized by low IgE, low IgG2 and/or low IgA levels. All patients had low levels of antibodies to *Streptococcus pneumoniae*. Low numbers of CD19+ B cells and CD4+ T cells were also typical as were normal numbers of NK cells (Paper III). The patients homozygous for early truncating mutations had relatively lower IgG2 levels than the others. Based on our results, a genotype-immunological phenotype

correlation can not be excluded. The characteristic pattern of immunological abnormalities is consistent with defects in isotype switch and V(D)J recombination in B cell and T cell development, and can be supportive of diagnosis prior to molecular genetic confirmation. IgA deficiency was not a consistent finding in our patients, and we suggest that the ESID diagnostic criteria for A-T should be revised to also include the other typical immunological findings. There was a discrepancy between the abundance of immunological laboratory abnormalities and the patients' more modest clinical symptoms. The immune defect was **not** age related and cannot be the sole explanation for the increased frequency of pulmonary infections in older A-T patients. Vaccination with the pneumococcal vaccines PCV7 plus PPV23 resulted in a significant increase in the patients' pneumococcal antibody titres, but lower than those observed in the controls (Paper IV). The results of the vaccination study are valuable in planning the care of A-T patients, using PCV7 to trigger and PPV23 to booster the immune response and possibly prevent severe pneumococcal disease.

Serum AFP increases with age in A-T and may be useful as surrogate marker in clinical trials. Our study showed a possible link between AFP and general A-T disease progression including hepatic pathology (Paper VII). In our cohort of patients a link between AFP level and the degree of neurological disease was not confirmed, and no correlation between serum AFP levels and *ATM* genotypes was found. All our patients had a classical A-T phenotype and 20 of 24 mutations were truncating. Genotype-phenotype correlations that we were unable to detect because of nature of our population, may well exist.

Low MBL levels have an additive effect on the immunodeficiency in A-T. MBL deficiency explains why our patient in Paper VII had more severe and recurrent pulmonary problems than previously reported in other patients with proximal 14q deletions and/or *TITF1* haploinsufficiency. The increased MBL levels and complement activation found in familial CMC with hypothyroidism (APECED excluded) may be part of a compensatory response to immunological defects.

We were not able to find an underlying immunological defect in our CMC patients (Paper VI). In contrast to APECED patients, our patients in family A had no autoantibodies. TPO-Antibodies and TG-Antibodies were only found in the patients with newly diagnosed hypothyroidism in family B. Decline in autoantibodies with time is an explanation we have not be excluded. The patients' immunoglobulins, various lymphocyte subclasses and monocyte numbers were all within normal references limits. Candidiasis, onycomycosis and seborrhoeic dermatitis were not accompanied by classical signs of T-cell deficiency such as warts, molluscum contagiosum, other viral or opportunistic infections. APECED studies in mice suggest immunological defects within the monocyte derived APCs, but an HLA association was excluded in our families. The general mechanisms involved in protective immunity to fungi are still poorly understood, and we still do not know why CMC patients (APECED included or excluded) are particularly susceptible to candida.

Characterizing the immunological phenotype in all PIDs thoroughly, both in the clinic and in the laboratory, provides increased diagnostic precision in general, better understanding of the individual patient's immunodeficiency and may allow more targeted treatment for PID subgroups in the future.

Future Perspectives

Establishment of a permanent national PID registry

With continuous registration of PID cases in Norway, it will be possible to obtain population-based knowledge of the natural course of PIDs and contribute to the ongoing and expanding international research effort. National and international data are important for future recommendations for treatment such as Ig supplementation and bone marrow transplantation (23). A permanent national registry of PID patients will be of great value to healthcare professionals and families alike. A likely spin-off will be increased awareness of PID among pediatricians and other physicians. We need to establish a network linking all involved parties, professionals and patients, in order to increase sharing of information and to improve diagnostics and management. We should strive to assure that all patients have access to the best possible follow-up and treatment. This means shortening time to specific PID diagnosis with the aid of immunologic and molecular genetic analyses.

Ethical considerations in conjunction with a future PID registry

Informed consent from patients or parents was not obtained directly before inclusion in our previous, temporary national PID registry. However, the registry questionnaire was distributed to members of the Norwegian PID patient organization, who subsequently consulted their physicians to ensure that they were included. A substantial proportion of patients were registered via this route. Future medical quality of care registries will require informed consent from all participants. All individuals will have the right to withdraw from the registry and remove his / her personal information at any time. How deceased patients, patients without the ability to give consent and first degree relatives should be registered must be discussed. First degree family relatives may give consent for living individuals who are not competent to give informed consent themselves, i.e. parents may sign on behalf of their children. For deceased PID patients, however, informed consent may be difficult to obtain via distant relatives. Information concerning deceased PID patients can be very important for living relatives. Confidentiality for registered individuals, secure data storage and access are important issues. It is important to ensure that information is accessible only to authorized individuals. Semi-identification or pseudo-anonymization rather than complete anonymization of personal information will facilitate updating data on each patient and allow for longitudional studies of subgroups of PID patients. Easily accessible, frequent reports with non-identifiable/non-personalized/anonymous data from the registry can be made available for research and patient management. Obtaining informed written consent, registering patients with the correct PID diagnosis, and revising and adding data over time, will require involvement of local physicians and pediatricians. Systematic longitudinal registration should increase collaboration between primary, secondary and tertiary healthcare levels and increase general awareness of PID. National established registries should have a coordinating role, and transfer patient data to the ESID registry or other international PID databases. Local physicians may report their PID patients directly to ESID either via the internet based registry or via a national PID registry and mechanisms for avoiding registering the same patient more than once are required. International mutation registries linked to the ESID registry represent a basis for future PID cohort studies. The PID registries are gold mines for the discovery of new diseases. A nation-wide registry may function as a medical quality registry, by facilitating evaluation of different treatment methods and securing equal access to appropriate follow-up and treatment regimens irrespective of place of residence.

A permanent national PID registry would:

- increase knowledge and awareness of PID and promote clinical and basic research of PID disorders
- be an important support for clinical research assuming that semi-identification of the patients is permitted. Semi-identification gives clinical researchers the opportunity to go back to individual patients and to offer participation in clinical research projects.
- allow for more systematic collection of information, including detection of true disease incidences and prevalences
- be made known to all diagnosed PID patients who would be offered the opportunity to register.
- include a quality of care registry which would allow us to keep track of the current numbers of patients on special treatments and to evaluate results and effects of longterm treatments or lack of treatment.
- allow longitudinal collection of data.
- be continuously updated. Treatment and diagnostics change. New treatment methods need to be evaluated and compared with results from other countries because individual PIDs may be exceedingly rare. International collaboration and pooling of data is vital for both research and good patient care in rare disorders.
- provide updated, accessible and reliable information for health care providers.
- allow transfer of relevant information to international patient registries such as the internet based ESID Registry (222) or the international neutropenia registry SCNIR (http://www.severe-chronic-neutropenia.org/). The questionnaire for our former epidemiological study was a simple two-page form which was easy to complete. Updating information on individual patients can be achieved with one reminder every year, or via internet registration of new data. The Euro Huntington Disease Network (EHDN) is a good example of a multi-national collaborative treatment and research network. (http://www.euro-hd.net/). Active patient and family interaction via internet is an integrated feature of EHDN.
- be linked to a team of professionals with clinical experience in caring for PIDs. The registry would facilitate arranging meetings and conferences, establishing local and regional networks and disseminating evidence-based knowledge on all aspects of diagnosis and treatment.
- hopefully promote collaboration instead of competition between medical centers and departments caring for PID patients.
- allow for detection of factors with possible clinical implications. Hypotheses can then be tested in larger cross-sectional studies.

For some rare, multisystem disorders, such as A-T, not only collection of medical data, but closer follow-up with collaboration between medical professionals across health care regions is needed. Creation of a Norwegian A-T Clinical Care Network may be an alternative which can secure equal access and state-of-the-art management of all patients irrespective of home address. Finally, longitudinal studies and systematic medical follow-up of cohorts of few patients with rare disorders are valuable because they provide knowledge about the natural course of rare diseases. This information is much in demand among families and professionals alike.

"Not only add years to life, also add life to the years"

Quality of life studies are needed in patients with PID diagnoses other than antibody deficiencies. In particular, there is a major need for psychosocial and behavioural studies in **children**. To perform this type of necessary study in patient groups with neurodegenerative disorders such as ataxia-telangiectasia is especially challenging. Triangulation of methods, combining quantitative and qualitative studies and finding coherence with theories of human behaviour and attitude may prove fruitful in future studies. Future studies and interventions should be guided by the philosophy: "Add not only years to life, but life to the years"

Availability of genetic testing must improve and the immunological phenotype must be characterized.

Research has begun to clarify the genetic defects underlying the different immunodeficiencies and mechanisms predisposing to autoimmunity and cancer. Clinical applications of research findings will include the identification of the causative genetic defect which in turn may allow for the options of prenatal testing and testing of relatives at risk. The availability of genetic tests must be improve so that delays in diagnosis are reduced. As of 2007 the Norwegian genetic laboratories only offer diagnostic sequencing of coding regions of following genes: *BTK, RAG1/RAG2, IL2RG, JAK3, ELA2, WAS, NEMO, AIRE* and *ATM*. Intragenic mutation analysis for other PIDs must be performed at laboratories abroad, and availability of genetic testing is dependent on the individual physician. In families with more than one affected member, genetic linkage analyses, SNP base genome-wide scans and microsatelite analyses may be helpful in ruling in or excluding candidate genes. In the future it will become increasingly important to study the immunological mechanisms in PIDs with known genetic causes, and to perform appropriate molecular genetic testing in all patients, including adults. Mode of inheritance, susceptibility to infections with specific pathogens, as well as immunological profile, are clues to the type of immunodeficiency and should guide genetic testing. Even single families with only a few affected members may be valuable for genetic linkage analyses in the search for PID associated genes. Case reports and reports of small families (193), demonstrate that PIDs are "spotlight disorders", point to the important role of specific factors within the immune system, and show how important genetic verification can be for patient care and family planning. Inherited immunological diseases with specific genetic causes, may be amenable to targeted therapy (317). There are evidences that mutated genes in rare disorders may represent important target genes for treatment of other common or rare disorders. Therefore, rare monogenic disorders, in particular, may be of interest for drug development (318). With the advent of clinical implementation of microarray CGH, a substantial number of new microdeletion /-duplication syndromes are expected to be discovered (191). In patients with constitutional chromosomal abnormalities, the chromosome abnormality often encompasses many genes of which only one or a few contribute significantly to the phenotype (319). The laboratory immunological findings and characteristics of the clinical immunodeficiency may determine which genes are putative. Careful immunological characterisation of patients with chromosome abnormalities and susceptibility to infection may uncover new PID genes. And the other way around, further studies of patients with single mutations in genes within the specific chromosomal regions may clarify genotype-phenotype correlations, not forgetting that sequence variations in other genes located outside the particular chromosomal region also may influence the phenotype.

Future perspectives A-T immunological research

The immunological findings in the A-T patients are valuable in planning care. Characterizing the immunodeficiency in A-T should continue to be a part of the diagnostic process.

We have developed new recommendations for vaccination in A-T. The clinical effects (reduction in frequency of infections/ nasopharyngeal carrier status) of pneumococcal vaccines remain to be studied. We also need to determine the immunological mechanism underlying low immune responses to lipopolysaccharides and *Pneumococcus*, in particular. Studies of the presumed restricted V-region usage and oligoclonality of T and B cells in response to pneumococcal vaccine in A-T are needed. We should also study the effect of early childhood PCV7 vaccinations as part of the national vaccination programme and degree of persisting pneumococcal antibodies in new A-T patients. There is a general need to define absolute protective levels for specific antibodies to other, various microbial antigens in humans. The immunodeficiency alone does not explain increasing lung problems as a function of age. ATM deficiency and a possible direct, local effect on lung tissue need to be studied. We know that susceptibility to aspiration may increase the risk for and consequences of lung infections. Early management and monitoring of lung function is necessary to minimize lung damage(320): We should in the future follow lung function closely and treat pneumonia promptly.

Future genotype-phenotype A-T studies

Our patients who were homozygous for proximal truncating mutations (i.e. two null mutations) had relatively lower IgG2 and earlier debut of telangiectasias. The relationship between null mutations and severity of immunodeficiency had not been detected before. To confirm our results larger studies are needed: Genotype-phenotype comparisons for immunological phenotype, neurological phenotype, eye findings including debut of telangiectasias and AFP levels should be performed between patients with classical A-T with null mutations and those with atypical/milder A-T disease and leaky/missense mutations. The spectrum of *ATM* mutations is limited in Norway, but there is a large cohort of patients with atypical/milder A-T disease in UK who will be interesting to study.

Detect factors early that can predict increased risk for leukaemia, lymphoma and solid tumors in A-T

- relevance for detecting mechanisms leading to cancer in general.

Immunological status should be assessed regularly in all A-T patients in order to check for development of haematological malignancies or hyperimmunoglobulinopathy. It would have been interesting to see if immunological findings have predictive value relative to cancer risk or other longterm outcomes. Advanced immunological tests like molecular genetic characterisation of BCR and TCR in various clones may be required to document the mechanisms behind oncogenic BCR and TCR translocations in A-T, and could also elucidate mechanisms leading to leukemia and lymphoma in general. Studies of incidence and risk of other cancers including solid tumours in patients and of cancer risk in mutation carriers are necessary. We have shown that coexistence of MBL deficiency exacerbates the clinical immunodeficiency in A-T patients. The same phenomenon was seen in the patient with a constitutional 14q deletion. Similarly, sequence variation in other genes, such as MDM2, may modify the risk of solid tumors in A-T and cancer in A-T carriers.

A-T is a multisystem, pleiotropic disorder and ATM deficiency may have different or similar effects on epithelial derived cells, hepatocytes in the liver, alveolar cells in the lungs and neurons in cerebellum. The relationships between ATM deficiency, ROS, TP53 and cell damage have been studied in *in vitro* and in *Atm -/-* mice, and need to be studied in various tissues from human patients. Promising treatments will be developed that aim to either 1) increase ATM levels, or 2) reduce ROS and subsequent DNA damage, or 3) circumvent the

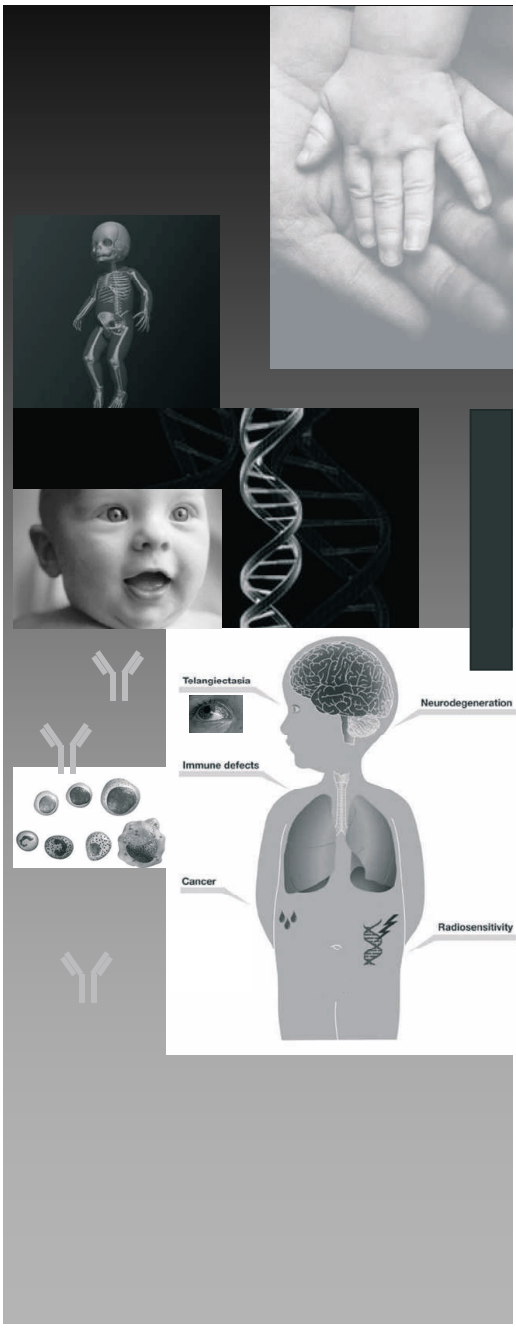
ATM repair mechanism to increase DNA repair. Effects *in vitro* and in mouse studies have been promising, but the various compounds remain to be tested in patients.

Develop standardized neurological assessments. AFP may be a surrogate marker.
Repeated standardized observations of neurological function over years are needed to chart disease progression. Longitudinal studies of gait function correlated with other neurological findings in larger cohorts of A-T patients should be done. Some standardized clinical measurements of the neurological deterioration in A-T exist (321) or are being developed, e.g. instrumental tracking of eye movements. There is a pressing need to standardize neurological assessment for use in international collaborative drug intervention studies.

One of the main difficulties in clinical research in A-T has been the lack of markers of disease progression. The immunologic variables are relatively constant, the neurologic markers difficult to validate, and occurrence of cancer is a seemingly random event. Thus, the elevation of AFP over time, even if only an epiphenomenon, may have potential as surrogate marker if correlates with a clinical outcome such as disease progression. The cause of AFP elevation remains to be determined. A possible connection between lower AFP levels and milder neurological disease needs to be studied. Measuring ATM protein levels and functional activity should be done in future studies to compare patients with classical A-T and those with milder disease.

An optimal strategy would be to study effects in mice and humans in parallel.
It is currently possible to study treatment effects, of for example antioxidants such PARP inhibitors(322), on *Atm -/-* mice with human mutations knocked-in combined with luciferase linked to genes of interest, i.e. genes involved in oxidative stress or apoptotic pathways. Bioluminescent assays with luciferase transgenic murine models permit demonstration of gene expression in an *in vivo* (323). Mouse APECED studies have suggested a primary monocyte/APC defect causing autoimmunity. *In vitro* functional autoimmunity studies of CMC patients' monocytes and dendritic cells are currently underway. The unexplained susceptibility to candidial infections requires study in human cells, since APECED mice do not develop candidiasis. There are numerous examples of differences between humans and knock-out mice as well as mice knocked-in for human mutations. Information from mouse models has contributed and will continue to contribute substantial knowledge to every medical field, especially to the study of immunodeficiency and DNA repair defects (305).

Finally, research collaboration between clinical scientists and laboratory scientists has brought us far and will continue to be fruitful in many aspects of PID research. Studies linking basic medical research and molecular genetics to clinical practice will play an increasing role in the future.


Telangiectasia
Neurodegeneration
Immune defects
Cancer
Radiosensitivity

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Supplement

Supplement 1

Presented here is a comprehensive list of various PIDs (Supplement Tables 1–9). The PID diagnoses can be classified into eight categories based on the immunologic phenotype. The tables are a modified version of information presented in the IUIS PID ESID-PAGID report (1;2). In addition, an algorithm which is helpful in diagnostic workup of SCID is included (Supplement Table 2). All SCID variants either lack T lymphocytes or have dysfunctional T cells. Some of the SCID variants can be classified by the number of B cells and/or NK cells present (Supplemental Table 2)

Supplement Table 1 Combined T- and B-cell immunodeficiencies

	Disease	Circulating T cells	Circulating B cells	Serum Immunoglobulins	Associated features	Inheritance	Genetic defects/presumed pathogenesis
1.	**T- B+NK- SCID**						
A	**γc deficiency**	Markedly decreased	Normal or increased	Decreased	Markedly decreased NK cells	XL	Defect in γ chain of receptors (IL2RG) for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21
B	**JAK3 deficiency**	Markedly decreased	Normal or increased	Decreased	Markedly decreased NK cells	AR	Defect in JAK3 signaling kinase
C	**IL-7Rα deficiency**	Markedly decreased	Normal or increased	Decreased	Normal NK cells	AR	Defect in IL-7 receptor α chain
D	**CD45 deficiency**	Markedly decreased	Normal	Decreased	Normal γ/δ T cells	AR	Defect in CD45
E	**CD3δ/CD3ε deficiency**	Markedly Decreased	Normal	Decreased	Normal NK cells	AR	Defect in CD3δ or CD3ε chains of T-cell antigen receptor
2.	**T−B−NK+ SCID**						
A	**RAG 1/RAG2 deficiency**	Markedly decreased	Markedly decreased	Decreased	Defective VDJ recombination	AR	Complete defect of recombinase activating gene (RAG) 1 or 2
B	**Artemis deficiency**	Markedly decreased	Markedly decreased	Decreased	Defective VDJ recombination, radiation sensitivity	AR	Defect in Artemis DNA recombinase-repair protein. Defect DNA double strand breaks repair.
C	**DNA ligase IV**	Decreased	Decreased	Decreased	Microcephaly, facial dystrophy, Defective VDJ recombination, radiation sensitivity	AR	DNA ligase IV defect, impaired nonhomologous end joining. Defect DNA double strand breaks repair.
D	**Cernunnous-XLF-NHEJ1**	Decreased	Decreased	Decreased	Microcephaly, facial dystrophy, Defective VDJ recombination, radiation sensitivity	AR	Impaired nonhomologous end joining, *NHEJ1* defect Defect DNA double strand breaks repair.
3.	**Omenn syndrome T(+)B-NK+ SCID**	Present; restricted heterogeneity	Normal or decreased	Decreased, except increased IgE	Erythroderma, eosinophilia, adenopathy, hepatosplenomegaly	AR	Missense mutations allowing residual activity, usually in RAG1 or RAG2 genes but also in Artemis, Cernunnous XLF, Ligase IV, IL-7Rα , and RMRP genes
4.	**Adenosine deaminase deficiency (ADA)**	Absent from birth (null mutations) or progressive decrease	Absent from birth or progressive decrease	Progressive decrease	Costochondral junction flaring Decreased NK cells	AR	Absent ADA, increased lymphotoxic metabolites (dATP, S-adenosyl homocysteine)
5.	**Purine nucleoside phosphorylase deficiency**	Progressive decrease	Normal	Normal or decreased	Autoimmune haemolytic anemia, neurologic impairment	AR	Absent PNP, T-cell, and neurologic defects from increased toxic metabolites (eg, dGTP)
6.	**Reticular dysgenesis**	Markedly decreased	Decreased or normal	Decreased	Granulocytopenia, thrombocytopenia Deafness	AR	Defective maturation of T, B, and myeloid cells (stem cell defect) Neutropenia
7.	**Winged helix deficiency (nude)**	Markedly decreased	Normal	Decreased	Alopecia, abnormal thymic epithelium (resembles nude mouse)	AR	Defects in forkhead box N1 transcription factor encoded by FOXN1, the gene mutated in nude mice
8.	**ORAI-1**	Normal levels, defect function	Normal	Normal, no response to vaccines	Ectodermal dysplasia, nonprogressive myopathia.	AR	Defect in calcium release-activated calcium channel function
9.	**MHC class II deficiency**	Normal number, decreased CD4 cells	Normal	Normal or decreased		AR	Mutation in transcription factors for MHC class II proteins (C2TA, RFX5, RFXAP, RFXANK genes)
10.	**Lymphocyte specific tyrosine kinase**	Lymphopenia, decreased CD4	Normal	Decreased	SCID-like phenotype	AR	Skipping of exon 7 in Lck transcript (in one SCID patient). Lck associates with CD4, and Lck is involved in T cell development (β chain rearrangement) and positive selection
11	**Trichothiodystrophy**	Decreased CD4 cells	Normal	Normal	Brittle hair, ichtyosis, mental retardation	AR	Defect nucleotide excision repair of DNA single strand breaks. (*ERCC2/XPD* linked to immunodeficiency, Other TTD genes are: *ERCC3/XPB, TTD-A, TTDN1)*
12.	**CD3γ deficiency**	Normal (reduced TCR expression)	Normal	Normal		AR	Defect in CD3γ
13.	**CD8 deficiency**	Absent CD8, normal CD4 cells	Normal	Normal		AR	Defects of CD8 α chain
14.	**ZAP-70 deficiency**	Decreased CD8, normal CD4 cells	Normal	Normal		AR	Defects in ZAP-70 signaling kinase
15.	**TAP-1/2 deficiency**	Decreased CD8, normal CD4	Normal	Normal	Vasculitis	AR	Mutations in TAP1 or TAP2 gene giving MHC classI deficiency
16.	**CD40 ligand deficiency Hyper IgM syndrome 1**	Normal	IgM and IgD B memory cells present, but others absent	IgM increased or normal, other isotypes decreased	Neutropenia, thrombocytopenia, hemolytic anemia, opportunistic infections, sclerosing cholangitis caused by cryptosporidiosis	XL	Defects in CD40 ligand (CD40L), defective B- and dendritic cell signalling
17.	**CD40 deficiency Hyper IgM syndrome 3**	Normal	IgM and IgD B cells present, other isotypes absent	IgM increased or normal, other isotypes decreased	Neutropenia, gastrointestinal and liver disease, opportunistic infections	AR	Defects in CD40, defective B- and dendritic cell signalling

SCID, Severe combined immunodeficiency; *XL*, X-linked inheritance; *JAK*, Janus-associated kinase; *IL-7Rα*, IL-7 receptor α; *AR*, autosomal recessive inheritance; *NK*, natural killer cells; XLF, XRCC4 like factor; NER, Nucleotide excision repair of single strand breaks in DNA; *dATP*, deoxyadenosine triphosphate; *dGTP*, deoxyguanosine diphosphate; *ZAP-70*, Zeta-associated protein of 70 kd; *TAP*, transporter associated with antigen processing.

*Atypical cases of severe combined immunodeficiency might present with T cells because of hypomorphic mutations or somatic mutations in T-cell precursors, or alloimmune T cells from the mother in Omenn syndrome.

Supplement Table 2 **Severe Combined Immunodeficiency SCID**

Gene	T	B	NK	Ig	Locus	Mode of inheritance	OMIM No
IL2RG	-	+	-	-	Xq13	XL	308380
JAK3	-	+	-	-	19p13.1	AR	600173
RAG1/RAG2*	-	-	+	-	11p13	AR	179615/179616
Artemis*	-	-	+	-	10p	AR	605988
DNALigase IV*	-	-	+	-	13q22-q34	AR	601837
Cernunnos-XLF-NHEJ1*	-	-	+	-	2q23	AR	79840
Reticular dysgenesis	-	-	-	-	Unknown	AR	267500
ADA	-	-	-	(+)/-	20q13.11	AR	102700
PNP	-	+/-	+	+/-	14q13.1	AR	164050
IL7Rα*	-	+	+	-	5p13	AR	146661
Nude WHN	-	+	+	-	17q11-q12	AR	600838
CD45	-	+	+/-	-	1q31-q32	AR	151460
CD3δ/CD3ε	-	+	+	-	11q23	AR	186790/186830
Lck	CD4 ↓	+	-	-	1p35-p34.3	AR	153390
MHC class II	CD4 ↓	+		+/-	16p13, 19p12,13q14 1q21.1-q21.3	AR	209920
Trichothiodystrophy	CD4 ↓	+	-	+	19q13.2-q13.3 2q21, 6p25.3, 7p14	AR	601675
CD3ξ	CD8 ↓	+		+	1q22-q23	AR	186780
CD3γ	CD8 ↓	+		+	11q23	AR	186740
ZAP70	CD8 ↓	+	+	+	2q12	AR	176947
TAP1/2	CD8 ↓	+		+	6p21.3	AR	170260/ 170261
ORAI1	+	+	-	+	12q24.31	AR	610277
*OMENN phenotype of RAG1/RAG2, Artemis, DNALigase IV, NHEJ1, IL7Rα, RMRP	+ oligo-clonal	-	+	IgE↑	Several: 11p13, 10p, 13q22-q34, 2q23, 5p13, 9p21-p12	AR	603554

Supplement Table 3 Predominantly antibody deficiencies

	Disease	B-cell numbers	Pro-B	Serum Ig	Associated features	Inheritance	Genetic defects /presumed pathogenesis
1.	**Severe reduction in all serum Ig isotypes with absent B cells**						
A	**Btk deficiency** **Bruton's agammaglobulinemia**	Profoundly decreased or absent.	Normal	All isotypes decreased	Severe bacterial infections	XL	Mutations in *BTK*
B	**m heavy chain deficiency**	Absent	Normal	All isotypes decreased	Severe bacterial infections	AR	Mutations in μ heavy chain
C	**lambda 5 deficiency**	Profoundly decreased or absent	Normal	All isotypes decreased	Severe bacterial infections	AR	Mutations in λ5
D	**Igα deficiency**	Absent	Normal	All isotypes decreased	Severe bacterial infections	AR	Mutations in Igα
E	**Igβ deficiency**	Absent	Normal	All isotypes decreased	Severe bacterial infections		
F	**BLNK deficiency**	Profoundly decreased or absent	Normal	All isotypes decreased	Severe bacterial infections	AR	Mutations in *BLNK*
G	**Thymoma with immunodeficiency**	Profoundly decreased or absent	Decreased	All isotypes decreased	Infections	None	Unknown
H	**Myelodysplasia**	Profoundly decreased or absent	Decreased	All isotypes decreased	Infections	Variable	Monosomy 7, trisomy 8 or dyskeratosis congenita
2.	**Severe reduction in at least 2 serum Ig isotypes with normal or low numbers of B cells**						
	Common variable immunodeficiency disorders[I]	Normal or decreased		Decrease in IgG and IgA; IgM might be normal	All have recurrent bacterial infections. Clinical phenotype vary: autoimmune, lymphoproliferative, and/or granulomatous disease	AR or AD	*TACI, BAFFR, Msh5* contributing polymorfisms[II]
A	**ICOS deficiency**	Normal or decreased		Decrease in IgG and IgA; IgM might be normal	Recurrent bacterial infections	AR	Mutation in *ICOS*
B	**CD19 deficiency**	Normal		Decrease in IgG and IgA; IgM might be normal	Recurrent bacterial infections	AR	Mutation in *CD19*
C	**X-linked lymphoproliferative syndrome 1**[III]	Normal		All isotypes may be low	Some patients have antibody deficiency, although most present with fulminant EBV infection or lymphoma	XL	Mutations in *SH2D1A*
3.	**Severe reduction in serum IgG and IgA with increased IgM and normal numbers of B cells**						
A	**CD40L deficiency**[IV] **Hyper IgM syndrome 1**	Normal or increased		IgG and IgA decreased; IgM increased or normal	Neutropenia, thrombocytopenia, hemolytic anemia and other autoimmune diseases, opportunistic infections, sclerosing cholangitis caused by cryptosporidiosis	XL	Mutations in *CD40L*
B	**CD40 deficiency**[IV] **Hyper IgM syndrome 3**	Normal		IgG and IgA decreased; IgM increased or normal	Neutropenia, gastrointestinal and liver disease, opportunistic infections	AR	Mutations in *CD40*
C	**AID deficiency**[V] **Hyper IgM syndrome type 2**	Normal		IgG and IgA decreased; IgM increased	Enlarged lymph nodes and germinal centers	AR or AD	Mutation in *AICDA.* Defect BER
D	**UNG deficiency**[V] **Hyper IgM syndrome type 5**	Normal		IgG and IgA decreased; IgM increased	Enlarged lymph nodes and germinal centers	AR	Mutation in *UNG.* Defect BER
4.	**Isotype or light chain deficiencies with normal numbers of B cells**						
A	**Ig heavy chain deletions**	Normal		IgG1, IgG2, or IgG4 absent; IgA1 and IgE can be absent	May be asymptomatic	AR	Chromosomal deletion at 14q32
B	**κ Chain deficiency**	Normal		All immunoglobulins have lambda light chain	Asymptomatic or recurrent viral-bacterial infections	AR	Mutation in Kappa constant gene
C	**Isolated IgG subclass deficiency**	Normal		Reduction in one or more IgG subclass	Recurrent bacterial infections	Variable	Unknown
D	**IgA with IgG subclass deficiency**	Normal		Reduced IgA, decrease in one or more IgG subclass;	Asymptomatic or recurrent infections with or without poor antibody response to carbohydrate antigens, allergies or autoimmune disease	Variable	Unknown
E	**Selective IgA deficiency**	Normal		IgA decreased	Some cases progress to CVID, others coexist with CVID in the same family	Variable	Mutation in TACI in few cases
5.	**Specific antibody deficiency with normal Ig concentrations and numbers of B cells**	Normal		Normal	Inability to make antibodies to specific antigens	Variable	Unknown
6.	Transient hypogammaglobulinemia of infancy	Normal		IgG and IgA decreased	Recurrent moderate bacterial infections	Variable	Unknown

Abbreviations to Supplement Table 3:

L, X-linked inheritance; *AR*, autosomal recessive inheritance; *Btk*, Burton tyrosine kinase; *BLNK*, B-cell linker protein; *AD*, autosomal dominant inheritance; *TACI*, transmembrane activator and calcium-modulator and cyclophilin ligand interactor; *BAFFR,* B-cell activating factor receptor; *Msh5,* homolog of *E.coli* MutS; *ICOS*, inducible costimulator; *AID*, activation-induced cytidine deaminase; BER, Base excicion repair of DNA single strand breaks, *UNG*, uracil-DNA glycosylase; *Ig(κ)*, immunoglobulin of κ light-chain type.

[I]Common variable immunodeficiency: there are several different clinical phenotypes, probably representing distinguishable diseases with differing immunopathogeneses

[II]Alterations in *TACI, BAFFR* and *Msh5* sequences represent contributing polymorphism or disease-modifying alterations. A disease-causing effect has been identified for homozygous C140R, S144X, and A181E *TACI* mutations.

[III]XLP1 (X-linked lymphoproliferative syndrome) is also included in Supplemental Table V.

[IV]CD40L deficiency (X-linked hyper IgM syndrome 1) and CD40 deficiency are also included in Supplemental Table I.

[V]Deficiency of activation-induced cytidine deaminse or uracil-DNA glycosylase present as forms of the HIGM syndrome but differ from CD40 ligand and CD40 deficiencies in that the patients have large lymph nodes with germinal centers and are not susceptible to opportunistic infections.

Supplement Table 4 — Other well-defined immunodeficiency syndromes

	Disease	Circulating T cells	Circulating B cells	Serum Ig	Associated features	Inheritance	Genetic defects/presumed pathogenesis
1.	**Wiskott-Aldrich syndrome**	Progressive decrease	Normal	Decreased IgM: antibody to polysaccharides particularly decreased; often increased IgA and IgE bacterial and viral infections	**Thrombocytopenia**; small platelets; eczema; lymphomas; autoimmune disease; bacterial infections	XL	Mutations in *WASP* gene; cytoskeletal defect affecting haematopoietic stem cell derivatives
2.	**DNA repair defects (other than those in Table I and Table II)**						
A	**Ataxia-telangiectasia**	Decreased	Normal	Often decreased IgA, IgE, and IgG subclasses; increased IgM monomers; antibodies variably decreased	Ataxia, telangiectasis, increased AFP levels, lymphoreticular and other malignancies, increased x-ray sensitivity	AR	Mutation in A-T gene (*ATM*); disorder of cell cycle check-point pathway leading to chromosomal instability and defect in DNA double strand breaks repair
B	**Ataxia-like syndrome**	Decreased	Normal	Often decreased IgA, IgE, and IgG subclasses; increased IgM monomers; antibodies variably decreased	Moderate ataxia, microcephaly, severely increased radiosensitivity, normal AFP levels, no telangiectasis	AR	Mutation in *MRE11*; disorder of cell-cycle checkpoint and defect in DNA double strand breaks repair
C	**Nijmegen breakage syndrome**	Decreased	Normal	Often decreased IgA, IgE, and IgG subclasses; increased IgM monomers; antibodies variably decreased	Microcephaly, lymphomas, ionizing radiation sensitivity, chromosomal instability, normal AFP levels, no telangiectasis	AR	Hypomophic mutation in *NBS1* (*Nibrin*); disorder of cell-cycle checkpoint and defect in DNA double strand breaks repair
D	**Bloom syndrome**	Normal	Normal	Reduced	Chromosomal instability, marrow failure, leukemia, lymphoma, short stature, bird-like face, sensitivity to the sun, telangiectasias	AR	Mutation in *BLM*, a RecQ-like helicase
3.	**Thymic defects**						
	Di George anomaly	Decreased or normal	Normal	Normal or decreased	Hypoparathyroidism, conotruncal malformation; abnormal facies; partial monosomy of 22q11-pter or 10p in some patients	*De novo* defect or AD	Contiguous gene defect in 90% affecting thymic development; deletion or mutation in *TBX1* (22q11) or deletion involving the *NEBL* gene (10p)
4.	**Immuno-osseous dysplasias**						
A	**Cartilage hair hypoplasia***	Decreased or normal	Normal	Normal or reduced; antibodies variably decreased	Short-limbed dwarfism with metaphyseal dysostosis, sparse hair, anemia, neutropenia, susceptibility to cancer, impaired spermatogenesis; neuronal dysplasia of the intestine	AR	Mutation in *RMRP* *
B	**Schimke immunoosseus dysplasia**	Decreased	Normal	Normal	Short stature, spondiloepiphyseal dysplasia, intrauterine growth retardation, nephropathy	AR	Mutation in *SMARCAL1*
5.	**Hermansky-Pudlak syndrome type2**	Normal	Normal	Normal	Oculocutaneous albinism, **neutropenia**, defective cytotoxic activity of T and NK lymphocytes, bleeding diathesis	AR	Mutation in *AP3B1*
6.	**Hyper-IgE syndrome**						
A	**Job syndrome (AD HIES)**	Normal	Normal	Elevated IgE	Recurrent skin boils and pneumonia caused by *Staph aur*; pneumatoceles; exzema; nail candidiasis, facial dysmorphism, hyperextensible joints, delayed shedding primary teeth	*De novo* defect or AD	Mutation in *STAT3*
B	**AR HIES with mycobacterial and viral infections**	Normal	Normal	Elevated IgE	Susceptibility to fungi (*Candida),* intracellular bacteria (mycobacteria, *Salmonella*), and viruses	AR	Mutations in *TYK2*
C	**AR HIES with viral infections and CAN vasculitis/hemorrhage**	Normal	Normal	Elevated IgE	Susceptibility to bacterial, viral and fungal *Candida* infections; eczema; vasculitis; CNS hemorrhage		Unknown
7.	**Chronic mucocutaneous candidiasis, CMC isolated or with hypothyroidism**	Normal	Normal	Normal	Chronic mucocutaneous candidiasis, impaired delayed-type hypersensitivity to *Candida* antigens	AD, AR, sporadic	Unknown
8.	**APECED, autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy**	Normal, increased $CD4^+$ cells	Normal	Normal	Autoimmune disease of parathyroid, adrenal and other organs plus candidiasis, dental enamel hypoplasia and other abnormalities	AR	Defects in *AIRE*, encoding a transcription regulator needed to establish thymic self-tolerance
9.	**Hepatic veno-occlusive disease with immunodeficiency**	Normal (decreased memory T cells)	Normal (decreased memory B cells)	Decreased IgG, IgA, IgM	Hepatic veno-occlusive disease; *Pneumocystis jiroveci* pneumonia; thrombocytopenia, hepatosplenomegaly	AR	Mutation in *SP110*
10.	**Hoyerall-Hreidarsson syndrome**	Progressive decrease	Progressive decrease	Variable	Intrauterine growth retardation, microcephaly, digestive tract involvement, develops pancytopenia, reduced number and function of NK cells	XL	Mutation in *Dyskerin*

Abbreviations to Supplement Table 4:

WASP, Wiskott-Aldrich syndrome protein; *MRE11*, meiotic recombination 11; *XL*, X-linked inheritance; *AR*, autosomal recessive inheritance; *AD*, autosomal dominant inheritance; AFP, alpha fetoprotein, *RMRP*, RNA component of mitochondrial RNA-processing endoribonuclease; *SMARCAL1*, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily alpha-like 1; *AP3B1*, adaptor-related protein complex 3, β-1 subunit.

* Patients with cartilage-hair hypoplasia can also present with typical severe combined immunodeficiency syndrome or with Omenn syndrome.

Supplement Table 5 Diseases of immune dysregulation

	Disease	Circulating T cells	B cells	Serum Ig	Associated features	Inheritance	Genetic defects/presumed pathogenesis
1.	**Immunodeficiency with hypopigmentation**						
A	**Chediak-Higashi syndrome**	Normal	Normal	Normal	Partial albinism, giant lysosomes, low NK and CTL activities, acute-phase reaction, encephalopathic accelerated phase	AR	Defects in *LYST* gene, impaired lysosomal trafficking
B	**Griscelli syndrome, type 2**	Normal	Normal	Normal	Partial albinism, low NK and CTL activities, acute-phase reaction, might have encephalopathy	AR	Defects in *RAB27A* encoding a GTPase in secretory vesicles
C	**Hermansky-Pudlak syndrome, type 2**	Normal	Normal	Normal	Partial albinism, **neutropenia,** low NK and CTL activity, increased bleeding	AR	Mutations of *AP3B1* gene, encoding for the β subunit of the AP-3 complex
2.	**Familial hemophagocytic lymphohistiocytosis (FHL) syndromes**						
A	**Perforin deficiency**	Normal	Normal	Normal	Severe inflammation, fever, decreased NK and CTL activities	AR	Defects in *PRF1*; perforin, a major cytolytic protein
B	**Munc 13-D deficiency**	Normal	Normal	Normal	Severe inflammation, fever, decreased NK and CTL activities	AR	Defects in *MUNC13D* required to prime vesicles for fusion
C	**Syntaxin 11 deficiency**	Normal	Normal	Normal	Severe inflammation, fever, decreased NK and CTL activities	AR	Defects in *STX11*, involved in vesicle trafficking and fusion
3.	**X-linked lymphoproliferative syndrome**						
A	**XLP1**	Normal	Normal or reduced	Normal or low Igs	Clinical and immunologic abnormalities triggered by EBV infection, including hepatitis, aplastic anemia, lymphoma	XL	Defects in *SH2D1A* encoding adaptor protein regulating intracellular signals
B	**XLP2**	Normal	Normal or reduced	Normal or low Igs	Clinical and immunologic abnormalities triggered by EBV infection, including splenomegaly, hepatitis, hemophagocytic syndrome, lymphoma	XL	Defects in *XIAP* encoding an inhibitor of apoptosis
4.	**Syndromes with autoimmunity**						
A	**Autoimmune lymphoproliferative syndrome (ALPS)**						
I	**CD95 (Fas) defects, type 1a**	Normal, increased double-negative ($CD4^-$ $CD8^-$) $\alpha\beta^+$T cells	Normal	Normal or increased	Defective lymphocyte apoptosis, splenomegaly, adenopathy, autoimmune blood cytopenias, increased lymphoma risk	AD (rare severe AR cases)	Defects in *TNFRSF6*, cell-surface apoptosis receptor
II	**CD95L (Fas ligand) defects, ALPS type 1b**	Normal, increased double-negative ($CD4^-$ $CD8^-$) $\alpha\beta^+$T cells	Normal	Normal	Defective lymphocyte apoptosis, splenomegaly, adenopathy, autoimmune blood cytopenias, lupus	AD	Defects in *TNFSF6*, ligand for CD95 apoptosis receptor
III	**Caspase 10 defects, ALPS type 2a**	Normal, increased $CD4^-$ $CD8^-$ $\alpha\beta^+$T cells	Normal	Normal	Adenopathy, splenomegaly, defective lymphocyte apoptosis, autoimmune disease	AD	Defects in *CASP10*, intracellular apoptosis pathway
IV	**Caspase 8 defects, ALPS type 2b**	Normal, slightly increased $CD4^-$ $CD8^-\alpha\beta^+$T cells	Normal	Normal or decreased	Adenopathy, splenomegaly; defective lymphocyte apoptosis and activation; recurrent bacterial and viral infections	AD	Defects in *CASP8*, intracellular apoptosis, and activation pathways
V	**NRAS gain-of.function, ALPS type 3**	Normal, increased double-negative ($CD4^-$ $CD8^-$) $\alpha\beta^+$T cells	Normal	Normal	Defective lymphocyte apoptosis, splenomegaly, adenopathy, multiple autoantibodies, increased leukaemia and lymphoma risk	AD	*NRAS* gain-of –function mutation augments RAF/MEK/ERK signaling which decreases the proapoptotic protein BIM and attenuates intrinsic, nonreceptor-mediated mitochondrial apoptosis.
B	**APECED, autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy***	Normal, increased $CD4^+$ cells	Normal	Normal	Autoimmune disease of parathyroid, adrenal and other organs plus candidiasis, dental enamel hypoplasia and other abnormalities	AR	Defects in *AIRE*, encoding a transcription regulator needed to establish thymic self-tolerance
C	**IPEX, immune dysregulation, polyendocrinopathy, enteropathy (X-linked)**	Normal, lack of $CD4^+$ $CD25^+$ $FOXP3^+$ regulatory T cells	Normal	Increased IgA, IgE	Autoimmune diarrhea, early-onset diabetes, thyroiditis, hemolytic anemia, thrombocytopenia, eczema	XL	Defects in *FOXP3*, encoding a T-cell transcription factor
D	**CD25 deficiency**	Normal to modestly decreased, impaired T cell proliferation	Normal	Normal	Lymphoproliferation (lymphadenopathy, hepatosplenomegaly), autoimmunity as in IPEX syndrome.	AR	Defects in IL2Rα chain
E	**STAT5b**	Modestly decreased, impaired T cell proliferation	Normal	Normal	Growth hormone insensitive dwarfism, dysmorphic features, eczema, lymphocytic interstitial pneumonitis, low NK activity	AR	Defects in STAT5B gene

Abbreviations to Supplement Table 5:

NK, Natural killer; *CTL*, cytotoxic T-lymphocyte; *AR*, autosomal recessive inheritance; *XL*, X-linked inheritance; *AD*, autosomal dominant inheritance; *LYST*, lysosomal trafficking regulator; *RAB27A*, Rab protein 27A; *PRF1*, perforin 1; *SH2D1A*, SH2 domain protein 1A; *TNFRSF6*, tumor necrosis factor receptor soluble factor 6; *APECED*, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; *AIRE*, autoimmune regulator; *IPEX*, immune dysregulation–polyendocrinopathy–enteropathy–X-linked; *FOXP3*, Forkhead box protein 3.
*APECED is also presented in Supplement Table 4

Supplement Table 6 Congenital defects of phagocyte number, function, or both

	Disease	Affected cells	Affected function	Associated features	Inheritance	Genetic defects/presumed pathogenesis
1.-3.	**Severe congenital neutropenias**	N	Myeloid differentiation	Subgroup with myelodysplasia	AD	*ELA2*: mistrafficking of elastase
		N	Myeloid differentiation	B/T lymphopenia	AD	*GFI1*: repression of elastase
4.	**Kostmann syndrome**	N	Myeloid differentiation		AR	HAX1: control of apoptosis
5.	**Cyclic neutropenia**	N		Oscillations of other leukocytes and platelets	AD	*ELA2*: mistrafficking of elastase
6.	**X-linked neutropenia /myelodysplasia**	N + M		Monocytopenia	XL	*WASP:* Regulator of actin cytoskeleton (loss of autoinhibition)
7.	**P14 deficiency**	N + L + melanocytes	Endosome biogenesis	Neutropenia, Hypogammaglobulinemia, ↓CD8 cytotoxicity, Partial albinism, Growth failure	AR	*MAPBPIP:* Endosomal adaptor protein 14
8.	**Leukocyte adhesion deficiency type 1**	N + M L + NK	Adherence Chemotaxis Endocytosis T/NK cytotoxicity	Delayed cord separation, Skin ulcers, Periodontitis, Leukocytosis	AR	*ITGB2:* Adhesion protein CD18
9.	**Leukocyte adhesion deficiency type 2** = Congenital disorder of glycosylation type IIc	N + M L + NK	Rolling, Chemotaxis	Recurrent bacterial infections periodontitis, pneumonia, cellulites without pus formation, leukocytosis, hh-blood group, short stature, mental retardation	AR	*SLC35C1*: FUCT1 GDP-fucose transporter
10.	**Leukocyte adhesion deficiency type 3**	N + M + L + NK + platelets	Adherence	Infections, leukocytosis, bleeding tendency	AR	*RASGRB2*: Rap1- mediated activation of β1-3 integrins
11.	**Rac 2 deficiency**	N	Adherence Chemotaxis O_2^- production	Poor wound healing, Leukocytosis	AD	*RAC2*: Regulation of actin cytoskeleton
12.	**β-Actin deficiency**	N + M	Motility	Mental retardation, Short stature	AD	*ACTB*: Cytoplasmic actin
13.	**Localized juvenile periodontitis**	N	Formylpeptide-induced chemotaxis	Periodontitis only	AR	*FPR1*: Chemokine receptor
14.	**Papillon-Lefèvre syndrome**	N + M	Chemotaxis	Periodontitis, palmoplantar hyperkeratosis	AR	*CTSC*: Cathepsin C activation of serine proteases
15.	**Specific granule deficiency**	N	Chemotaxis	N with bilobed nuclei	AR	*C/EBPE*: myeloid Transcription factor
16.	**Shwachman-Diamond syndrome**	N	Chemotaxis	Pancytopenia, exocrine pancreatic insufficiency, Chondrodysplasia	AR	*SBDS*
17.	**X-linked chronic granulomatous disease (CGD)**	N + M	Killing (faulty O_2^- production)	Subgroup: McLeod phenotype	XL	*CYBB*: Electron transport protein (gp91phox)
18.-20.	**Autosomal CGDs (3 forms)**	N + M	Killing (faulty O_2^- production)		AR	*CYBA*: Electron transport protein (p22phox)
						NCF1: Adapter protein (p47phox)
						NCF2: Activating protein (p67phox)
21.	**Neutrophil G-6PD deficiency**	N + M	Killing (faulty O_2^- production)	Hemolytic anemia	XL	*G-6PD*: NADPH generation
22.	**IL-12 and IL-23 receptor β1 chain deficiency**	L + NK	IFN-γ secretion	Susceptibility to *Mycobacteria* and *Salmonella*	AR	*IL-12Rβ1*: IL-12 and IL-23 receptor β1 chain
23.	**IL-12p40 deficiency**	M	IFN-γ secretion	Susceptibility to *Mycobacteria* and *Salmonella*	AR	*IL-12p40* subunit of IL12/IL23: IL12/IL23 production
24.	**IFN-γ receptor 1 deficiency**	M + L	IFN-γ binding and signaling	Susceptibility to *Mycobacteria* and *Salmonella*	AR, AD*	*IFNGR1*: IFN-γR binding chain
25.	**IFN-γ receptor 2 deficiency**	M + L	IFN-γ signaling	Susceptibility to *Mycobacteria* and *Salmonella*	AR	*IFNGR2*: IFN-γR signaling chain
26.	**STAT1 deficiency (2 forms)**	M + L	IFN α/β/γ signaling	Susceptibility to *Mycobacteria, Salmonella*, and viruses	AR	*STAT1*
			IFN-γ signaling	Susceptibility to *Mycobacteria* and *Salmonella*	AD*	*STAT1*

N, Neutrophils; *AD*, autosomal dominant; *AR*, autosomal recessive inheritance; *M*, monocytes-macrophages; *XL*, X-linked inheritance; *L*, lymphocytes; *NK*, natural killer cells; *LAD*, leukocyte adhesion deficiency; *FUCT1*, fucose transporter 1; *GDP*, guanosine diphosphate; *SBDS*, Schwachman-Bodan-Diamond syndrome; *STAT1*, signal transducer and activator of transcription 1.

*The AD form of IFNGR1 deficiency or of STAT1 deficiency is caused by dominant negative mutations.

G-CSFR mutations are no longer in the list causing severe congenital neutropenia since mutations in G-CSFR are now regarded as aquired somatic mutations linked to G-CSF refractory neutropenia and cancer development (324)

Supplement Table 7 Defects in innate immunity

Disease	Affected cells	Functional defects	Associated features	Inheritance	Genetic defects
Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) **Hyper IgM syndrome 4**	L + M	NF-κB signaling pathway	Anhidrotic ectodermal dysplasia + specific antibody deficiency (lack of antibody response to polysaccharides), various infections (*Mycobacteria* and pyogens)	XR	*NEMO*
Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID)	L + M	NF-κB signaling pathway	Anhidrotic ectodermal dysplasia + T-cell defect + various infections	AD	*IKBA* gain-of-function
IL-1 receptor–associated kinase 4 (IRAK4) deficiency	L + M	TIR-IRAK signaling pathway	Bacterial infections (pyogens)	AR	*IRAK4*
WHIM (warts, hypogammaglobulinemia infections, myelokathexis) syndrome	N + L	Increased response of the CXCR4 chemokine receptor to its ligand CXCL12 (SDF-1)	Hypogammaglobulinemia, reduced B-cell number, severe reduction of neutrophil count, warts/HPV infection	AD	*CXCR4* gain-of-function
Epidermodysplasia verruciformis	Keratinocytes + leukocytes		Human papilloma virus (group B1) infections and cancer of the skin	AR	*EVER1, EVER2*
Herpes simplex encephalitis	CNS resident/ Epithelial/ Dendritic cells + leukocytes	UNC-93B-dependent IFN-α, IFN-β and IFN-λ induction	Herpes simplex virus 1 encephalitis and meningitis	AR	*UNC93B1*
Herpes simplex encephalitis	CNS resident/ Epithelial/ Dendritic cells + CTL	TLR3-dependent IFN-α, IFN-β and IFN-λ induction	Herpes simplex virus 1 encephalitis and meningitis	AD	*TLR3*

L, lymphocytes; *M*, monocytes-macrophages; *NF-κB*, Nuclear factor κB; *XR*, X-linked recessive; *NEMO*, NF-κB essential modulator; *AD*, autosomal dominant inheritance; *AR*, autosomal recessive inheritance; *IRAK4*, IL-1 receptor-associated kinase 4; *N*, Neutrophils; *SDF-1*, stromal-derived factor 1; *EVER*, epidermodysplasia verruciformis; *TIR*, Toll and IL-1 receptor; *HPV*, human papilloma virus; CNS, central nervous system; CTL, cytolytic lymphocytes; TLR, Toll-like receptor.

Supplement Table 8 Autoinflammatory disorders

Disease	Affected cells	Functional defects	Associated features	Inheritance	Genetic defects
Familial Mediterranean fever	N + M	Decreased production of pyrin permits ASC-induced IL-1 processing and inflammation after subclinical serosal injury; macrophage apoptosis decreased	Recurrent fever, serositis, and inflammation responsive to colchicines; predisposes to vasculitis and inflammatory bowel disease	AR	*MEFV*
TNF receptor–associated periodic syndrome (TRAPS)	N + M	Mutations of 55-kd TNF receptor leading to diminished soluble cytokine receptor available to bind TNF	Recurrent fever, serositis, rash, and ocular or joint inflammation	AD	*TNFRSF1A*
Hyper-IgD syndrome		Mevalonate kinase deficiency affecting cholesterol synthesis; pathogenesis of disease unclear	Periodic fever and leukocytosis with **high IgD** levels	AR	*MVK*
Muckle-Wells syndrome*	N + M	Defect in cryopyrin, involved in leukocyte apoptosis and NF-κB signaling and IL-1 processing	Urticaria, SNHL, amyloidosis; responsive to IL-1R/antagonist (Anakinra)	AD	*CIAS1* (also called PYPAF1 or NALP3)
Familial cold autoinflammatory syndrome*	N + M	Same as above	Nonpruritic urticaria, arthritis, chills, fever, and leukocytosis after cold exposure; responsive to IL-1R/antagonist (Anakinra)	AD	*CIAS1*
Neonatal-onset multisystem inflammatory disease (NOMID) or chronic infantile neurologic cutaneous and articular syndrome (CINCA)*	N + chondrocytes	Same as above	Neonatal-onset rash, chronic meningitis, and arthropathy with fever and inflammation responsive to IL-1R antagonist (Anakinra)	AD	*CIAS1*
Pyogenic sterile arthritis, pyoderma gangrenosum, acne (PAPA) syndrome	Hematopoietic tissues, upregulated in activated T cells	Disordered actin reorganization leading to compromised physiologic signaling during inflammatory response	Destructive arthritis, inflammatory skin rash, myositis	AD	*PSTPIP1* (also called C2BP1)
Blau syndrome	M	Mutations in nucleotide binding site of CARD15, possibly disrupting interactions with lipopolysaccharides and NF-κB signalling	Uveitis, granulomatous synovitis, camptodactyly, rash, and cranial neuropathies; 30% have Crohn's disease	AD	*NOD2* (also called CARD15)
Majeed syndrome	N and other bone marrow cells		Chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anemia with hypochromia and microcytosis, growth failure joint contractures, exzema, neutrophilic dermatosis (Sweet syndrome), Recurrent fevers (every 3rdweek)	AR	*LPIN2*

ASC, Apoptosis-associated speck-like protein with a caspase recruitment domain; *AR*, autosomal recessive inheritance; *MEFV*, Meditteranean fever; *PMNs*, polymorphonuclear cells; *AD*, autosomal dominant inheritance; *TNFRSF1A*, tumor necrosis factor receptor soluble factor 1A; *NF-κB*, nuclear factor κB; *N*, neutrophils; *M*, monocytes/macrophages; *L*, lymphocytes; *NK*, natural killer cells; *SNHL*, sensorineural hearing loss; *CIAS1*, cold-induced autoinflammatory syndrome 1; *PSTPIP1*, proline/serine/threonine phosphatase-interacting protein 1; *CD2BP1*, CD2 binding protein 1; *CARD*, caspase recruitment domain; *NOD2*, nucleotide-binding oligomerization domain protein 2.

*All 3 syndromes are associated with similar *CIAS1* mutations. Disease phenotype in any individual appears to depend on modifying effects of other genes and environmental factors.

Supplement Table 9 Complement deficiencies

Disease	Functional defects	Associated features	Inheritance	Genetic defects
C1q deficiency	Absent C hemolytic activity, defective MAC*; faulty dissolution of immune complexes; faulty clearance of apoptotic cells	SLE-like syndrome, rheumatoid disease, infections	AR	C1q
C1r deficiency[I]	Absent C hemolytic activity, defective MAC; faulty dissolution of immune complexes	SLE-like syndrome, rheumatoid disease, infections	AR	C1r*
C4 deficiency	Absent C hemolytic activity, defective MAC; faulty dissolution of immune complexes; defective humoral immune response	SLE-like syndrome, rheumatoid disease, infections	AR	C4
C2 deficiency[II]	Absent C hemolytic activity, defective MAC; faulty dissolution of immune complexes	SLE-like syndrome, vasculitis, polymyositis, pyogenic infections	AR	C2[†]
C3 deficiency	Absent C hemolytic activity, defective MAC; defective bactericidal activity; defective humoral immune response	Recurrent pyogenic infections	AR	C3
C5 deficiency	Absent C hemolytic activity, defective MAC; defective bactericidal activity	Neisserial infections, SLE	AR	C5
C6 deficiency	Absent C hemolytic activity, defective MAC; defective bactericidal activity	Neisserial infections, SLE	AR	C6
C7 deficiency	Absent C hemolytic activity, defective MAC; defective bactericidal activity	Neisserial infections, SLE, vasculitis	AR	C7
C8a deficiency[III]	Absent C hemolytic activity, defective MAC; defective bactericidal activity	Neisserial infections, SLE	AR	C8α
C8b deficiency	Absent C hemolytic activity, defective MAC; defective bactericidal activity	Neisserial infections, SLE	AR	C8β
C9 deficiency	Reduced C hemolytic activity, defective MAC; defective bactericidal activity	Neisserial infections[IV]	AR	C9
C1 inhibitor deficiency	Spontaneous activation of the complement pathway with consumption of C4/C2; spontaneous activation of the contact system with generation of bradykinin from high-molecular-weight kininogen	Hereditary angioedema	AD	C1 inhibitor
Factor I deficiency	Spontaneous activation of the alternative complement pathway with consumption of C3	Recurrent pyogenic infections	AR	Factor I
Factor H deficiency	Spontaneous activation of the alternative complement pathway with consumption of C3	Hemolytic-uremic syndrome, membranoproliferative glomerulonephritis	AR	Factor H
Factor D deficiency	Absent hemolytic activity by the alternate pathway	Neisserial infection	AR	Factor D
Properdin deficiency	Absent hemolytic activity by the alternate pathway	Neisserial infection	XL	Properdin
Mannose Binding Lectin (MBL)deficiency	Defective mannose recognition; defective hemolytic activity by the lectin pathway	Pyogenic infections with very low penetrance, mostly asymptomatic	AR	SNP variation in *MBL2*
MASP2 deficiency[V]	Absent hemolytic activity by the lectin pathway	SLE syndrome, pyogenic infection	AR	*MASP2*

MAC, Membrane attack complex; *SLE*, systemic lupus erythematosus; *AR*, autosomal recessive inheritance; *AD*, autosomal dominant inheritance; *XL*, X-linked inheritance; *MBL*, mannose-binding lectin; SNP, single nucleotide polymorphism; *MASP2*, mannose-binding protein–associated serine protease 2.

I C1r deficiency in most cases is associated with C1s deficiency. The gene for C1s also maps to chromosome 12 pter.

II Type 1 C2 deficiency is in linkage disequilibrium with HLA-A25, HLA-B18, and HLA-DR2 and complotype SO42 (slow variant of Factor B, absent C2, type 4 C4A, type 2 C4B) and is common in white subjects. It results from a 28-bp deletion in the C2 gene; C2 is synthesized but not secreted. Type 2 C2 deficiency is very rare and involves gene defects other than that found in type 1 C2 deficiency and a failure of C2 synthesis.

III C8a deficiency is always associated with C8 g deficiency. The gene encoding C8 g maps to chromosome 9 and is normal, but C8 g covalently binds to C8a.

IV Association is weaker than with C5, C6, C7, and C8 deficiencies.

V A single patient.

Appendices

Abbreviations

AFP	alpha fetoprotein
AID	activation-induced cytidine deaminase
ALPS	autoimmune lymphoproliferative syndrome
AOA1	ataxia oculomotor apraxia syndrome 1
AOA2	ataxia oculomotor apraxia syndrome 2
APC	antigen presenting cell
APECED	autoimmune polyendocrine candidiasis ectodermal dystrophy, caused by *AIRE* gene mutations
APS I	autoimmune polyendocrine syndrome type I
A-T	ataxia-telangiectasia
ATG	anti-thymocyte globulin
ATLD	A-T like disorder
ATM	ataxia telangiectasia mutated, the A-T gene
ATR	ATM and Rad3-related, member of PIKK family
AVED	ataxia with vitamin E deficiency
BCG	Mycobacterium bovis Bacille Calmette-Guerin
BCR	B cell receptor
BER	base excision repair
BTK	bruton tyrosine kinase
Bu	busulphan
C.albicans	*candida albicans*
C2-C9	complement factors 2-9
CD	cluster of differentiation, a protocol for leukocyte cell surface molecules
cDNA	complementary DNA
cer	centromere
CGD	chronic granulomatous disease
CGH	comparative genomic hybridization
CI	confidence interval
CMC	chronic mucocutaneous candidiasis
CMV	cytomegalovirus
CNS	central nervous system
ConA	concanavalin-A
CPS	capsular polysaccharide
CRM197	cross-reactive material 197 of diphtheria toxin
CSR	class switch recombination
CVID	common variable immunodeficiency
Cy	cyclophosphamide
DC	dendritic cell
del	deletion
DGGE	denaturing gradient gel electrophoresis
dHPLC	denaturing high performance liquid chromatography

DNA	deoxyribonucleic acid
ds	double substitution
DSBs	DNA double-strand breaks
EBMT	European Group for Blood and Marrow Transplantation
EBNA	anti-EBV nuclear antigen
EBV	Epstein-Barr virus
EHDN	Euro Huntington Disease Network
EIA	enzyme immunoassay
ELISA	enzyme linked immunosorbent assay
ENT	ear, nose, throat
ESID	European Society for Immunodeficiencies
FAP	familial adenomatous polyposis
FAT	FRAP, ATM, TRRAP
FATC	FRAP, ATM , TRRAP C-terminal
FISH	fluorescent in situ hybridization
FRAP	FKBP-12-rapamycin associated protein, also known as mTOR
fs	frame shift
GAD	glutamic acid decarboxylase
G-CSF	granulocyte-colony stimulating factor
GVHD	graft versus host disease
HCV	hepatitis C virus
Hib	*Hemophilus influenzae* type b
HLA	human leukocyte antigen
HR	homologous recombination
HSV	herpes simplex virus
HVG	host versus graft
IFNGR1	interferon gamma receptor deficiency type 1
Ig	immunoglobulin
IgA	immunoglobulin A
IGH	immunoglobulin heavy chain
IGK	immunoglobulin kappa light chain
IGL	immuoglobulin lambda light chain
IL-12	interleukin 12
Ins	insertion
IPEX	immune dysregulation, polyendocrinopathy, enteropathy, X-linked, caused by *FOXP3* mutations
IUIS	International Union of Immunological Societies
IvIg	intravenous immunoglobulin
JCS	Jalowiec Coping Scale
LAD1	leukocyte adhesion defect type 1
LPS	lipopolysaccharide
MBL	mannose binding lectin
MEIA	microparticle enzyme immunoassay

MHC	major histocompatibility complex
microarray CGH	microarray-based comparative genomic hybridization
MIM	Mendelian Inheritance in Man, the online version is called OMIM
MLPA	multiplex ligation-dependent probe amplification
MMR	mismatch repair
ms	missense
mTOR	mammalian target of rapamycin, also known as FRAP, member of PIKK family
MTX	methotrexate
MUD	matched unrelated donor
NAPOS	Norwegian Porphyria Centre
NBS1	Nijmegen breakage syndrome
NER	nucleotide excision repair
NHEJ	nonhomologous end joining
NHS	Nowotny Hope Scale
NK cells	natural killer cells
ns	nonsense
OMIM	Online Mendelian Inheritance in Man, a database of human genes/genetic disorders
p	short arm of the chromosome
PAGID	Pan American Group for Immunodeficiency
PCR	polymerase chain reaction
PCV7	seven-valent pneumococcal conjugated vaccine
PHA	phytohemagglutinin
PI3K	phosphoinositide 3-kinases
PIDs	primary immunodeficiency diseases
PIKK	phosphatidylinositol 3-kinase-like kinase
PPV23	23-valent pneumococcal polysaccharide vaccine
PTT	protein truncating test
PWM	pokeweed mitogen
q	long arm of the chromosome
QLI	Ferrans and Powers Quality of Life Index
ROS	reactive oxygen species
RPP Scale	scale for measuring resources and pressures in the past
RR	relative risk
SCAN1	spinocerebellar ataxia with neuropathy
SCAR1	spinocerebellar ataxia recessive of non-Friedreich type 1
SCC	side-chain cleavage enzyme
SCID	severe combined immunodeficiency
Sclg	subcutaneous immunoglobulin
SCN	severe congenital neutropenia
SCNIR	Severe Chronic Neutropenia International Registry
SCT	stem cell transplantation
SF-36	Short Form-36

SHM	somatic hypermutation
SI	stimulation index; counts per min divided with the spontaneous proliferation
SIR	standardized incidence rate
SNP	single nucleotide polymorphism
SP-A	surfactant protein A
SP-D	surfactant protein D
ss	splice site mutation.
TCR	T cell receptor
TDP1	tyrosyl-DNA phosphodiesterase 1
ter	terminal
TG-Ab	thyroglobulin antibodies
TPO-Ab	thyroperoxidase antibodies
TRA	T cell receptor alpha
TRC	T cell receptor beta
TRD	T cell receptor delta
TRG	T cell receptor gamma
TSH	thyroid stimulating hormone
TSHR-Ab	thyroid-stimulating hormone receptor antibodies
TTPA	alpha-tocopherol transfer protein
UNG	uracil-DNA glycosylase
UT	untranslated region
V(D)J	variable (diversity) joining
VCA	anti-EBV virus capsid antigen
VZV	varicella-zoster virus
WAS	wiskott aldrich syndrome
XL EDA-ID;	x-linked ectodermal dysplasia anhidrotic immunodeficiency, caused by *NEMO* gene mutations
XLP	x-linked lymphoproliferative syndrome
AADC	aromatic L -amino acid decarboxylase
17OH	17a-hydroxylase
21OH	21-hydroxylase

Errata

1. 14p11 corrected to 14q11 (Fig.7)
2. the spelling of following words were corrected: opportunistic, tortuosity, seborrhoeic
3. HLADR changed to HLA-DR (p.31)
4. AutoDELPFIATM changed to AutoDELFIA™ (p. 61)
5. Brutons agammaglobulinemia (pp.10,11,66,71) and Bruton agammaglobulinemia (p.84) changed to Bruton's agammaglobulinemia.
6. trombo- changed to thrombo- (pp.71,74,82)
7. Due to black/white print,  with a red spot was changed to in the Figure 20 legend.
   Ex.58
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II

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Coping, quality of life, and hope in adults with primary antibody deficiencies

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Abstract

Background: Living with a chronic disease, such as primary antibody deficiency, will often have consequences for quality of life. Previous quality-of-life studies in primary antibody deficiency patients have been limited to different treatment methods. We wanted to study how adults with primary antibody deficiencies manage their conditions and to identify factors that are conducive to coping, good quality of life and hope.

Methods: Questionnaires were sent to all patients ≥20 years of age with primary antibody deficiencies who were served by Rikshospitalet University Hospital. The questionnaires consisted of several standardized scales: Ferrans and Powers Quality of Life Index (QLI), Short Form-36 (SF-36), Jalowiec Coping Scale (JCS), Nowotny Hope Scale (NHS), and one scale we devised with questions about resources and pressures in the past. Of a total of 91, 55 patients (aged 23–76 years) answered the questionnaires. The questionnaire study were supplemented with selected interviews of ten extreme cases, five with low and five with high quality of life scores.

Results: Among the 55 patients, low quality of life scores were related to unemployment, infections in more than four organs, more than two additional diseases, or more than two specific occurrences of stress in the last 2–3 months. Persons with selective IgA deficiency had significantly higher QLI scores than those with other antibody deficiencies. An optimistic coping style was most frequent used, and hope values were moderately high. Based on the interviews, the patients could be divided into three groups: 1) low QLI scores, low hope values, and reduced coping, 2) low QLI scores, moderate hope values, and good coping, and 3) high QLI scores, moderate to strong hope values, and good coping. Coping was related to the patients' sense of closeness and competence.

Conclusion: Low quality of life scores in adults with primary antibody deficiencies were linked to unemployment and disease-related strains. Closeness and competence were preconditions for coping, quality of life and hope. The results are valuable in planning care for this patient group.

1. Background

Primary immunodeficiency diseases represent a heterogeneous group of rare disorders characterized by an increased susceptibility to infections and autoimmune diseases. Primary antibody deficiencies (PAD) constitute the largest subgroup and include: Common Variable Immunodeficiency, X-linked (Brutons) Agammaglobulinemia, Selective IgA deficiency, IgG subclass deficiency, and Hyper IgM syndrome [1]. Some patients need lifelong replacement therapy with immunoglobulins and/or frequent courses of antibiotics as treatment and/or prophylaxis. Patients with PAD have increased incidence of autoimmune diseases and experience long-term complications of infections and/or treatment [2]. Living with a chronic disease, such as PAD, will often have consequences for quality of life. Previous quality-of-life studies in PAD patients have been limited to different treatment methods. After initiation of subcutaneous replacement therapy, increased health-related function and improved self-rated health have been reported [3]. We wanted to study wider aspects of quality of life among adults with PAD: How do they manage their condition? Which factors are conducive to coping, good quality of life, and hope?

Coping, quality of life, and hope are important aspects when the effects of a disease from infancy to old age are examined. There are various partially overlapping perspectives on, and definitions of coping, quality of life, and hope [4]. Coping reflects a process and includes active involvement over a period of time [5,6]. Hope and quality of life describe outcomes rather than processes. Hope and quality of life are concepts which have several dimensions. Coping also includes different strategies, but the total sum of the strategies does not constitute a global definition of the concept. Choice of strategies can influence outcome variables such as hope or quality of life positively or negatively. Coping is of importance for quality of life, and hope can be regarded as a coping strategy [7]. Hope can be seen as a variable that positively contributes to the experience of quality of life.

Coping is defined by Lazarus and Folkman [[5]; p.141] as "Constantly changing cognitive and behavioural efforts to manage, reduce or tolerate external and/or internal demands that are appraised as taxing or exceeding the resources of the person". The coping process depends on the situational context in which it occurs [5]. According to Lazarus and Folkman's theory [5,6], resources and pressures are linked to coping. We used resources and pressures as concepts in the present study. Resources can be divided in two groups; personal and socio-ecological resources. Pressures, such as disease-related experiences, may lead to stress and to reduced coping ability.

Locus of control is seen as a crucial factor in coping [8]. An internal locus of control is present when a person explains events by referring to causes within themselves. The person perceives that the event is contingent upon his/her own behavior or his/her own relatively permanent characteristics. An external locus of control is present when a person explains events by referring to causes in the situation or environment. Events and circumstances are typically perceived as the result of luck, chance, fate, under the control of powerful others, or unpredictable because of the great complexity of the forces surrounding the person given an external locus of control.

Resilience predisposes for successful coping [9,10]. Longitudinal studies in high risk children have showed positive correlation between resilience and overcoming difficult social circumstances as adolescents. Sommerschild [11] developed a theoretical model which sums up of the key points of resilience theory (see Figure 1). Resilience refers to the person's latent resources which can be mobilized in defense of the self in stressful situations. Resilience is based on self confidence. Closeness and competence contribute to self confidence. Figure 1 shows the concept closeness explained at three levels; the dyadic relationship with one competent adult, family, and the social network. Competence encompasses the person's skills and experience of usefulness.

In the present study, quality of life was defined as a person's overall satisfaction with life: "A person's sense of well-being that stems from satisfaction or dissatisfaction with areas of life that are important to him/her" [[12]; p. 15]. The global concept of quality of life is represented by four domains: A health and functioning domain, a socio-economic domain, a psychological/spiritual domain, and a family domain. Health-related quality of life is defined as a person's satisfaction or happiness within areas of life that are affected by health or health care. Health-related quality of life includes eight domains: physical functioning, role physical, bodily pain, general health perceptions, vitality, social functioning, role emotional, and mental health.

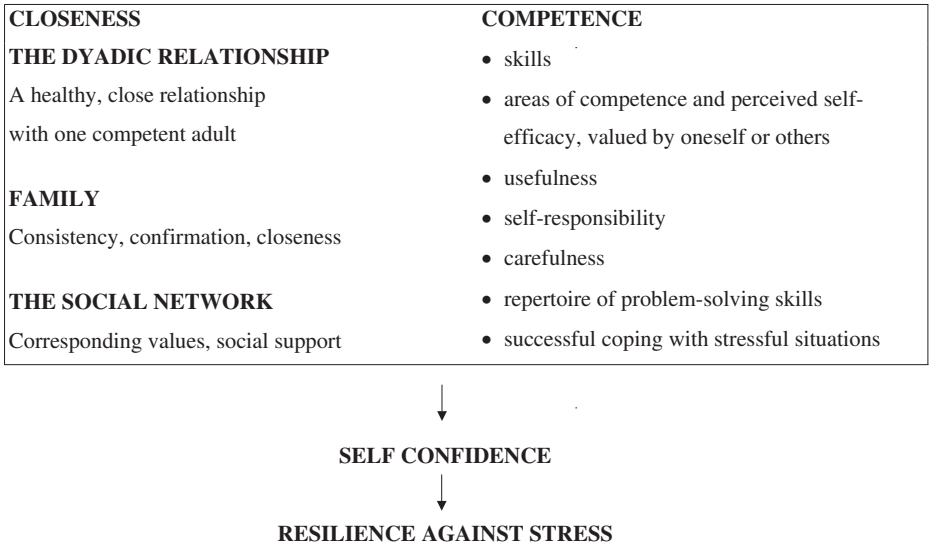

Figure 1
Closeness and competence: preconditions for resilience against stress. (Sommerschild, 1998 [11]).

Hope is future-oriented and described as a feeling, an emotion, an experience, a need and a dynamic attribute [13,14]. "A six-dimensional, dynamic attribute of the person which orients to the future includes: active involvement by the individual, comes from within, is possible, relates to or involves others or a higher being, and relates to meaningful outcomes to the individual" [[15]; p.89].

Two questions have been central in the present study:

1. How do adults with PAD manage their condition?

2. What kinds of factors influence their coping, quality of life and hope?

2. Methods

The survey was the main investigation in this study, and was analyzed quantitatively. The interviews were included as a supplement to the survey, and did not represent a traditional qualitative study.

The survey

Sample

As of 2000, 122 patients with PAD were registered in Norway [1]. All PAD patients ≥ 20 years and served by Rikshospitalet, in total 91 persons, received the questionnaires, after excluding one cognitively impaired person. The cohort included 50 men and 41 women, aged 20–82 years, with various PAD diagnoses: Common variable immunodeficiency (n = 66), X-linked Agammaglobulinemia (n = 8), Selective IgA deficiency (n = 16) and Hyper IgM syndrome (n = 1).

55 of the 91 adults we approached completed the questionnaires (60% response rate), 31 men and 24 women. The mean age was 41.6 (median 38, range 20–76) years.

The sample included 29 young adults, aged 20–39 years and 26 older adults, aged 40–76 years. Distribution of the specific PAD diagnoses was: Common Variable Immunodeficiency (n = 43), X-linked Agammaglobulinemi (n = 3), Selective IgA deficiency (n = 8) and Hyper IgM syndrome (n = 1). Nine of the responders reported previous infection with Hepatitis C virus (HCV). Specific PAD diagnoses, gender, and mean age, 47.2 (44, 20–82) years, were similar and not significant different, when responders and non-responders were compared. Responders (n = 55) were reasonably representative of the original cohort (n = 91).

Measures

The survey included questions about the individual's past and present situations, and about his/her thought concerning plans for the future. Demographic variables including age, gender, education, employment and marital status were requested. Information was elicited concerning the following disease-related variables: specific diagnosis, duration of clinical immunodeficiency state, frequency of infections, in which organs the infections (acute and chronic) occurred, other medical complications (including Hepatitis C virus infection), additional diseases (including auto-immune diseases), treatment with subcutaneous (ScIg) versus intravenous immunoglobulin (IVIg), other treatments (antibiotics) and stressful events in the previous 2–3 months. Disease-related strains were defined as the most problematic strains linked to the PAD diagnosis, medical complications or additional diseases.

Five different scales were incorporated into one comprehensive 30-page questionnaire. Four of the standardized scales had previously been translated and tested in Norwegian populations [7,16,17]. The standardized scales were: Ferrans and Powers Quality of Life Index (QLI) [18], Short Form-36 (SF-36) [19], Jalowiec Coping Scale (JCS) [20], and Nowotny Hope Scale (NHS) [13]. An additional scale designed for this project (RPP Scale), focused on resources and pressures in the past. Factor analyses were used to assess the empirical support for each subscale in all instruments. Internal consistency was estimated using Cronbach's alpha coefficient.

Ferrans and Powers Quality of Life Index (QLI) has been designed to measure quality of life in both ill and healthy individuals, and this was based on Ferrans' definition of quality of life [12,18]. The global construct quality of life is represented by four underlying subscale domains/subscales:

- Health/Functioning
- Socio-economic
- Psychological/Spiritual
- Family

The QLI consists of two sections. One section measures satisfaction within various domains. The other section measures the importance of each domain for the subject. The items are scored according to a 6-point Likert scale ranging from "very satisfied" to "very dissatisfied" for the satisfaction items, and from "very important" to very unimportant" for the importance items. The overall score is the product of the satisfaction responses and the importance responses. The possible range for the overall and subscale scores is 0–30, the higher the score the better quality of life.

The validity and reliability of QLI has previously been evaluated. Content validity of the original version was assessed on the basis of a review of the literature [12,18]. Concurrent validity of the QLI was provided by a correlation (r = 0.80) between the QLI and a measure of satisfaction with life [12]. Construct validity was found to be satisfactory in different patient populations, and was confirmed by factor analysis ("the maximum-likelihood method" and "the direct oblimin method of rotation") [12,21]. A four-factor solution had the best fit with the data. Internal consistency reliability was 0.95 for the global score, ranging from 0.66 to 0.93 for the subscales. The test-retest reliability varied from 0.87 at two weeks to 0.81 at one month [18].

QLI has been translated and tested in a Norwegian population of newly diagnosed cancer patients [7]. 131 cancer patients participated in the test, 103 in the retest. The Cronbach's alpha coefficient for the QLI was 0.93 for test and 0.95 for retest. The coefficients for the subscales in both tests ranged from 0.79 to 0.91 [7]. Test-retest-reliability was r = .78 within three-four weeks (Pearsons correlation coefficient). The correlation coefficients ranged from r = .65 to r = .83 for the different subscales. Construct validity was analysed by "the maximum-likelihood method" and "direct oblimin method" of rotation (factor analysis). Eight factors had an "Eigenvalue greater than 1". Four factors explained only 45.4% of the variance in this cancer patient cohort, in contrast to 91% in the study of Ferrans and Power [21].

Reliability analyses in the present study with 55 PAD patients showed a Cronbach's alpha of 0.81 for the total QLI, and ranged from 0.54 to 0.92 for the four subscales. The Family subscale had the lowest alpha value, and the Health/Functioning subscale had the highest. Factor analyses based on the four subscales were done by "the maximum-likelihood method", non-rotated method and "direct oblimin method", rotated. Non-rotated method

with "Eigenvalue greater than 1", resulted in one cluster where only one of the factors appear, (2.571). All the subscales could be related to this factor. The factor explained 64.3% of the variance in our sample. This result supported a total scale with a common component.

The Short Form-36 (SF-36), one of several generic questionnaires developed to assess health-related quality of life [19], consists of 36 items which measure eight conceptual domains:

- Self-reported General Health (GH)
- Physical Functioning (PF)
- Bodily Pain (BP)
- Mental Health (MH)
- Role limitations (Physical) (RP)
- Role limitations (Emotional) (RE)
- Vitality (VT)
- Social Functioning (SF)

In addition, one item assesses change in health in the past year (HT). The scores in each domain are transformed into 0–100 scales. The higher score the better health-related quality of life.

The reliability of the eight scales has been estimated using both internal consistency and test-retest methods [22]. Reliability coefficients for each of the eight scales were equal or greater than .80 (ranging from .81 in General Health to .93 in Physical Functioning) with the exception of Social Functioning, which had a reliability of .68. The content validity of the SF-36 has been compared to that of other widely used generic health surveys. Systematic comparisons reveal that the SF-36 includes eight of the most frequently represented health concepts.

SF-36 has previously been translated and tested in 2323 persons from the general Norwegian population [17,23]. Reliability analyses (Cronbach's alpha) showed values from 0.80 to 0.93 for the eight subscales, Role limitations (Emotional) had the lowest and Bodily Pain the highest value. Correlations between the SF-36-scales ranged from r = .29 (Mental Health and Physical Functioning) to r = .68 (Mental Health and Vitality).

Reliability analysis (Cronbach's alpha) of the SF-36 in the present study (n = 55) yielded values from 0.74 to 0.92, Role limitations (Emotional) had the lowest and Social Functioning the highest value. Factor analysis is not usually performed when using SF-36. In spite of a relatively small sample size, factor analysis was done in this study by "Principal Component Analysis", non-rotated method and "Varimax with Kaiser Normalization" rotated method of the eight subscales. The analyses revealed two main factors. The scales which contributed the sum score of Physical Health, were one factor. The scales which contributed the sum score of Mental Health, constituted the other factor. Compared with the original SF-36, there was one finding of note in the present study: the Social Functioning subscale was correlated with the sum score of Physical Health. The Social Functioning subscale in the SF-36 is originally included in the sum score of Mental Health.

The Jaloviec coping scale (JCS) is based on Lazarus and Folkman's theory of stress and coping [5,6,20]. The JCS has been designed to measure how people cope with various types of physical, emotional and social stressors. The JCS measures the use and effectiveness of 60 cognitive and behavioural coping strategies in a stressful situation. The items describe cognitive and behavioural efforts in response to stress. In our questionnaire, stress was specified as stress induced by living with PAD. The strategies are grouped into eight coping dimensions:

- Confrontive – "tried to change the situation"
- Evasive – " put off facing up to the problem"
- Optimistic – "tried to think positively"
- Fatalistic – "accepted the situation because very little could be done"
- Emotive – "worried about the problem"
- Palliative – "tried to keep busy and work harder"
- Supportive – "depended on others to help out"
- Self-reliant – "preferred to work things out yourself"

Item responses are rated on a 4-point scale from 0 (never used) to 3 (often used), and a scale of helpfulness from 0 (not helpful) to 3 (very helpful). The higher score, the more coping effort involved. The higher total coping score the more alternation between different coping strategies.

The JCS has previously been tested in several studies [20,24]. Its content validity has been assessed by an expert panel and is supported by a broad theoretical and empirical base. Construct validity has been evaluated. The 60 items are classified into eight subscales, with an agreement ranging from 94% on the Supportive subscale to

54% on the Emotive subscale. Reliability of the JCS, assessed with Cronbach's alpha coefficients and based on results from 24 different studies ranged from 0.48 to 0.81 for the use subscales and from 0.48 to 0.82 for the effectiveness subscales.

JCS has previously been translated and tested in a Norwegian population of 273 patients with psoriasis [16]. Correlations between the eight subscales in JCS ranged from r = .39 ($p < .001$) to r = .73 ($p < .001$). Reliability analyses (Cronbach's alpha) of the eight subscales ranged from 0.55 to 0.88. The construct validity of the JCS was analysed by "Principal Component Analysis" with orthogonal rotation (factor analysis). The analyses resulted in three coping dimensions with sufficient internal consistency: confrontive problem-solving coping, normalizing / optimistic coping and combined emotive engagement. 37 % of the total variation in the Norwegian version of JCS was attributed to these three factors [16].

Because few patients responded to the coping effectiveness part of the JCS in the present study, we have only included the coping strategy use part. Reliability analyses (Cronbach's alpha) ranged from 0.41 to 0.75 for the eight subscales (use-scores). The Confrontive, the Evasive and the Optimistic subscales yielded the highest values from 0.73 to 0.75, and the Fatalistic subscale the lowest alpha at 0.41. Our finding of three subscales with the strongest internal consistency is in keeping with the results of Jaloviec et. al. [20]. Factor analyses based on subscales, done by "Principal Component Analysis", non-rotated method and "Varimax with Kaiser Normalization", rotated method, resulted in a three-factor-solution with factor 1: Evasive, Fatalistic and Self-reliant coping; factor 2: Confrontive, Emotive, Palliative coping; and factor 3: Optimistic coping. These analyses revealed that the Optimistic scale could be considered a separate contributing factor in the present study.

Nowotny Hope Scale (NHS) is designed to measure hope in a general adult population after a stressful event [13,15], and has been employed primarily in cancer patients. NHS is a 29-item scale with items scored on a 4-point Likert Scale ranging from 4, strongly agree, to 1, strongly disagree. It consists of six subscales:

- Confidence
- Relates to others
- Future is possible
- Spiritual beliefs
- Active involvement
- Comes from within

The total score range is from 29 to 116, with a high score indicating high hope. Cut-off scores are developed for four levels of hope.

The content validity of the NHS has been evaluated by an expert panel [13]. The concurrent validity was established with the Beck Hopelessness Scale (r = -.47). The construct validity of NHS was analysed by "Principal Components Analysis" with orthogonal rotation (factor analysis). The result supported the six dimensions and subscales of hope. Cronbach alpha's reliability coefficient for this instrument was 0.90. The concurrent validity has been found to be satisfactory [13].

NHS has been translated and tested in the above-mentioned Norwegian population of newly diagnosed cancer patients [25]. Correlations between different subscales ranged from r = -.16 to r = .73. Factor analysis done by "Principal Components Analysis", showed that the items of the "Spiritual beliefs" subscale appeared as one factor, and the "Comes from within" items as another factor analogous to Nowotny's subscale items. With the exception of these two factors, the results of the NHS factor analysis of Rustøen [25] diverged from Nowotny's original six dimensions. At three-four week test-retest of NHS correlation was high, Pearson's r = .81. Correlation coefficients ranged from r = .59 to r = .92 for the various subscales. Cronbach's alpha for NHS was 0.89 both in the test and the retest. The alpha coefficients for the subscales ranged between 0.53 and 0.95.

Reliability analysis of NHS in the present study (n = 55) showed a total Cronbach's alpha of 0.87. Cronbach's alpha of the six subscales ranged from 0.54 to 0.94. Our results were similar to Rustøen and Moum's results [25]. Factor analyses based on subscales were done by "Prinicipal Component Analysis", non-rotated method, and "Varimax with Kaiser Normalization", rotated method. Both the non-rotated and the rotated variant of factor analysis seemed to confirm only two explicit contributing factors: factor 1; the Spiritual subscale, and factor 2; the five other subscales.

The Resources and Pressures in the Past Scale (RPP Scale) was theoretically founded on Lazarus and Folkman's theory of coping [5,6] and consisted of 64 items divided into two main categories: Resources and Pressures. The past was defined as the period from adolescence to present time. The distinction between different domains within the person's resources was based on previous studies of coping [9,10,26-28]. The concept of Resources included both personal characteristics / temperament and social support resources. Pressures were defined as the person's individ-

ual perception of his/her experiences, including immunodeficiency-related experiences and general events.

Resources consisted of four subscales:

• Personal characteristics / temperament

• Family and supporting adults

• Supporting persons in school and social network

• Public Health Service

Pressures in our scale consisted of four subscales:

• Immunodeficiency-related events (for example: many hospitalizations because of the disease)

• General events

• Immunodeficiency-related experiences in school (for example: significant absence from school because of the disease)

• General experiences in school

The items were scored on a 5-point Likert Scale ranging from 5, strongly agree, to 1, strongly disagree. The total score range of Resources was from 29 to 145, and the total score range of Pressures was from 35 to 175. A high score indicated either a good availability of resources or a high level of strain.

In the present study, missing was handled by the same procedure as in the standardized scales JCS and NHS: when more than 50% of the subscale is answered, the missing value is replaced by the mean score of the rest of the subscale.

This scale was evaluated by reliability analyses (Cronbach's alpha) and factor analyses. The aim behind using reliability analyses was to evaluate in what degree the individual items correlated with the main concepts in the scale. Some items were excluded to attain highest possible consistency. The Cronbach's alpha was 0.89 for Resources and 0.90 for Pressures, with a range from 0.63 (Personal characteristics/temperament) to 0.90 (Family and supporting adults) in Resources, and a range from 0.59 (Immunodeficiency-related events) to 0.89 (Immunodeficiency-related experiences in school) in Pressures. Validity was tested by "Principal Component Analysis", non-rotated method and "Varimax with Kaiser Normalization" with rotation (factor analysis) based on the subscales. With "Eigenvalue greater than 1", the non-rotated method made only one contributing factor appear for the Pressures scale to which 55% of the total variance could be attributed. Likewise, the factor analysis (non-rotated method) yielded only one contributing factor from the Resources' scale, to which 46% of the total variance could be attributed.

Statistical analyses of the survey data

The SPSS-PC statistical program (v 9.0) was used for data analyses. Descriptive analyses were performed to assess the characteristics of the sample. The impact of demographic and clinical variables on the dependent variables was assessed by t-test for independent samples (two-tailed). The effect sizes were measured by the difference between the means of the samples divided by the mean of the standard deviations of the samples [29]. The effect size was defined by qualitative standardized values (small = .25SD, medium = .50SD, large = 1.00SD). Both correlation analyses and multiple regression analyses were performed to assess the relationship between variables.

The interviews

The interviews were included as a supplement to the survey to elucidate preconditions for coping, good quality of life, and hopefulness. The interview study was designed to probe and to aid in the interpretation of some of the results from the questionnaire. Cases were selected for interviews to detect possible patterns within two groups: patients with high QLI scores and patients with low QLI scores. The selected cases represented a strategic sample of patients with the lowest and highest QLI scores (n = 21). Originally, we wanted to interview all these extreme cases (n = 21). Ten patients consented to participate in the interview study. Ten cases were regarded as sufficient to detect patterns within the different groups. The qualitative interviews were based on significant results related to QLI in the survey. The interviews were semi-structured with a prewritten interview guide, and lasted nearly two hours.

The interview guide was based on the most central issues in the survey: previous resources and pressures, the interviewees' experience of coping and quality of life, and their hope for the future. Questions about coping included present challenges and choice of coping strategies. Questions about experience of quality of life were related to the four dimensions in QLI: Health/Functioning, Socio-economic, Psychological/Spiritual and Family. Questions concerning hope, dealt with the patients' general experience of hope. All concepts were related to a lifespan perspective as the interviewees were asked to evaluate their present situation related to their past. All interviews were done by the same person and tape-recorded.

In accordance with Kvale's methodology [30], the interviews were analyzed on a thematic and a theoretical level. Kvale distinguishes between three different contexts of

interpretation of the interview statements. The thematic level implies a condensed form of what the interviewees themselves understand to be the content of their statements. The interpretation is more or less based on the interviewees' self-understanding as understood by the researcher. The common sense level represents a critical common sense understanding. The interpretations may include a wider frame of understanding than those of the interviewees themselves. The interviewer should be critical of what is said, and may focus on the content of the statement. The theoretical level is a framework for interpreting the meaning of a statement. These interpretations are likely to go beyond the interviewees' self-understanding and to exceed common sense understanding. In this study, the theoretical level included the common sense understanding.

The interviews were analyzed to identify interesting and important themes. New themes appearing during the interviews were included in the analyses (thematic level). Similarities and differences were described within and between the extreme groups. The expressed meanings were summarized into shorter terms. Individual texts were further analyzed with respect to meaning of the texts, and to their respective categories. The categories were divided into groups defined by contrasting elements within and across the groups denoting high and low quality of life [31]. When thematic analyses showed differences within or between the extreme groups, further analyses were done. Since some of these variants seemed to be in accordance with previous studies in coping research, the results of the first thematic analyses were reanalyzed according to relevant theory about the constructs of coping, quality of life, and hope (theoretical level).

According to Kvale [30] reliability pertains to the consistency of the research findings, and validity to the truth and correctness of a statement. Kvale emphasizes that issues of verification do not belong to a separate stage of an investigation, but should be addressed throughout the entire process. Validation is done at seven stages in the interview process: 1. *Thematizing* based on the logic of derivations from theory to the research questions of the study, 2. *Designing* dependent on the adequacy of the design and the methods used for the purpose of the study, 3. *Interviewing* based on trustedness of the interviewees reports and the quality of the interviewing itself, 4. *Transcribing* dependent on the quality of the translation from oral to written language, 5. *Analyzing* dependent on whether the questions in the interview text are valid and whether the interpretations are logical, 6. *Validitating*, based on reflective consideration of what forms are relevant to a specific study and 7. *Reporting* dependent on to what degree a given report is a valid account of the main findings of a study.

3. Ethical aspects

The study was approved by the Norwegian Regional Committee for Medical Research Ethics and the Norwegian Social Science Data Services. Participants were guaranteed anonymity and the right to withdraw from the study at any time. An information letter to respondents provided information about the potentially sensitive items.

4. Results

The survey

t-tests were done on selected demographic and disease-related variables and the results are presented in table 1(see Additional file 1). Other results and comparisons are only presented in the text. Table 1 includes results of total scores (means and standard deviations) of all scales in the present study with one exception: the most significant differences in SFscores were found in four out of the eight domains. Only these are presented in the table (BP, MH, RP and SF).

On the RPP, the 55 adults with PAD reported good availability of *resources* in the past (personally and support from others) (mean 3.7, range 2.03–4.93, out of a possible total score of 5). Parents and other supporting adults had been of major importance as social support (mean 3.8, range 1.69–5.00 out of a possible score of 5). Adults with PAD had experienced moderate *pressures* (2.6, 1.27–4.66, out of a possible total score of 5). Pressures related to their immunodeficiency were the most burdensome in the school context (3.09, 1.00–5.00). Four conditions in present time implicated significantly more pressures in the past: younger age (20–39 years) (p = .024), (Effect Size (ES) = .77SD), living alone (p = .015), (ES = .84SD), having more than two additional diseases (p = .005), (ES = 1.07SD), or suffering from infections in more than four organs (p = .038), (ES = .69SD) (t-tests, two-tailed) (Table 1).

The mean score in global QLI (*quality of life*) was moderate at 20.0 (range 12.3–27.6, out of a possible total score of 30.0). In addition to immunodeficiency, the following conditions were associated with significantly lower QLI scores: unemployment (p = .008), (ES = .80SD), infections in more than four organs (p = .020), (ES = .79SD), the presence of more than two other diseases (p = .001), (ES = 1.55SD), or more than two specific occurrences of stress in the last 2–3 months (p = .007), (ES = 1.15SD) (t-tests, two-tailed). These results are presented in table 1. Unemployed men had lower QLI scores compared to employed men (p= .020). The term "unemployed" was defined to include currently/previously unemployed, never employed and recipient of disability pension. Men working full-time achieved significantly higher QLI scores than men working part-time or unemployed men (p = .016). These differences did not exist among the women.

Variables without impact on QLI were: length of education, type of treatment (subcutaneous Ig versus intravenous Ig), frequency of treatments, self-administration of treatment at home (ScIg), hospital based treatment (IVIg), and HCV infection.

Compared to a Norwegian sample of newly diagnosed cancer patients (n = 131) [7], the PAD patients (n = 55) had a significantly lower total QLI score ($p < .05$), along two dimensions: Health and Functioning ($p < .05$) and Socio-economic ($p < .01$).

Health-related quality of life in different conceptual domains on the SF-36, revealed that the adults with PAD had their lowest mean score in General Health (37.8, range 5.0–87.0, of a possible total score of 100.0) and their highest mean score in Physical Functioning (81.1, 10.0–100.0, of a possible total score of 100.0) (SF-36). The most significant differences in SF scores were found in four of the eight domains, and these are presented in table 1. Gender, employment and disease-related pressures/strains had significant influence. Men had a significantly higher score than women in Bodily Pain, Social Functioning and Vitality, respectively ($p = .029$), (ES = .64SD); ($p = .016$), (ES = .68SD); and ($p = .004$), (ES = .85SD) (t-tests, two-tailed). Unemployed men and women, had significantly lower health-related quality of life, compared to employed adults. Low health-related quality of life was found in Bodily Pain ($p = .006$), (ES = .81SD); General Health ($p = .002$), (ES = .88SD); Mental Health ($p = .021$), (ES = .68SD); Physical Functioning ($p = .001$), (ES = 1.01SD); Role limitations (Physical) ($p = .000$), (ES = 1.16SD); and Social Functioning ($p = .004$), (ES = .91SD). The disease-related strains were infections in more than four organs, infections more than eight times yearly, more than two other diseases and/or more than two specific occurrences of stress in the last 2–3 months. Hepatitis C infection did not have a negative influence on health-related quality of life.

Compared to a control group with a normal distribution (n = 2323) [17], our PAD patients (n = 55) showed significantly lower functional ability scores in all areas of health-related quality of life (SF-36). The finding reached statistical significance (.001) in four areas: General Health, Role limitations (Physical), Social Functioning and Vitality. Compared to a sample of psoriasis patients (n = 283) [4], these PAD patients showed different scores in two areas (SF-36): Bodily Pain, where adults with PAD scored higher ($p < .01$), and General Health, where adults with PAD scored lower ($p < .001$).

Of the eight coping strategies measured by the JCS, an optimistic coping strategy was most frequently used (mean item rating 2.28, range 0.44–3.00, on a 4-point scale of 0–3). A palliative coping strategy was rarely used (1.18, 0.20–2.29). The total score for all coping strategies used showed a mean item rating of 1.64 (1.13–2.32). This reflects the extent of use of all coping strategies measured [20]. Being unemployed was associated with high coping scores among adults with PAD ($p = .013$), (ES = .78SD) (t-tests, two-tailed). Full-time employment was associated with lower coping scores compared to part-time employment, housework or unemployment ($p = .021$), (ES = .67SD) (Table 1).

The PAD patients had moderate hope values on the NHS with a mean score of 84.9 (range 52–102, of a possible total score of 116). Having more than two additional diseases in addition to PAD was associated with a lower hope value among responders ($p = .015$), (ES = 1.02SD) (t-tests, two-tailed). The results are presented in table 1. There was positive correlation between being hopeful about the future and quality of life (QLI) in the present, $r = .454$ ($p < .001$) (Pearson correlation). Regression analysis with quality of life (QLI) as the dependent variable and hope (NHS) as one of the independent variables, showed $R^2 = .206$ ($p < .001$), which suggests that hope explains 20.6% of the total variance in the quality of life.

Compared to a sample of newly diagnosed cancer patients (n = 131) [7], our PAD patients (n = 55) had a significantly lower total hope value ($p < .05$) visualized in two dimensions of NHS: Relates to others ($p < .01$), and Future is possible ($p < .05$).

The interviews

During thematic analysis of the ten interviews, certain categories appeared to be of particular importance. These categories were used as the main categories in the theoretical analysis: quality of life, closeness and competence as resilience, locus of control, and hope. The five responders with high QLI scores showed more homogeneous results than the five responders with low QLI scores for the interview themes resources and pressures in past, coping ability, quality of life, and hope for future. The latter group was split into two subgroups based on criteria related to experience of closeness and competence, and locus of control. Locus of control was determined by evaluating the responders' answers about their own experience of internal control over external occurrences. In spite of a low QLI score, three of the responders seemed to have strong resilience combined with an internal locus of control.

Based on the theoretical analysis, the subjects with a low QLI score were divided into two groups (Group 1 and Group 2). The subjects with a high QLI score were defined as Group 3. Persons in Group 1 (n = 2) had low scores in all four subscales of QLI (Health/Functioning, Socio-economic, Psychological/Spiritual, Family). They had prob-

lematic psychological bonds to their mothers, and less experience of closeness or/and competence (Fig 1). They experienced difficulties in coping (self-reported), they had low hope values and either an internal or an external locus of control. In addition, persons in Group 1 had needed various forms of social support. However, they expressed reluctance to receive such support. Persons in Group 2 (n = 3) had low scores in two subscales of QLI: Health/Functioning and Socio-economic. They had especially close relationship to their mothers, but a positive experience of closeness and competence. They were coping successfully (self-reported), had a moderate hope values and an internal locus of control. The persons in Group 2 also needed additional social support, but received such help according to their own wishes. Persons in Group 3 (n = 5) had high scores in all four subscales of QLI. They had experienced closeness and competence, and they were coping successfully. They had moderate to strong hope values, an internal locus of control, and reported no need for additional support.

5. Discussion

The purpose of the present study was to study how adults with PAD manage their condition and to identify factors that are conducive to coping, good quality of life, and hopefulness. Low scores in quality of life were linked to unemployment and disease-related strains among adults with PAD. Closeness and competence were preconditions for coping, good quality of life and hope.

The survey showed that parents and other supporting adults were the most important caregivers (*Resources*) in adolescence. This is in accordance with findings in previous coping studies [32]. Not surprisingly, the interviews confirmed the family as the best caregivers during childhood. In cases where the parents did not fulfill their function as caregivers, other people such as neighbors and health personnel functioned as caregivers. In addition, social support was not only associated with positive experiences among the responders with low QLI score. Those with a low QLI score reported a complicated relationship to their mothers. They wanted to be accepted as adults, but did not experience that they were.

Experiences related to immunodeficiency (*Pressures*) were of major importance, for example: episodes of illness, absence from school, psychosocial consequences of the disease, self-respect and respect from other people. These results came from the survey. The interviews confirmed that occurrences related to the immunodeficiency were the most chronic problems. This is in accordance with Ogden's conclusions [33]. Ogden classified painful school experiences as a long-term element of risk. Many studies have emphasized the importance of the impact of previous pressures on later development [32,34-36]. In accordance with their findings, the results of the present study point to previous resources and pressures as crucial factors for future coping ability and maturation.

A high degree of immunodeficiency-related strain as well as unemployment had a negative impact on Health and Functioning on the QLI in this sample (n = 55) (*Quality of life*). In order to achieve a high total QLI score, a low score in one dimension has to be compensated by higher scores in the other dimensions. To be satisfied with one's own achievement as an experience of coping is seen as crucial to achieving a high quality of life [37]. Consequently, unemployment requires that one is able to compensate for lack of employment with another meaningful activity. This may be interest in interpreting the finding in this study that unemployed men reported lower quality of life than men with a steady job.

Persons with Selective IgA-deficiency achieved a higher global QLI score than other PAD patients. Patients with symptomatic selective IgA deficiency are usually healthier than other PAD patients [2]. Surprisingly, the QLI differences were not found in the health/function domain, but in the socioeconomic, family and psychological/spiritual domains.

We observed a difference in QLI between those who treated themselves (ScIg) compared to those who were treated by others (IVIg), but the difference was not statistically significant. However, the PAD patients who treated themselves (ScIg) had a significantly higher score in Social Functioning (SF-36) compared to the others. SF-36 measured *health-related quality of life*. Our study focused on global quality of life, not specific in relation to treatment, and the results did not elucidate all aspects of these different treatment methods. Gardulf [3] found a significantly increased health-related function, and improved self-rated health among patients with PAD after initiation of ScIg infusions. However, our study was not designed to detect differences before and after introduction of a specific treatment method.

Nine of the 55 responders had experienced Hepatitis C virus infection due to contaminated IVIg [38]. Surprisingly, HCV infection had no influence on the QLI scores or scores of SF-36 in this study.

The interviewees with a low QLI score were in poor health and reported some limitations in daily life functioning. Still, these patients showed obvious differences within the group related to other quality of life conditions, as some of them (Group 2) seemed to fully adjust their experience of quality of life. Well-being and satisfactory social support were reported. This was not anticipated because of low QLI scores from the questionnaire. Wilson and

Cleary's research studies [39] suggest that there is no direct correlation between serious limitation in health and loss of quality of life. On the other hand, positive self-esteem, ability to be active and to use one's abilities are elements of crucial significance [37,40,41] in achieving a high quality of life score. In our interview study, there was a lack of such characteristics in Group 1 who had low scores in all domains of the QLI. Insufficient involvement, dependence on others, low self-esteem, and lack of happiness and well-being were characteristic interview responses in this group.

There were some differences between the findings in the survey and the interviews: The global QLI scores and SF-scores did not give a good description of social network. Interviews indicated that a good network was important for resilience. The interviews may have described more comprehensively the responders' experience of different types of support. Mutual relationships were identified as important by interview, but had not been included among the specific items in the survey. Psychological characteristics, external living conditions and relationships have been evaluated by others [37] as essential factors for quality of life. The responders with a low QLI score in Group 2 (the interview study) confirmed that a good social network had contributed to increasing their quality of life.

Compared to a Norwegian sample of newly diagnosed cancer patients [7], our 55 PAD patients had a lower global QLI score, along dimensions: Health and functioning and socio-economic. By nature of their disease, the PAD patients have had a life-long course, in contrast to the cancer patients. Perhaps the chronicity of PAD explains some of the divergence.

Compared to the cancer cohort [17], our PAD patients scored lower on functional ability in all areas of health-related quality of life (SF-36). Other quality-of-life studies confirm significant negative implications of physical health limitations [42,43]. Compared to a sample of psoriasis patients [16], our PAD patients scored differently in two areas (SF-36): Bodily Pain, where adults with PAD had a higher score, and General Health, where adults with PAD had a lower score. Bodily pain is not characteristic for PAD patients and can be a possible explanation for the high score in SF-36. Adults with psoriasis have better general health, compared with adults with PAD.

An optimistic *coping* strategy was most frequently used in dealing with the illness (survey). This is a consistent finding in other studies of groups with other chronic diseases [16,44-46]. Employment is linked to competence and may predispose for successful coping (Fig 1) [11]. According to Sommerschild [11], coping depends on competence in various areas, e.g. perceived self-efficacy, usefulness, and problem-solving skills. Unemployment requires that one is able to compensate in other spheres in order to achieve a feeling of competence [32]. Competence and closeness are areas which may be amenable to psychosocial interventions aimed at increasing quality of life in adults with PAD. Prospective interventions can be designed for patients with low quality of life scores or may include all PAD patients.

The PAD patients had moderate *hope* scores on the NHS. Having more than two other diagnoses in addition to PAD was associated with a lower hope score. Less hopefulness correlated positively with a high degree of disease-related strain. We found a definite positive correlation between being hopeful about the future and quality of life (QLI) in the present. Hope seemed to be responsible for 1/5 of the total variance in the quality of life. Increasing hope may have an impact on enhancing quality of life.

Compared to the cohort of newly diagnosed cancer patients [7], our PAD patients had a significantly less global hope score in two dimensions of NHS: "Relates to others", and "Future is possible". Studies confirm that persons with a cancer diagnosis, thought to be in a hopeless situation, often have a positive and hopeful vision of the future [47]. The PAD patients include people with a congenital chronic disease for which there is no curative treatment. All of the newly diagnosed cancer patients had been diagnosed within the previous year. They were aware that cancer is a terminal illness, but they may have retained hope for curative treatment.

Another study, which focused on sources of hope among people with chronic diseases, emphasized hope as a main coping strategy [48]. Evangelista [49] found that hope strongly correlated with quality of life in a cohort of female heart transplant recipients. The Group 2 and Group 3 patients in our interviews had plans and dreams, and were optimistic about the future, and their plans were related to other people. But in Group 1, thoughts about the future were characterized by fatalism, less faith in the future and lack of involvement with others.

The low response rate (60%) may be considered a limitation of the external validity of the survey. Including subjects with other immunodeficiency disorders would have increased sample size at the cost of homogeneity. Homogeneity was chosen to enhance validity. Simple statistical analyses were employed. We were able to use the t-test after controlling for statistical assumptions (independent samples, normal distribution, and homogeneity of variance). The findings reported as significant reached, with one exception, levels of significance of .01 and .001. Effect size varied between moderately large to a large [29]. In

spite of some concerns regarding statistical power, we consider that our results have relatively good *statistical validity* [50].

Five scales were used; four of them standardized and well tested by others, and in Norwegian populations. One scale was specially designed for this study. Seen in a lifespan perspective, related to previous pressures/strains and availability of resources, we showed that the results of this scale also were important for the evaluation of the results from the other scales. In spite of some variation between the subscales, the five scales showed good reliability generally (Cronbach's alpha) in keeping with the main constructs of the study. The four standardized scales were internally consistent and test-retest reliability was satisfactory. Despite a thorough testing of the RPP Scale, the construct validity of this scale may be more uncertain. Nevertheless, in spite of some limitations, the total construct validity appears robust.

If the group is too special, it may not be correct to generalize the results to other kinds of persons or other diseases. However, this sample was characterized by a wide age span (23–76 years), different subgroups of PAD, various types of treatment, age at diagnosis and number of disease-related strains. Analysis of the non-responders showed that the responders in this survey (n = 55) were representative for the entire cohort (n = 91), and they constitute 75% of all patients registered with PAD in Norway [1]. Therefore, this survey was has relatively good *external validity* relative to this patient group.

The results of the interviews were evaluated in according to Kvale's methodology [30] for qualitative research interviewing, and the reliability and validity were found to be reasonably good. An evaluation during the analysis process of the interviews concluded that the size of the sample was sufficient to detect different patterns in these two groups of extreme cases. And similar patterns have been found in previous studies on coping [9,10,51]. Such a confirmation is expected to strengthen the results, and to some extent compensate for the limited sample size. However, the low number necessitates a tentative and explorative use of the study findings.

Since the sample of the ten interview subjects was in accordance with the strategic sample concerning the most central variables impacting on quality of life, *the generalizability* of the interview study could be evaluated as relatively good.

The interviews were included to elucidate nuances in the knowledge from the survey results. Triangulation of different methods is considered an appropriate strategy for strengthening the validity of research findings [52,53]. The interview study supplemented the survey, supported the constructs in the survey and, as a result, strengthened the validity of the findings. In this project, the results from the survey were thoroughly examined during the interviews. However, the interview study also raised some new hypotheses, which were not registered in the survey. The interviews have added new aspects, and functioned as a supplement to the survey, increasing the relevance of the project and its results. Combining the findings from both the survey and the interviews strengthens the validity of the project's end result.

There may be ethical concerns when inviting patients to complete questionnaires about coping, quality of life and hope. In this study, the information letter gave an opportunity to prepare for potentially sensitive questionnaire items. Moreover, the interviews made ethical demands on the interviewer. In an interview, the interviewer is a part of the method, and has a responsibility to manage separating roles, having focus on the interview and taking care of the interviewees in a professional way. In the present study, to get the interviewees' confidence, the interviewer had to define the content of the interview precisely, and explain the interviewers' role. The interviewer referred the interviewees to other health personnel when needed.

6. Conclusion

Low scores in quality of life were linked to unemployment and disease-related strain among adults with PAD. Coping was closely linked to the patients' sense of closeness and competence. The results are in accordance with previous studies of other groups with chronic diseases. Closeness and competence are areas where psychosocial interventions may contribute to better quality of life in adults with PAD. The findings are relevant also for other groups of patients. Medical interventions should reduce the patient's strain, and support his or her ability to be employed.

Additional material

Additional File 1

Table 1. Mean scores (SD) in PAD patients on resources, strains, quality of life, functioning, coping, and hope
Click here for file
[http://www.biomedcentral.com/content/supplementary/1477-7525-3-31-S1.doc]

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Table 1. Mean scores (SD) in PAD patients on resources, strains, quality of life, functioning, coping, and hope

Scales	n	RPP		QLI	SF-36				JCS	NHS
		Resources	*Pressures*		*Bodily Pain*	*Mental Health*	*Role limitation (Physical)*	*Social Functioning*		
All responders	55	3.7 (0.6)	2.6 (0.6)	20.0 (3.5)	66.0 (27.7)	73.0 (18.6)	56.5 (42.7)	70.8 (28.8)	1.6 (0.3)	84.9 (9.9)
Age groups										
>39 years	26	3.6 (0.7)	2.4 (0.6)	20.6 (3.3)	65.3 (31.4)	75.3 (18.0)	62.0 (43.4)	76.0 (30.4)	1.6 (0.3)	86.3 (9.6)
≤39 years	29	3.7 (0.6)	2.8* (0.6)	19.4 (3.7)	66.5 (24.4)	71.0 (19.2)	51.7 (42.2)	66.1 (27.0)	1.7 (0.3)	83.3 (10.2)
Gender										
Men	31	3.6 (0.7)	2.6 (0.7)	20.5 (3.6)	73.6 (21.8)	75.7 (19.5)	62.5 (40.3)	79.2 (23.8)	1.6 (0.3)	85.1 (10.5)
Women	24	3.7 (0.6)	2.6 (0.5)	19.3 (3.4)	56.4* (31.6)	69.5 (17.0)	49.0 (45.1)	60.4* (31.6)	1.7 (0.3)	84.5 (9.2)
Diagnosis										
Selective IgA deficiency	8	3.8 (0.6)	2.5 (0.3)	22.5 (2.4)	62.3 (29.7)	80.5 (18.7)	71.9 (26.8)	68.8 (35.4)	1.8 (0.3)	89.7 (7.4)
Other immunodeficiencies	45	3.7 (0.6)	2.6 (0.6)	19.6* (3.6)	67.1 (26.5)	71.0 (18.4)	54.0 (42.8)	71.9 (26.8)	1.6 (0.3)	84.3 (10.3)
Number of infected organs										
0-4 organs	46	3.8 (0.5)	3.0 (0.8)	20.4 (3.4)	69.5 (26.2)	74.5 (17.4)	61.1 (41.8)	75.8 (24.3)	1.6 (0.3)	85.9 (9.3)
> 4 organs	9	3.2 (0.9)	2.5* (0.5)	17.7*(3.4)	48.3*(30.1)	65.8 (23.5)	33.3 (41.5)	45.8* (37.5)	1.7 (0.2)	80.0 (11.7)
Number of infections										
>8 yearly	18	3.7 (0.5)	2.8 (0.6)	19.2 (3.6)	64.2 (27.0)	71.2 (18.9)	38.9 (39.5)	61.8 (30.1)	1.7 (0.3)	87.2 (8.5)
0-8 yearly	35	3.6 (0.7)	2.5 (0.6)	20.7 (3.3)	68.2 (28.4)	75.4 (17.8)	67.1* (41.0)	77.1 (26.7)	1.6 (0.3)	83.8 (10.4)
Number of other diseases										
0-2	47	3.8 (0.6)	2.5 (0.6)	20.6 (3.4)	70.6 (26.1)	76.4 (17.8)	61.4 (42.1	77.4 (24.2)	1.6 (0.3)	86.3 (9.2)
>2	8	3.1**(0.6)	3.2*(0.6)	16.4***(2.0)	39.3**(28.9)	53.5***(8.3)	28.1*(36.4)	32.8***(24.0)	1.7 (0.3)	76.5*(10.0)
HCV infection										
Yes	9	3.5 (0.8)	2.8 (0.6)	21.1 (3.9)	71.8 (20.7)	79.1 (22.3)	55.6 (44.7)	75.0 (28.6)	1.7 (0.3)	85.2 (11.8)
No	44	3.7 (0.6)	2.6 (0.6)	19.9 (3.5)	66.3 (28.4)	72.8 (17.6)	56.4 (43.0)	71.5 (27.7)	1.7 (0.3)	85.5 (9.2)
Treatment										
By themselves (ScIg)	36	3.6 (0.7)	2.6 (0.6)	20.1 (3.4)	70.5 (25.9)	73.7 (18.0)	60.7 (42.6)	77.4 (23.9)	1.6 (0.3)	84.5 (10.5)
By others (IVIg)	11	3.7 (0.4)	2.8 (0.7)	18.6 (3.6)	57.6 (27.1)	67.3 (17.9)	36.4 (40.9)	57.5* (28.4)	1.7 (0.3)	84.0 (7.2)
Cohabitation										
Living alone	11	3.3 (0.7)	2.9 (0.5)	19.6 (4.0)	75.2 (31.6)	70.5 (19.5)	59.1 (43.7)	73.8 (36.5)	1.6 (0.3)	79.3 (14.7)
Living with someone	43	3.8* (0.6)	2.52* (0.6)	20.1 (3.5)	63.4 (26.9)	74.4 (17.9)	57.1 (42.5)	70.6 (27.4)	1.7 (0.3)	86.2 (7.9)
Employment										
Full time/Part time/Work at home	37	3.7 (0.7)	2.6 (0.6)	20.8 (3.3)	72.8 (25.1)	76.9 (17.3)	69.1 (40.0)	78.7 (25.0)	1.6 (0.3)	84.4 (9.9)
Unemployed	17	3.6 (0.6)	2.7 (0.7)	18.1**(3.4)	51.1**(28.0)	64.5*(19.0)	26.6**(33.5)	53.7*(29.9)	1.8**(0.3)	85.9 (10.1)
Stressful events the last 2-3 months										
0-2	36	3.7 (0.7)	2.6 (0.6)	20.7 (3.3)	68.8 (28.5)	74.3 (18.8)	57.1 (41.8)	74.6 (29.2)	1.6 (0.3)	85.2 (9.1)
> 2	8	3.6 (0.6)	2.9 (0.8)	16.7**(3.7)	59.5 (25.7)	56.5*(16.5)	43.8 (50.0)	50.0*(26.7)	1.8 (0.4)	85.9 (10.5)

Levels of significance: *p=. 05, **p=. 01, ***p=. 001 (t-tests, two-tailed)

Abbreviations:
RPP Scale - Resources and pressures in the past. 1-5 scales. The higher score the higher level of pressures/strains or a better availability of resources.
QLI, Quality of Life Index: 0-30 scales. The higher score the better quality of life;
SF-36, Short Form-36 (Health-related quality of life): 0-100 scales. The higher score the higher functioning.
JCS, Jalowiec Coping Scale: 0-3 scales. The higher total score the more different coping strategies alternated.
NHS, Nowotny Hope Scale: 1-4 scales. The total score range as 29-116, with a high score indicating high hope.

III

IV

V

VI

VII

Choreoathetosis, developmental delay and severe pulmonary infections due to interstitial microdeletion 14q and homozygosity for MBL2 variant alleles

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Summary:

We report a twelve years old boy with developmental delay, ataxia, choreoathetosis, muscular hypotonia, thyroid dysfunction and severe pulmonary problems characterised by recurrent episodes of respiratory distress and pneumonia. He had a heterozygous interstitial microdeletion of proximal chromosome 14q resulting in haploinsufficiency of multiple genes including *TITF1 (NKX2.1)* and *PAX9*. The karyotype was 46,XY, del(14)(q13.1q21.1). He was also homozygous for the mannose-binding lectin (MBL) haplotype B, a variant in codon 54 in the *MBL2* gene on chromosome 10q causing absence of functional serum MBL. The 14q deletion was confined to the index patient, while the MBL deficiency and corresponding *MBL2* genotypes were also found in his mother and his younger sibling.

List of abbreviations:

TITF1	Thyroid transcription factor 1
PAX9	Paired box gene 9
GERD	gastro-oesophageal reflux
SP-A	Surfactant protein A
SP-D	Surfactant protein D
MBL	Mannose-binding lectin

Introduction:

Deletions of the proximal chromosome 14q region have been reported in patients with various neurological and dysmorfic features [1]: midline defects of the central nervous system, feeding problems, growth retardation, muscular hypotonia, developmental delay, mild to severe mental retardation and craniofacial findings. Respiratory distress and thyroid dysfunction are also common findings [1-4] as well as gastroesophageal reflux. Within the 14q13-q21 region some important genes with documented clinical implications are located, such as the *TITF1 (NKX2.1)* gene, which encodes for thyroid transcription factor 1. This gene is important for the development and function of thyroid, forebrain, basal ganglia and lung [5-9]. Hypothyroidism, neurological abnormalities and respiratory distress are described in humans with heterozygous deletions or mutations in the *TITF1* gene [8] [10-13]. TITF1 induces promotor activity of surfactant protein A [14;15], surfactant protein C [16;17] and surfactant protein B genes [18;19]. Surfactant proteins are important not only for enhanced lung compliance and low alveolar surface tension, but play an important part of the innate immunity of the lungs (SP-A and SP-D)[20]. Paired box gene 9 *(PAX9)* locus is located close to *TITF1.* PAX9 is important for craniofacial skeletogenesis and oral cavity patterning. Haploinsufficiency of PAX9 in humans is associated with autosomal dominant hypodontia [21]. Here we report a patient with interstitial 14q microdeletion and severe, recurrent pulmonary infections probably related to a concomitant MBL deficiency. Even in reports of larger 14q deletions, clinical and laboratory signs of immunodeficiency have not been described in the patients.

CASE REPORT

Clinical Course

Second child of healthy, unrelated parents. He was born at term with cleft lip-palate and pes adductus. In addition, short nose, long philtrum, flat broad nasal root and micrognathia were observed (Figure 1). He had normal birth weight 3790g, length 52 cm and large head circumference 39cm (97.5 th centile). He was first hospitalised 5 weeks old with *RSV* infection necessitating respiratory support for one week. Due to persisting feeding difficulties with acute respiratory problems despite operative correction of his cleft lip-palate, he received a gastrostomy at 16 months of age and a fundoplicatio ad modum Nissen eight months later. He eats some meals per os, but has mainly been fed through the percutaneous endoscopic gastrostomy. At present, twelve years old he still has recurrent pulmonary infections, on average one per month. The last five years he has been followed by the Cystic Fibrosis medical team, intensively treated with active chest physiotherapy including use of cough assist machine, inhalations four times a day with N-Acetylcysteine/Salbutamol, periodically Fluticason aerosol, and BIPAP (Bilevel Positive Airway Pressure) breath assist machine every night. The two last years he has during the autumn/winter seasons been treated with Tobramycin inhalations; four week on/four weeks off, resulting in less intercurrent infections. Sputum/expectorate samples are collected every 3rd-4th week for routine microbiological investigation. Bacteriae including *Staphylococcus aureus, Haemophilus influenzae, group B streptococcus, Streptococcus pneumoniae, Pseudomonas aerginosa and Stentrophomonas maltophilia* have regularly been found in his expectorates. He also has had episodes of diarrhoea requiring hospitalisation. He has since early childhood had bilateral chronic otitis, frequent skin infections around the gastrostomy, repeated chalazions of the eyelids and suffered chickenpox twice. After the MMR vaccination he developed a skin abscess.

His neuropsychological development has been delayed. At two years of age he started to walk with an ataxia that has developed into more choreoatetoid movements during the years. He has signs of muscular hypotonia with joint laxity, tendon reflexes are normal. He has macroglossia, involuntary tongue movements and dysartria. The last years he has been followed by the dentist because of crowded teeth and persisting primary teeth.

By neuropsychological testing at the age of 6 years 9 months he functioned intellectually 2½ years behind his age both according to Wechsler Preschool and Primary Scale of Intelligence - Revised (WPPSI-R) and information from his teacher and his parents. Scores of Developmental Test of Visual-Motor Integration (VMI) and Grooved Pegboard tests corresponded to 4 years when he was 6¾. Nine years old he started to have absence seizures. MR imaging of his brain was normal. EEG showed focal spike-wave complexes in the left parieto-temporo-occipital regions and also in the right temporal region. Benign epilepsy of childhood with Rolandic spikes was diagnosed and since then he has been treated with 450 mg daily doses of natrium valproate retard tablets. The treatment has reduced the frequency of the absence seizures. By neuropsychological testing at the age of 10 years of age he functioned 3-4 years behind his age according to Wechsler Intelligence Scale for Children (WISC-III), Raven's progressive matrices and information from his teacher. At 10 years of age he had difficulties performing the Visual-Motor Integration (VMI) and Grooved Pegboard tests involving fine movements, and these scores were lower than scores from the pure intelligence tests. He cooperated well during the tests, but had signs of attention deficit and hyperactivity; ADHD. The locomotor behavior included both voluntary "restlessness" and involuntary movements such as tics. He is a social and humoristic person, without signs of autism.

He had elevated TSH levels for many years (10 mU/L, reference 0,4-3,5) while T4 levels (13-17pmol/L) were within normal range and thyroid autoantibodies had never been detected. Thyroxin substitution was started when he was seven years old, but no clinical including neurological improvement was achieved. Other hormones such as FSH, LH, prolactin, IGF-1, growth hormone and cortisol were normal. He had normal serum levels of alpha fetoprotein. And urinary excretion of purines and pyrimidines analysed by high-performance liquid chromatography was normal. Ultrasound showed a normal thyroid and thymus was present. When he was seven years old his height was 5 cm below the 2.5^{th} centile, weight was at 10^{th} centile and normal head circumference at 50^{th} centile (Figure 2). Twelve years old his height is at 2.5^{th} centile, weight is at 25^{th} centile.

Relatives

His father and his older brother were healthy, while his mother and his younger brother had a history of frequent respiratory infections, but fewer and less severe than seen in the index patient. The mother had frequent lower respiratory tract infections, and the younger brother had episodes of wheezing. They were included in the genetic and immunolgical study to further elucidate the specific 14q deletion phenotypes.

Methods

Immunoglobulins, specific antibodies and lymphocytes

Methods and reference values for quantitation of total IgG, IgA, IgM, IgD, IgE and IgG subclasses are fairly described in [22] as are detection of diphtheria antibodies and tetanus antitoxin[23-25]. Antibodies to *Streptococcus pneumoniae* were tested against the 23-valent polysaccharide vaccine in an ELISA [26;27] as were antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b [28]. Enzyme immunoassay (EIA) techniques were used for anti-varicella-zoster virus (VZV) IgG, anti-herpes simplex virus (HSV) IgG, anti-measles IgG, anti-Epstein-Barr virus (EBV) nuclear antigen (EBNA) and anti-EBV virus capsid antigen (VCA) IgG, and the microparticle enzyme immunoassay (MEIA) technique for anti-rubella virus IgG and anti-cytomegalovirus (CMV) IgG. Flowcytometric immunophenotyping of peripheral blood leukocytes was performed according to [22].

Vaccine responses

Diphtheria and tetanus vaccines (0,5 mL containing 15Lf Diphtheria toxin and 3.8 Lf Tetanus toxin) and the conjugated pneumococcal vaccine, Prevenar® (Wyeth-Lederle), were used. Antibodies were measured prior to and > 6 weeks after the vaccination (Table 1).

Mannose-binding lectin (MBL)

Serum concentrations of MBL were quantified using ELISA (Antibodyshop/Statens Serum Institut, Copenhagen, Denmark) with detection limit at 5 nanogram/mL. Low MBL levels were defined as <400 ng/mL and extremely low if <100 ng/mL. To further characterise the role of MBL, a functional ELISA measuring the lectin pathway activation capacity of exogenously added purified complement C4b-binding capacity was performed as described by

Petersen et al [29;30]. Detection of gene mutations and promoter polymorphisms in the MBL gene was performed by polymerase chain reaction according to [31]. The following polymorphisms were studied: three point mutations in the promoter region (position -550 (H/L variants), -221 (X/Y variants), -70 (nt C or T)), one point mutation in the 5' untranslated (UT) region at position +4 (P/Q variants) and three point mutations located at codons 52, 54 and 57 in exon 1, at nucleotide positions 223, 230 and 239.

Surfactant protein D (SP-D)

SP-D was measured by ELISA as described in [32]. Briefly, microtitre wells were coated with $F(ab')_2$ prepared from rabbit anti-SP-D antibody, incubated with dilutions of test serum, later incubated with monoclonal antibody anti-human SP-D, and then peroxidase was added. Serum samples from the family were tested simultaneously in 1:5 and 1:10 dilutions and the mean value calculated. Reference values for SP-D are 370-1300ng/mL according to [33]. Three biallelic polymorphisms at the SP-D codons for the amino acids in position 11, 160, 270 in the mature SP-D were genotyped using an in house PCR-sequence specific priming method.

Genetic investigations

FISH

Cosmids covering *TITF1* and one covering *PAX9* [3]were biotinylated by nick translation. Labelling and fluorescent in situ hybridisation (FISH) were performed as described [34].

Array-CGH

Microarray-based comparative genomic hybridisation (array-CGH) was performed on a Human Genome CGH Microarray 44K (Agilent Technologies, USA) with a resolution of

approximately 0.36 Kb. Array-CGH analysis was done according to the supplier´s instructions (for details, see www.agilent.com). Briefly, after digestion with Alu I and Rsa I, patient DNA and normal reference DNA (1 µg each) were labeled with Cy3-dCTP and Cy5-dCTP, respectively. Commercial reference DNA was used consisting of a pool from either ten normal female donors or ten normal male donors (Promega GmbH, Germany). The DNA´s were together with 50 µg human Cot-1 DNA (Invitrogen) hybridized onto the array. The arrays were scanned in an Agilent G2565B scanner (Agilent Technologies), and images were analysed, using the "Agilent Feature extraction software (v9.5)" (Agilent Technologies). Array-CGH results were visualised in the "Agilent CGH Analytics Software 3.4" (Agilent Technologies). Imbalances were identified and located using the ADM-2 algoritm in the software with a moving average on 1 Mb, while mapping of the breakpoints of the imbalances were manually determined from the individual oligo ratio intensities. The genomic location of the oligos is based on the Agilent hg build 18 which is based on the NCBI build 36.2.

Results:

Genetics

The cytogenetic investigations had revealed a microdeletion 14q. Fluorescence in situ hybridisation (FISH) analysis with a cosmid containing the *NKX2.1* gene yielded a signal on only one chromosome 14 homologue and confirmed the presence of a deletion encompassing the *TITF1 (NKX2.1)* locus (Figure 3). In addition, FISH for the *PAX9* gene confirmed the presence of a deletion of this gene as well (Figure 3). Array-CGH analysis with 44.000 oligos (Agilent Technologies) located the deletion breakpoints to 14q13.1 and 14q21.1. The breakpoints were mapped to be located at (33,74 Mb – 33,94 Mb) and (40,83 Mb – 41,12 Mb) distal from 14qter, giving a minimum size of the deletion of 6,9 Mb and a maximum size of

7,4 Mb. The deletion spands a region of 36 predicted Ensembl genes: *C14orf147, EAPP, SNX6, CFL2, BAZ1A, SRP54, C14orf24, PPP2R3C, KIAA0391, PSMA6, NFKBIA, INSM2, GARNL1, BRMS1L, MBIP, XR_017793.1, Q8IWV4, TITF1, YN001_HUMAN, NKX2-8, Q96HD_HUMAN, PAX9, SLC25A21, MPOL1, C14orf25, FOXA1, TTC6, SSTR1, CLEC14A, SEC23A, SIP1, TRAPPC6B, PNN, MA2, CTAGE5, FBXO33,* plus 3 pseudogenes and 9 novel genes without known function. Within the deleted area only 5 genes are yet known to be associated with human disease: *NFKBIA, CFL2, MPOLI, PAX9* and *TITF1*. Within the deleted region is also another interesting gene *FOXA1*, a transcription activator for liver genes such as AFP and albumin. Based on the array-CGH result a more exact karyotype were defined: 46,XY, del(14)(q13.1q21.1) *de novo*. His parents and his siblings had normal karyotypes and no deletions were found.

Immunology

Our patient had low IgG2 (0.38 g/L) and IgE (<3 kU/L), while total IgG, IgA, IgM and IgD were normal. Total numbers of lymphocyte subpopulations and proliferative response to mitogens were within reference values, with T cells within the lower normal range. He had antibodies to measles, mumps, HSV, chickenpox and Hib, but not to diphtheria, tetanus, CMV and EBV and low levels of pneumococcal polysaccharide antibodies (Table 1). We performed a Diphtheria/Tetanus vaccination, and six weeks later he showed normal antibody levels (Table 1). He also responded to the conjugated pneumococcal vaccine with anti-pneumococcal antibodies. His siblings and his parents had normal immunoglobulin levels and protective antibodies to the bacterial antigens tested (Table 1).

Collectins:

Collectin results are presented in Table 2. The patient and also his mother and his younger brother had extremely low serum MBL levels (<30ng/mL). Complement C4b-binding capacity was measured in the patient and the mother, and both had values < 5%. The patient and the youngest brother were homozygous for the MBL mutant variant B, variation in codon 54, associated with absence of MBL [35]. The mother was compound heterozygous with two different MBL variant alleles B and C, which in combination also cause MBL deficiency. The oldest brother and the father were heterozygous for the MBL variant allele B. However, in front of their normal (wild-type) *MBL2* gene a high producing promotor type was found (designated HYP) [31]. None of the family members had reduced serum levels of SP-D. The index patient, the mother and the youngest brother had no mutation in the SP-D gene. The father and the oldest brother were heterozygous for Thr11Met in the SP-D gene, not associated with reduced SP-D levels [36].

Discussion:

The boy has various neurological problems, thyroid dysfunction and severe pulmonary problems. His neurological symptoms correspond very well to those described for *TITF1 (NKX2.1)* haploinsufficiency [11;12]. The microdeletion of 14q also involves other genes located close to *TITF1*. However, Krude et al and Doyle et al found that mutations strictly within the *TITF1* gene also resulted in the complex phenotype with affections of brain, thyroid and lung, suggesting that the phenotype of our patient could entirely be due to *TITF1* haploinsufficiency [11;12]. However, compared to others with *TITF1* haploinsufficiency [2;8;11;12] our patient had a higher frequency of severe respiratory infections. In addition, he had diarrhoea and failure to thrive, which are often seen in patients with MBL deficiency [37]. The low IgG2 levels may also have contributed to his recurrent respiratory infections. A link between IgG2 deficiency, low MBL levels and low levels of spontaneous pneumococcal antibodies may exist [38], homozygosity for certain MBL variant alleles is previous shown to correlate with an increased frequency of invasive pneumococcal disease [39;40].

Other children reported with haploinsufficiency of proximal 14q, as well as the patient reported by Schuffenhauer with de novo deletion 14(q11.2q13)[4], exhibited cleft lip and palate, dysphagia and reduced oesophageal peristalsis [1]. These abnormalities and gastro-oesophageal reflux with aspiration may have contributed to our patient's lung infections. Stockton et al found that haploinsufficiency of the *PAX9* gene only, exclusively was associated with hypodontia and not with dysmorfic features, developmental delay, hypotonia or cleft lip/palate [21]. The deleted 14q chromosomal area contains almost 30 genes and other genes than *TITF1* and *PAX9* may be responsible for our boy's feeding difficulties and respiratory problems. The different pattern of malformations observed in the patients with

deletion of proximal 14q, may be due to different chromosomal breakpoints with varying number of genes involved [41].

Based on the characteristics of the boy's recurrent infections, T cell abnormalities were not expected, and he had normal T cell counts. Progressive T cell deficiencies are described in PNP (purine nucleoside phosphorylase) deficiency, which is inherited autosomal recessive and NP gene located to 14q13.1 close to the deleted area. Our patient had normal urinary excretion of purines and pyrimidines, which excludes PNP deficiency. A heterogeneity of neurologic abnormalities including ataxia and mental retardation similar to our patient have been observed in PNP deficient patients [42;43]. Located to 14q13 within the deleted area is another immunodeficiency gene where heterozygosity may lead to immunodeficiency. Courtois et al. (2003) described a patient with an autosomal dominant form of anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) associated with a heterozygous mutation in the NKBIA(IKBA) gene (OMIM 164008)[44;45]. The specific mutation was shown to be a gain-of-function mutation enhancing the inhibitory capacity of I-kappa-B-alpha by preventing its phosphorylation and degradation, and resulting in impaired NF-kappa-B activation. Deletions more likely results in loss-of-function or "half-the-function".

We found that serum MBL levels corresponded well to the MBL haplotype and clinical phenotype of the relatives as well (Table 2). A strong correlation between MBL haplotype and serum MBL levels has also been described by others [31;46]. Previous MBL genotyping of 100 Norwegian blood-donor controls showed that 3 % of them were homozygous defective for MBL [47]. Both the mother and the youngest brother had low MBL and no *TITF1* deletion. Based on our results from this little family we propose that expression

of serum MBL and SP-D levels are not influenced by *TITF1* haploinsufficiency, even if the collectin genes *MBL2, SP-A (SFTP1, SFTP2)* and *SP-D (SFTP4)* are clustered within the 10q21-q23 region (OMIM*154545, *178630, *178642, *178635). Our suggestions are supported by Y. He and collaborators who reported that, despite finding regions of homology to the thyroid transcription factor 1-binding site, SP-D promoter activity did not require TITF1[48].

The mild elevation of TSH with normal thyroid hormone secretion points to a functional thyroid defect. A functional thyroid defect can be explained by the reduced activation of TITF1 targets, such as the thyroglobulin promoter in the thyroid follicular cells [49]. Other TITF1 targets are TSH receptor and thyroid peroxidase gene [50;51]. Thyroid dysgenesis have been found in other patients with *TITF1*haploinsufficiency [12], but ultrasound of the thyroid gland in our patient showed a present thyroid of normal size and shape.

Conclusions:

In conclusion, we suggest that the MBL deficiency and probably also the IgG2 deficiency explain why he had more severe and recurrent pulmonary problems than previously reported for *TITF1* haploinsufficiency. His mother and younger brother had MBL deficiency and a history of frequent respiratory infections, although fewer and less severe than in the index patient. His 14q deletion involved *PAX9* and probably also other genes that may have caused his cleft lip-palate, clubfeet (as case 777 in [52]) and feeding difficulties with gastrooesophageal reflux. Further studies of patients with single mutations in genes within this region may further elucidate genotype-phenotype correlations of proximal 14q deletions,

although polymorphisms in other genes located outside this chromosomal region may influence the clinical phenotype.

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Table 1 Specific antibodies

Identity	**Age in years**	**Anti-Hib μg/mL**	**Received Hib vaccine***	Anti-diphtheria toxin U/ml	Anti-tetanus toxin IE/mL	Received DT vaccines*	Anti-pneumococcal polysaccaride U/ml	Anti-CMV	Anti-VZV	Anti-HSV	Anti-EBV EBNA	Anti-EBV VCA	Anti-measles	Anti-rubella	Received MMR vaccine
Patient	6.6	1,39	Y	**0,0025 L**	**<0,1**	Y	**1,5 L**	**-**	**+**	**+**	**-**	**-**	**+**	**+**	**Y**
	7.5	NT		⇒ 5,12	⇒ 5,9		⇒ 11,3	NT	NT	NT	NT	NT	NT	NT	
Mother	32	NT	N	10,3	4,2	Y	16,9	-	+	+	+	NT	+	+	N**
Father	34	NT	N	2,56	1,9	Y	9,0	-	+	+	+	NT	-	-	N
Oldest brother	10.5	NT	Y	0,32	0,8	Y	14,2								Y
Youngest brother	2.5	NT	Y	0,64	4,9	Y	3,5								Y
Protective level		>1		>.01	>.1										

* Received vaccines as part of the National Children Vaccination Program
** Only received Rubella vaccine

Abbreviations: NT, Not tested; N/Y, No/Yes; +, Antibodies detected; - , Antibodies not detected; ⇒, After new vaccines (Diphtheria/Tetanus and Conjugated Pneumococcal vaccine)
Abnormal values are in **bold**

Table 2 Collectins: Mannose-binding Lectin and Surfactant Protein D

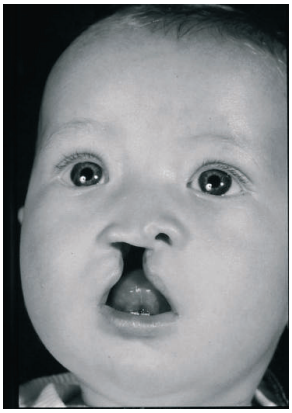
Identity	*Age in years*	Mannose - binding lectin						Surfactant protein D			
		Serum MBL ng/mL	*MBL function (%C4b-binding capasity)*	*MBL deficiency*	*MBL genotype*[1]	*Gene variants with MBL deficiency*	*MBL gene promotor*[2]	*Serum SP-D ng/mL*	*SP-D deficiency*	*SP-D genotype*[3]	*SP-D gene variants*
Patient	**8**	**22**	**<5%**	**Yes**	**B/B**	**Homozygous**	**YLP**	**2132,8**	**No**	**TT**	**No mutation**
Mother	**33**	**14**	**<5%**	**Yes**	**B/C**	**Compound heterozygous**	**YLPQ**	**1251**	**No**	**TT**	**No mutation**
Father	**35**	**860**	**50%**	**No**	**HYPA/B**	**Heterozygous**	**AB YHLP Compensating with a high producing promotor**	**1351,8**	**No**	**CT**	**Heterozygous Thr11Met**
Oldest Brother	**11**	**975**	**36%**	**No**	**HYPA/B**	**Heterozygous**	**AB YHLP Compensating with a high producing promotor**	**1137,1**	**No**	**CT**	**Heterozygous Thr11Met**
Youngest Brother	**3**	**18**	**<5%**	**Yes**	**B/B**	**Homozygous**	**YLP**	**603,8**	**No**	**TT**	**No mutation**

1) MBL genotype
A: normal or wild-type MBL gene variant
B: variation in codon 54
C: variation in codon 57
HYPA high producing MBL haplotype on a normal chromosome

2) MBL gene promotor
H or L : base substitution position –550
X or Y : base substitution position –221
P or Q : base substitution position +4

3) SP-D genotype
T: no changes in 11Met, 160Ala or 270Ser in the SP-D gene. Normal or wild-type SP-D gene variant
C: Thr11Met variation in SP-D gene

Three biallelic polymorphisms at the SP-D codons for the amino acids in position 11, 160, 270 in the mature SP-D were genotyped. Thr11Met genotyping was performed according to {DiAngelo, Lin, et al. 1999 288 /id}. Ala160Thr and Ser270Thr genotyping were performed according to {Lahti, Lofgren, et al. 2002 287 /id}. Especially variation in amino acid 11 in the mature SP-D, Thr11Met, is interesting since methionin mutated to threonin seems to change the protein polymerisation. 15-20% of the Danish population (very similar to the Norwegian population) is homozygous for this variant giving reduced levels of SP-D (personal com. Holmskov). Heterozygous Thr11Met seems to have no clinical relevance {Lahti, Lofgren, et al. 2002 287 /id}.



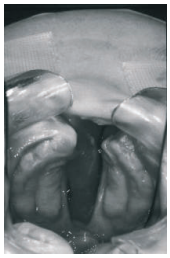
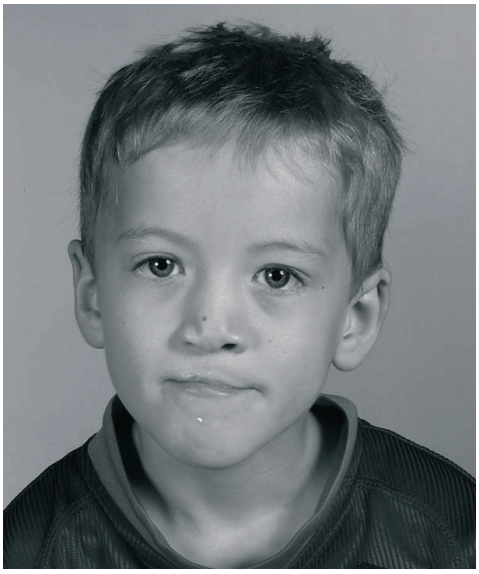


Figure 1



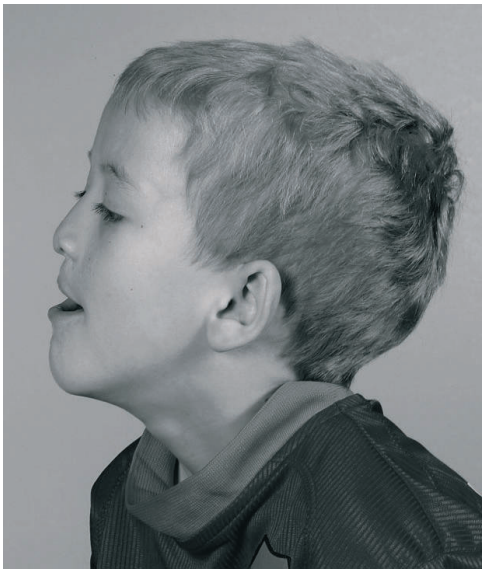


Figure 2

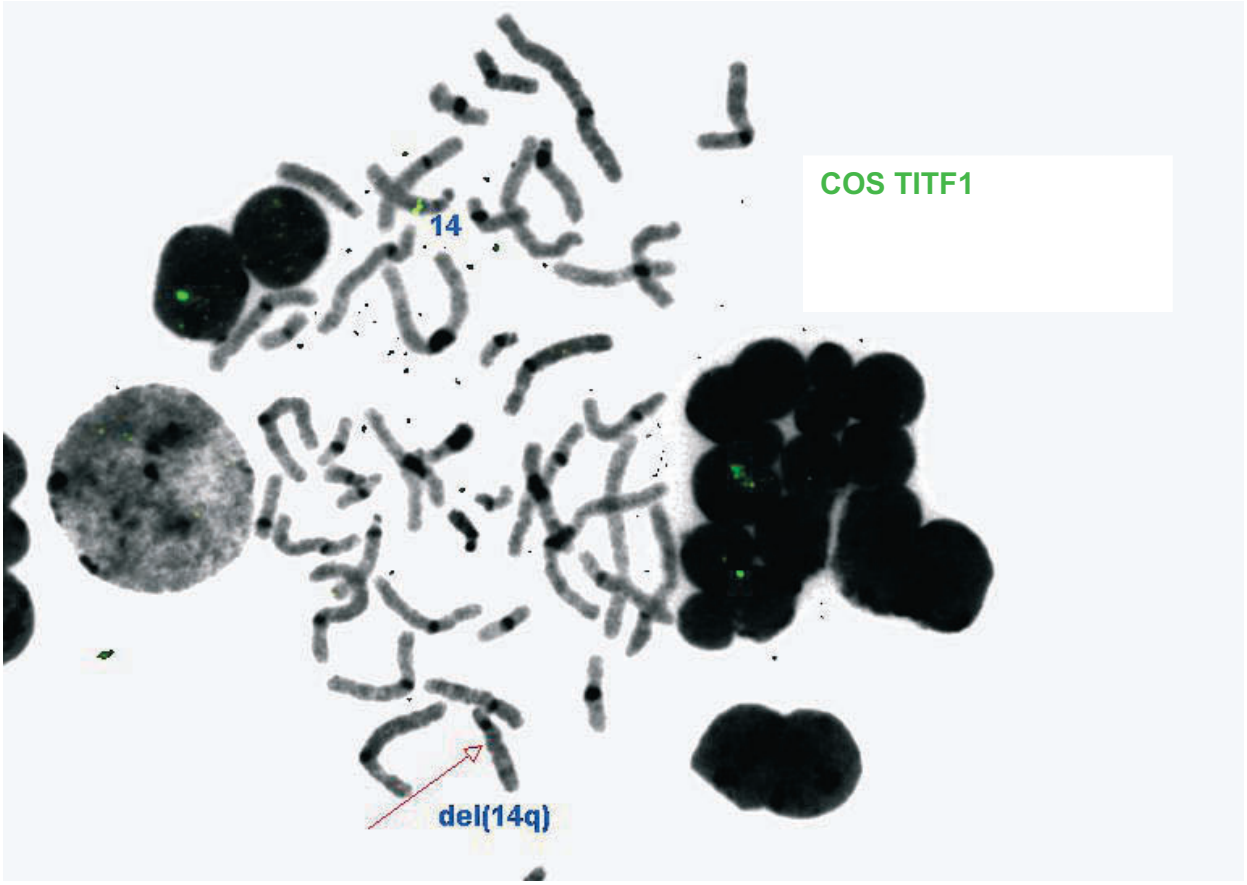


Figure 3

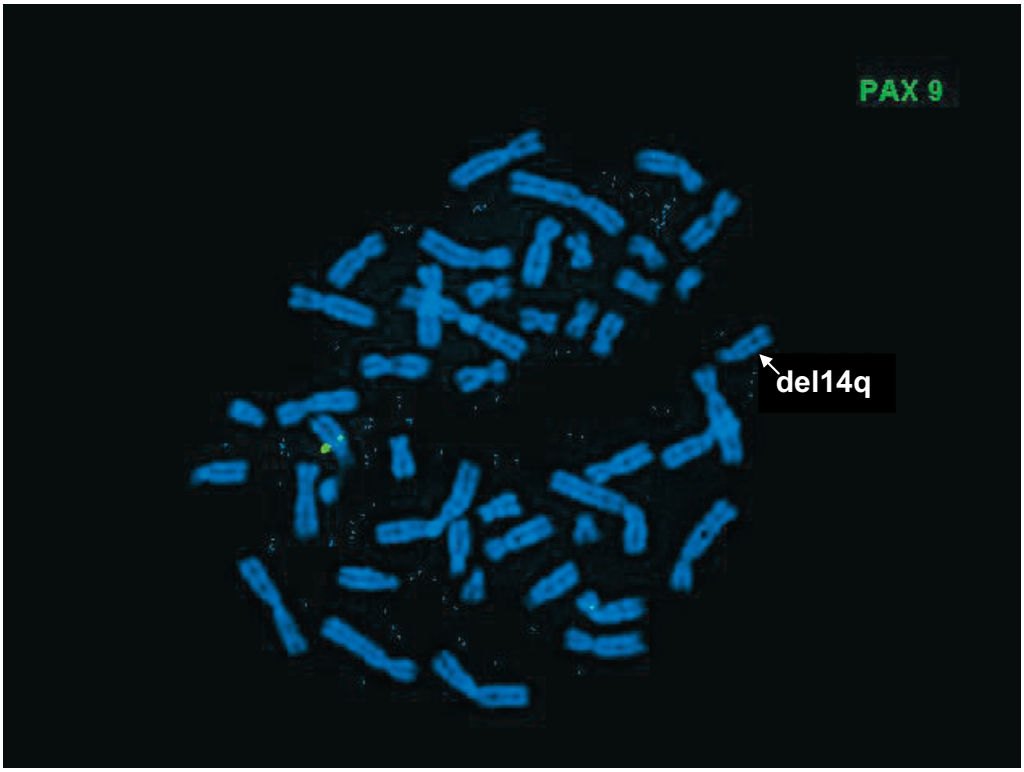


Figure 4

Figure Legends

Figure 1 Photos
Photos of the patient 9 months old, including one photo of the cleft lip-palate

Figure 2 Photos
Photos of the patient 7 years old

Figure 3 FISH *TITF1*
Fluorescence in situ hybridisation (FISH) analysis with a cosmid containing the TITF1 gene yielded a signal on only one chromosome 14 homologue and demonstrates the presence of a deletion encompassing this locus.

Figure 4 FISH *PAX 9*
Fluorescence in situ hybridisation (FISH) analysis for the PAX9 gene yielded a signal on only one chromosome 14 homologue, demonstrating the presence of a deletion of the PAX9 locus