RETT SYNDROME:

CLINICAL AND GENETIC ASPECTS

Dissertation for the degree of Philosophiae Doctor (PhD)

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ABBREVIATIONS

AED(s) = Anti-epileptic drug(s)

CG = a cytosine followed by a guanine, creating the CG dinucleotide

CH = non-CG = CA/CT/CC = A cytosine followed by

CNS = Central nervous system

DNA = Deoxyribonucleic acid

HTS = High Throughput sequencing

MBD = methyl-CpG-binding domain

MLPA = Multiplex Ligation-dependent Probe Amplification

NFRS = Norsk forening for Rett syndrom

NID = NCOR_SMRT Interaction Domain

NGS = Next Generation Sequencing

PCR = Polymerase Chain Reaction

RTT = Rett syndrome

RSSS = Rett Syndrome Severity Scale

TRD = Transcriptional Repression Domain

WES = Whole exome sequencing

1. INTRODUCTION AND BACKGROUND

1.1 RETT SYNDROME

Rett syndrome (RTT) is a disorder that influences all parts of life and affects girls almost exclusively. In its classic form it is characterized by an apparently normal development from birth followed by stagnation in development and then loss of acquired skills. The most affected skills are purposeful hand use and communication (Neul et al., 2010). In addition, social withdrawal and inconsolable screaming spells are often seen in this period. After months or years the situation stabilizes, and while hand function and language seldom are regained the child will usually be more interested in social interaction. Most will learn to walk, but the gait is almost always ataxic and unsteady (Neul et al., 2014). The individual with RTT will be highly dependent with both physical and intellectual disability throughout life, and experience challenging health issues like epilepsy, breathing disturbances, reflux, constipation, scoliosis, and sleep problems to varying degrees (Gold et al., 2018). In addition to the classic form several variant forms are described. The phenotype of these forms can mainly be divided into three groups. The preserved speech variant has a less severe phenotype and preserved or regained language. The early seizure variant is characterized by early onset severe epilepsy, where the first seizure often presents within the first five months of life. The congenital variant has a deviant development from birth. Both the congenital and the early seizure variants are characterized by a severe general phenotype (Neul et al., 2010).

A mutation in the gene *MECP2* on the X-chromosome is found in most individuals with RTT (Amir et al., 1999). However, in the last decade new technology in genomic investigation has increased the number of genes reported to be associated with a RTT or RTT-like disorders to more than a hundred (Ehrhart et al., 2018; Iwama et al., 2019; Vidal et al., 2017). In addition, mutations in *MECP2* are found in individuals with phenotypes far from the RTT phenotype, like mild ID, schizophrenia and autism (Klauck et al., 2002; Shibayama et al., 2004). The current diagnostic criteria for RTT are based on clinical characteristics, indicating that a mutation in *MECP2* is neither necessary nor

diagnostic. Neither does a mutation in another gene exclude RTT (Neul et al., 2010). It is important for both scientific and clinical reasons that diagnostic criteria are accurate. In clinical settings a diagnosis is informative for treatment planning and prognosis, and it can provide support to the affected through diagnosis-specific support groups. In a scientific setting accurate diagnosis is, among other things, important for the validity of the projects, to ensure that the sample studied is representative for the population the results are generalized to. In this context, and with the recent discovered large variation in genotypes in individuals with a RTT phenotype one can ask whether the current "RTT phenotype" with its variations includes more than one disorder, and if the current diagnostic criteria are accurate enough.

The first part of this thesis describes the phenotypic and genotypic variation in a sample of individuals with RTT. All individuals with RTT in Norway were invited to participate. Data collection consisted of interview with parents/other care givers, clinical examination, review of medical journals and genomic examination. Through this we could revisit the clinical diagnoses according to the 2010 criteria, describe both genotypes and important clinical characteristics. In addition, we compared individuals with and without *MECP2* mutations, to see if there were important differences in clinical characteristics between individuals with different genotypes.

Another important change in RTT in the last decades is the increased survival. In quite recent time, the longevity of people with intellectual disabilities was short (Carter et al., 1983). In RTT, like in other disorders with intellectual disability, survival has increased considerably during the last century. The latest survival data for RTT show that more than 70 percent of individuals with RTT will live past their 45th birthday, indicating a growing population of adults with RTT (Tarquinio et al., 2015a). Most research on RTT involves mainly children and adolescents, and the results may not apply to adults. More knowledge on adults, in particular older adults is important for understanding the course of the disease, as well as for clinical work and for planning of future structures in health services.

The last part of this thesis describes health in a sample of adults with RTT and compares the prevalence and burden of medical issues in children, adolescents and adults, and

between adults of different ages. The age range of the sample was wide, from 1 to 66 years, and more than half were 20 years or older, thus providing a good basis for exploring the issues related to health in adults with RTT.

1.2 THE HISTORY OF RTT

The history of RTT started in Vienna in 1965. Then the Austrian neuropaediatrician Andreas Rett observed two of his patients, two girls, sitting on their mothers' lap in the waiting room doing the exact same hand stereotypies. After a thorough examination he found their history and their clinical presentation amazingly alike. Together with his nurse Martha he found six more girls with the same history among his clinical population (Ronen et al., 2009). They assumed that this was the same disorder, and as far as they could see the condition had not yet been described. In 1966 Andreas Rett had examined 22 girls with this disorder, and published the article "Über ein eigenartiges hirnatrophisches Syndrom bei Hyperammonamie im Kindersalter"(Rett, 1966). This paper was only published in German and did not reach an international public.

At the same time the Swedish neuropaediatrician Bengt Hagberg had, unaware of Dr. Rett's publication, followed 16 patients with the same clinical picture. He called the condition "Vesslans disease", and presented his material at a European child neurology congress in Manchester in 1980. At this meeting he was both made aware of Dr. Rett's publications and established a collaboration with colleagues from France and Portugal. This collaboration ended in an article in 1983 describing 17 Swedish, 4 Portuguese and 14 French girls with what they called Rett syndrome, as a tribute to Andreas Rett (Hagberg et al., 1983). After this publication both diagnostic effort and research on RTT increased internationally. The first symposium on Rett syndrome was arranged by Andreas Rett in Vienna in April 1983. A small group of people from Europe and Japan attended this meeting, where Dr. Rett presented several girls with RTT (Nomura et al., 2005). Their similarities were striking. In 1985 the second symposium was arranged, and after this symposium the first official diagnostic criteria were published (Hagberg et al., 1985). This was the beginning of an era where RTT became internationally known,

and a large number of girls were diagnosed with RTT. Parents associations were established and several important research groups, both with a clinical and a basic research focus were established. Since then more than 3500 publications on RTT have been published (https://www.ncbi.nlm.nih.gov/pubmed/). Experiences from clinical work and results from clinical research lead to an extension of the diagnostic criteria in 1994 where atypical RTT or RTT-variants were included (Hagberg et al., 1994).
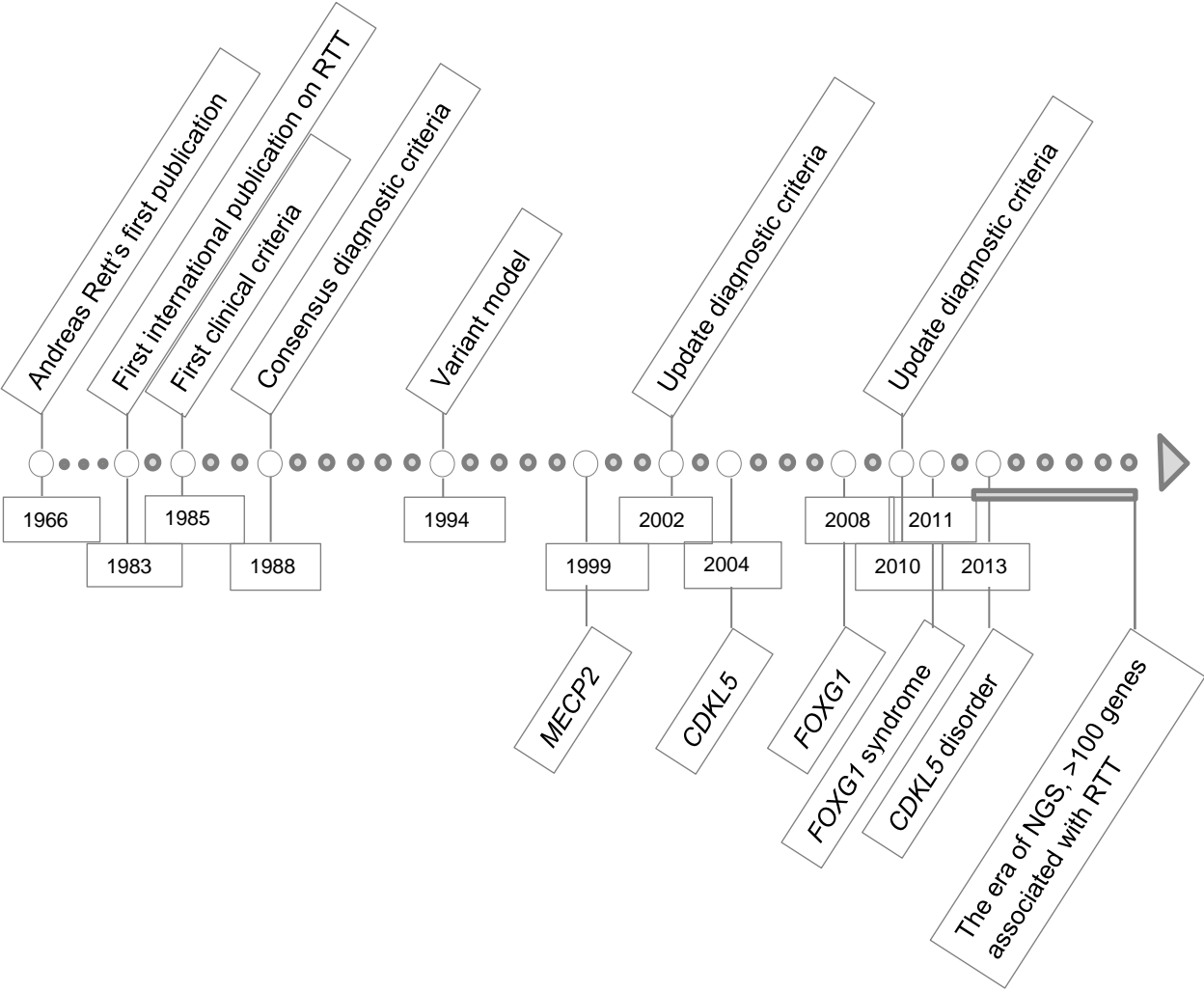
Figure 1. *Timeline of the evolution of the diagnostic criteria for RTT and the genotypes associated with RTT. (Mari Wold Henriksen)*

In Norway the first girl got diagnosed with RTT in 1983 by the two neuropaediatricians Ruth Bolstad and Ragnhild Kiil (Bostad et al., 1987). In 1987 Frambu, a Norwegian resource centre for rare diseases, arranged a seminar on RTT. Dr. Bengt Hagberg was present, and many girls got their suggested diagnosis of RTT confirmed. At this seminar the Norwegian Rett syndrome foundation was funded.

Internationally the search for a biological marker for RTT was intense. The almost exclusively female occurrence and the high concordance in monozygotic twins increased the suspicion of a genetic cause (Zoghbi, 2016). Already in 1983 Hagberg and colleagues proposed a dominant mutation on the X-Chromosome to be the major etiological cause (Hagberg et al., 1983). But the genetic technology was far from what it is today, and in spite of intense research the final breakthrough was not until 1999, when Amir and her colleagues found that RTT was related to mutations in the *MECP2* gene (Amir et al., 1999). In 2004 and 2008 associations between mutations in the genes *CDKL5* and *FOXG1*, respectively, and atypical RTT were described (Ariani et al., 2008; Tao et al., 2004).

In the last decade, however, both the phenotypic and the genotypic spectrum of RTT have extended. The number of genes associated with RTT has increased considerably (Ehrhart et al., 2018), and many individuals who share many characteristics but do not fulfil the diagnostic criteria of RTT are now included in the RTT spectrum via the term RTT-like disorders (Schonewolf-Greulich et al., 2017a).

1.3 MOLECULAR GENETICS

1.3.1 *MECP2*

The findings of Amir and her colleagues in 1999 was a milestone in RTT research. In the following years Mecp2-mutant mouse models and cell lines were developed, which have been invaluable in research of the pathophysiology of RTT (Leonard et al., 2017; Lombardi et al., 2015). *MECP2* is located on the X-chromosome, and over the years more than 95 percent of individuals with classic RTT and more than 75 percent of those with

atypical RTT have been found to have a pathogenic mutation in this gene (Neul et al., 2010). Mutations in *MECP2* give loss of function of the MeCP2-protein, which plays an essential role in the nervous system, including as a regulator of gene expression (Feldman et al., 2016). However, not all individuals with a mutation in *MECP2* have an RTT-phenotype. Other clinical presentations have been described, including neuropsychiatric disorders, non-syndromic autism, mild intellectual disability and Angelman syndrome (Klauck et al., 2002; Shibayama et al., 2004; Suter et al., 2014; Watson et al., 2001). Some of this phenotypic variation is explained by X-inactivation, since *MECP2* is located on the X-chromosome, and girls have two X-chromosomes. Thus, they will have both one affected allele and one normal. Only one is activated in each cell and the activation is random. While in some individuals around half of the cells will have the mutated allele active and half the non-mutated, others may have a less equitable distribution, resulting in a more severe clinical state (Ravn et al., 2011). In addition to the pathology caused by too little MeCP2 protein, too much protein is also pathogenic; the *MECP2*-duplication syndrome illustrates this with the presence of intellectual disability, seizures and respiratory tract infections (Giudice-Nairn et al., 2019).

Most mutations in *MECP2* are de novo, and RTT is sporadic in 99.5 percent of cases (Trappe et al., 2001). The mutations normally develop in the paternal germline, which may explain why so few boys have RTT (Trappe et al., 2001).

MECP2 consists of four exons and encodes for the two known isoforms of MeCP2: MeCP2-e1 and MeCP2-e2 (Figure 2). The two isoforms differ only in the n-terminus. MeCP2-e1 consists of exon 1 + 3 and 4, while MeCP2-e2 exon 2 + 3 and 4. They are believed to be functionally equivalent (Leonard et al., 2017). The MeCP2 protein has some areas that are important for its function. First the methyl-CpG-binding domain (MBD) which binds to modified cytokines, both CG and non-CG (CH), and is crucial to MeCP2s effect on DNA methylation. Second the transcriptional repression domain (TRD) including the NCOR-SMRT interaction domain (NID), which is important for the function MeCP2 has as a regulator for gene expression (Lyst et al., 2015). Most pathological mutations in *MECP2* lie within one of these two areas (Leonard et al., 2017). MeCP2 is expressed in most cells in the body, but animal studies have shown that animals with a

mutation only in central nervous system(CNS)-cells are indistinguishable from animals with mutation in all cells, indicating that it is the loss of MeCP2 in CNS that gives the symptoms of RTT (Lombardi et al., 2015). Furthermore it has been shown that most of the effect is in the neurons, although loss of MeCP2 function in astrocytes probably contributes somewhat to the RTT phenotype (Leonard et al., 2017). Morphological changes in neurons with MeCP2 loss of function include small neurons, less complex dendrites and reduced synaptic density (Leonard et al., 2017).
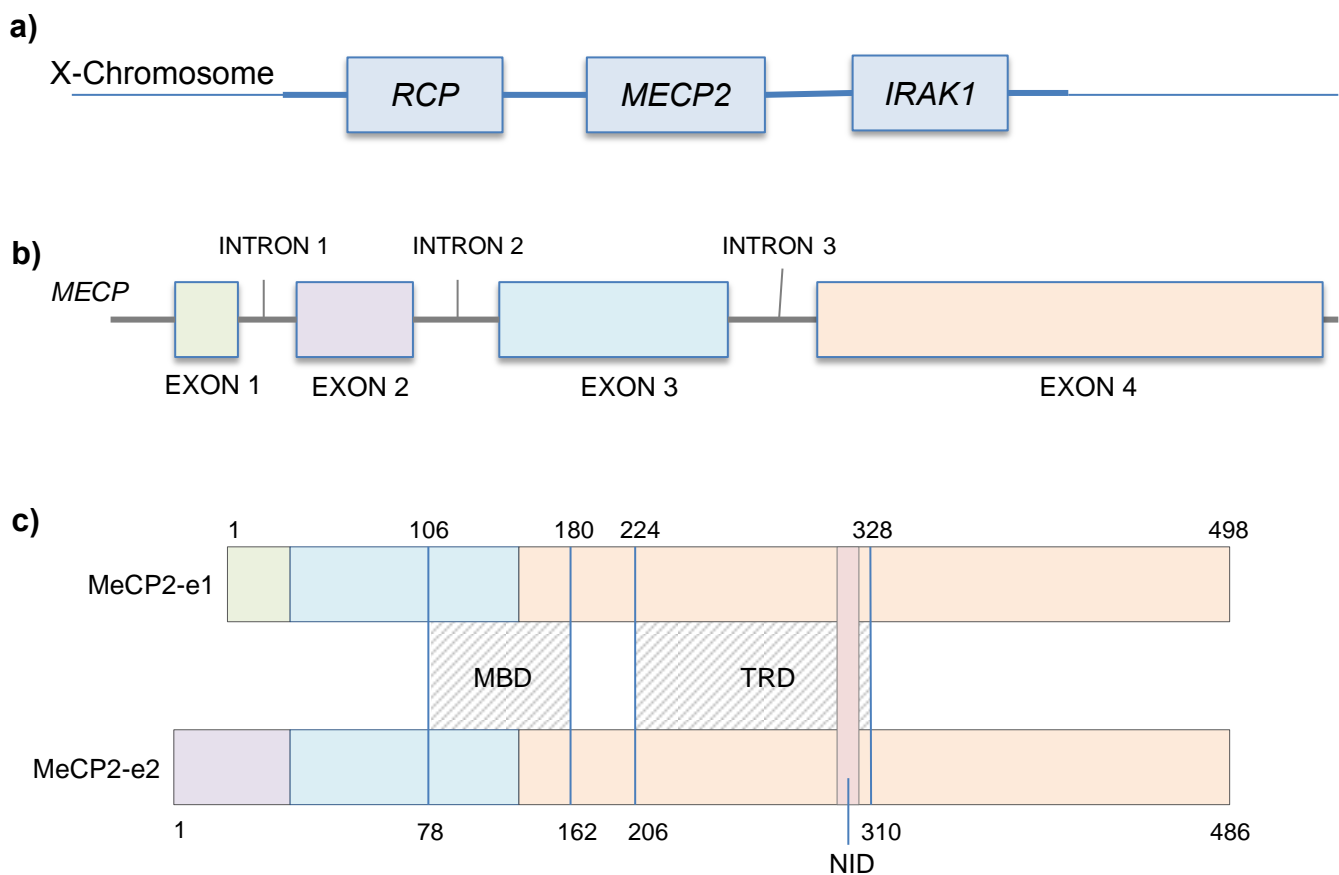
Figure 2.
a) MECP2 with its neighboring genes on the X-chromosome
b) Details of MECP2
c) The two MeCP2 isoforms, with MBD, TRD and NID. The number of the first and the last amino acid in the isoforms, as well as in MBD and TRD is marked. (Mari Wold Henriksen)

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Different kinds of mutations have been found in RTT: missense and nonsense point mutations, indels, intronic variants and large deletions (Maortua et al., 2013). Strong associations between genotype and phenotype in both classic and atypical RTT have been described (Cuddapah et al., 2014; Neul et al., 2008). On individual basis the phenotype may vary with the same genotype, probably caused by both X-inactivation and the genetic environment (Ehrhart et al., 2018). The genotypes associated with a milder phenotype are mostly point mutations, and truncating mutations located close to and in the c-terminal. Most nonsense mutations, splice sites and large deletions are associated with a more severe phenotype (Cuddapah et al., 2014).

1.3.2 *CDKL5*

In 2004 the first reports on mutations in *CDKL5* as a cause of atypical RTT were published (Tao et al., 2004; Weaving et al., 2004). Like *MECP2, CDKL5* is located on the X-chromosome, it is highly expressed in the brain, and it is important in the neuronal development (Mari et al., 2005). There is evidence that the MeCP2 protein and the CDKL5 protein belong to the same molecular pathway, which could explain the similarities in phenotype (Amendola et al., 2014; Mari et al., 2005; Sajan et al., 2017). Fehr et al. (2013) suggest that individuals with mutations in *CDKL5* should not be diagnosed with RTT, but with *CDKL5* disorder. They surveyed 86 individuals with a mutation in *CDKL5* and found that 74 percent did not fulfil the diagnostic criteria of RTT, mainly due to abnormal development from birth and absence of a period of regression. In addition, they compared the typical RTT-features in the cohort with *CDKL5* mutations with a cohort consisting of individuals with RTT and a *MECP2* mutation and found more epilepsy, less respiratory irregularities and less scoliosis in the group with *CDKL5* mutations. Similar findings were reported by Mangatt et al. (2016). Mutations in *CDKL5* are associated with early onset epilepsy, severe intellectual disability and motor impairment (Fehr et al., 2013).

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1.3.3 *FOXG1*

A third gene in which mutations are associated with RTT is *FOXG1*, a gene located on chromosome 14. Mutations in this gene were described in two individuals with congenital RTT in 2008 (Ariani et al., 2008). *FOXG1* codes for the Foxg1 protein which is essential in early development of the brain, and Foxg1 and MeCP2 seem to indirectly affect some common targets (Ariani et al., 2008). Kortum et al. (2011) have suggested that FOXG1 is a separate entity, just like *CDKL5*. They argue that the brain imaging abnormalities in individuals with mutations in *FOXG1,* the lack of regression and respiratory irregularities are sufficient to distinguish their symptoms from those of individuals with RTT, and to allow clinical recognition of the *FOXG1* syndrome (Kortum et al., 2011).

1.3.4 Other genes

As the approach in genetic testing has changed, the number of genes reported to be associated with RTT have increased considerably. Traditionally the genetic diagnosis in monogenic disorders like RTT was based on a phenotypic approach where suspected genes were tested one by one by first generation sequencing, i.e., Sanger-sequencing (Sanger et al., 1977). In 2003 the first article where use of MLPA revealed deletions in *MECP2* in individuals who tested negative on Sanger-sequencing was published (Erlandson et al., 2003). As a consequence MLPA was included in the genetic workup. The last decade, however, Next Generation Sequencing (NGS), a massive parallel sequencing of multiple genes, has become increasingly available. This technique is used in several ways: targeted sequencing (multiple specific genes), whole genome sequencing or whole exome sequencing, the latter frequently used with bioinformatic filtering for panels of genes of interest for a specific diagnostic group . The new technology has led to identification of novel disease genes, novel variants in known disease genes, and variants in other genes than those presumed by the phenotype (Koboldt et al., 2013).
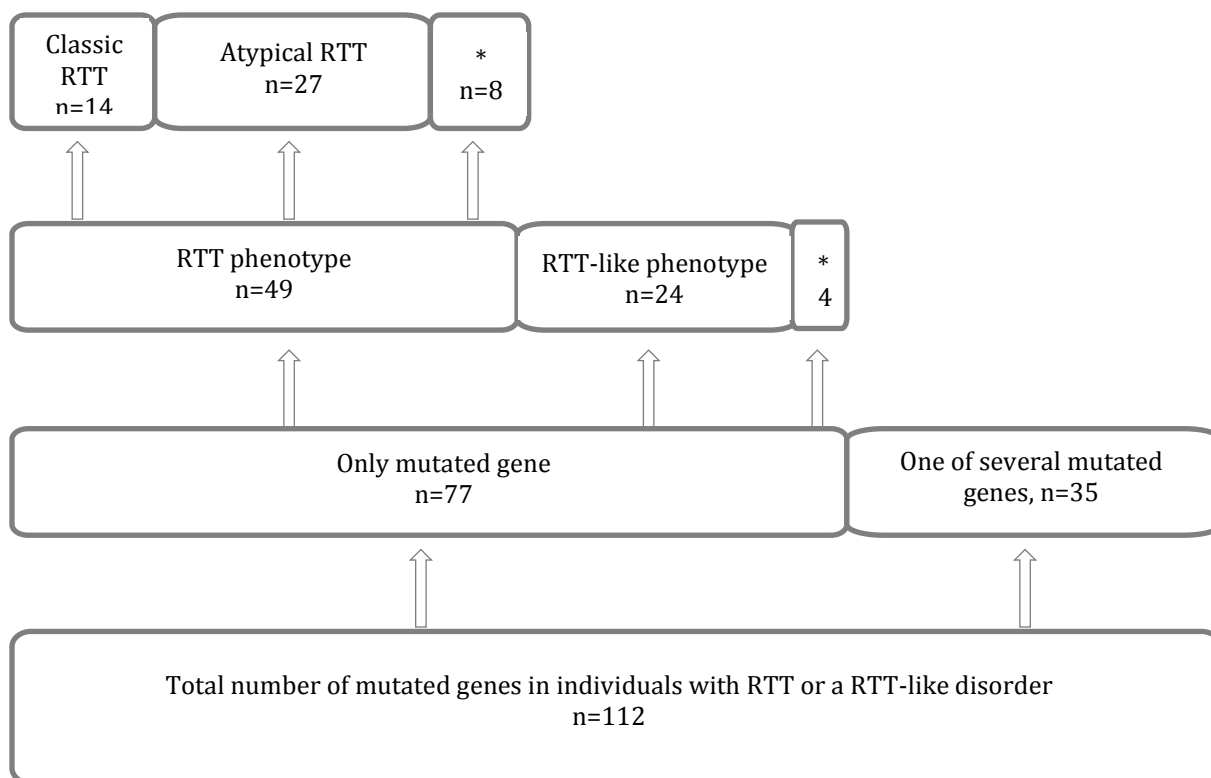
Figure 3. *Number of genes associated with different phenotypes in RTT and RTT-like disorder described in literature (references in text). * specific diagnosis not described (Mari Wold Henriksen)*

A review of the literature shows that mutations in 107 different genes have been revealed by NGS-analyses in individuals with RTT or a RTT-like disorder since 2014. When adding two more genes found with other methods and *MECP2, CDKL5* and *FOXG1,* which are already known, a total of 112 different genes have been associated with individuals with RTT or a RTT-like disorder (Allou et al., 2017; Baasch et al., 2014; Borg et al., 2005; Burger et al., 2017; Craiu et al., 2015; Epperson et al., 2018; Gilissen et al., 2014; Hara et al., 2015; Hoffjan et al., 2016; Huisman et al., 2017; Iwama et al., 2019; Jang et al., 2015; Kulikovskaja et al., 2018; Kyriakopoulos et al., 2018; Lee et al., 2016a; Lee et al., 2016b; Liang et al., 2017; Lopes et al., 2016; Lucariello et al., 2016; Nakamura et al., 2018; Ohba et al., 2014; Okamoto et al., 2015; Olson et al., 2015; Percy et al., 2018; Pescucci et al., 2003; Romaniello et al., 2015; Saez et al., 2016; Saitsu et al., 2014; Sajan et al., 2017; Schonewolf-Greulich et al., 2017a; Shimada et al., 2018; Srivastava et al., 2018;

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Vidal et al., 2017; Vrecar et al., 2017; Vuillaume et al., 2018; Williamson et al., 2015; Yoo et al., 2017; Yuge et al., 2018). In 35 of these genes the mutation revealed was one of several presumed pathological mutations in the same individual (Lopes et al., 2016; Lucariello et al., 2016; Sajan et al., 2017). In the remaining 77 it was the only pathological mutation revealed. The phenotypes associated are illustrated in Figure 3.

1.4 CLINICAL MANIFESTATIONS AND DIAGNOSIS

1.4.1 Diagnostic criteria and variant forms

The diagnostic criteria for RTT were last revised in 2010 (Table 1)(Neul et al., 2010). Regression, loss of hand function and language, gait abnormalities and hand stereotypies are required to diagnose classic RTT. In addition, no brain injury or grossly abnormal development in first six months of life should be present. For the diagnosis of atypical RTT regression is required, as well as presence of two of the four main criteria and five of the 11 supportive criteria. Three different variant forms are described in these criteria: preserved speech variant, early seizure variant and congenital variant. The preserved speech variant is known for its mild phenotype, in particular the presence of speech, which is lacking in classic RTT. It has a milder reduction of hand skills and autistic features are often present. Mutations in *MECP2* are usually found. The early seizure variant is, as the name indicates, characterized by early onset of epileptic seizures, often before five months of age. Mutations in *MECP2* are rare, but mutations are often found in *CDKL5*. The congenital variant is characterized by grossly abnormal development from birth, severe microcephaly and regression during the first five months of life. *MECP2* mutations are rarely found, but mutations in *FOXG1* may occur (Neul et al., 2010). If the *FOXG1* syndrome and *CDKL5* disorder become fully implemented, one may question whether these variant forms are still relevant. In many recent articles the individuals with atypical RTT are categorized into two groups based on clinical severity rather than specific variant forms (Neul et al., 2014; Tarquinio et al., 2017; Tarquinio et al., 2018).

Table 1. *RTT diagnostic criteria*

RTT diagnostic criteria 2010
Consider diagnosis when postnatal deceleration of head growth observed
Required for classic RTT
Required criteria, all main criteria and all exclusion criteria
Required for atypical RTT
Required criteria, at least 2 of the 4 main criteria and 5 out of 11 supportive criteria
Required criteria
A period of regression followed by recovery or stabilization
Main criteria
Partial or complete loss of acquired purposeful hand skills.
Partial or complete loss of acquired spoken language
Gait abnormalities: Impaired (dyspraxic) or absence of ability
Stereotypic hand movements such as handwringing/squeezing, clapping/tapping, mouthing and washing/rubbing automatisms
Exclusion criteria
Brain injury secondary to trauma (peri- or postnatally), neurometabolic disease, or severe infection that causes neurological problems
Grossly abnormal psychomotor development in first 6 months of life
Supportive criteria
Breathing disturbances when awake
Bruxism when awake
Impaired sleep pattern
Abnormal muscle tone
Peripheral vasomotor disturbances
Scoliosis/kyphosis
Growth retardation
Small cold hands and feet
Inappropriate laughing/screaming spells
Diminished response to pain
Intense eye communication - ''eye pointing''

Neul et al., 2010

1.4.2 Epidemiology and survival

RTT is a rare disorder which almost exclusively affects females. The reported prevalence and incidence have varied somewhat between countries, but the main reason for this is most likely methodological; some excluded individuals without *MECP2* mutations, others only included classic RTT (Bienvenu et al., 2006; Wong et al., 2007). The Australian Rett Syndrome Database is a population-based register which provides data for epidemiological studies. Laurvick et al. (2016) reported a cumulative incidence of RTT diagnosis by age 12 of 1.09 per 10,000 females born from 1980 to 1999 in Australia. The prevalence in 2004 was 0.88 per 10.000 in the age group 5-18 and 0.53 in those aged 19 and older (Laurvick et al., 2006). The prevalence is not affected by race, socio-economic status or geography (Kozinetz et al., 1993; Laurvick et al., 2006).
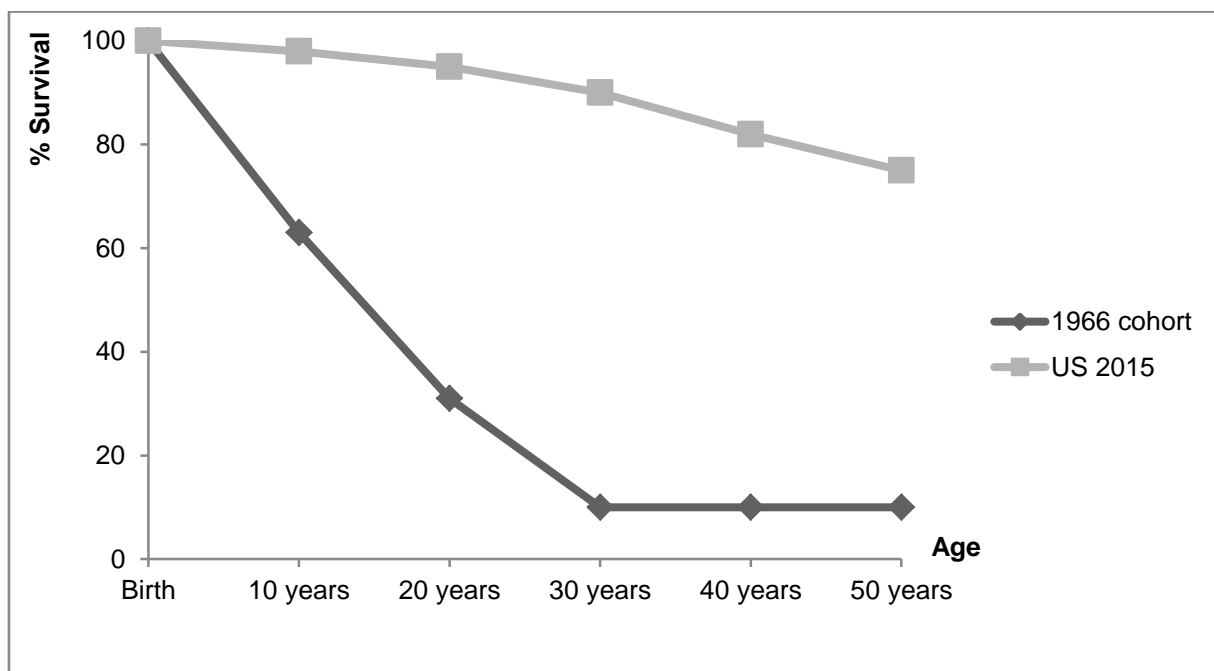
Figure 4. *Survival data from the cohort Andreas Rett described in 1966, n=22 (Freilinger et al., 2010) and from the North American Natural history study in 2015, n=1189 (Tarquinio et al., 2015a)*

Little was known about longevity in the 1980's. Several middle-aged women with RTT were identified, but the prevalence in adults was low compared to children (Haas, 1988). Hagberg et al. reported in 2001 a median age of death of 20 years (Hagberg et al., 2001). The survival data of the original cohort described by Andreas Rett in 1966 was published in 2010 and showed a 21 percent survival up to the age of 25 (Freilinger et al., 2010). The most recent survival data is from the North American Natural History Study, and shows that more than 70 percent live past their 45th birthday (Tarquinio et al., 2015a), indicating a considerable increase in longevity, like in individuals with intellectual disabilities in general (Glover et al., 2017). Figure 4 illustrates the large differences between the survival in Rett's original cohort and the recent American survival data.

The first mortality data reported in RTT was from Great Britain in 1997. Half of all deaths occurred in debilitated people and one quarter died suddenly and unexpectedly. Of the remaining deaths, half followed severe seizures and half had natural causes (Kerr et al., 1997). As in survival the causes of death in RTT have changed remarkably. In two recent reports death due to debilitation was only reported in one individual. The main reason of death in both studies was cardio-respiratory issues (Anderson et al., 2014; Tarquinio et al., 2015a).

1.4.3 Early development and regression

Andreas Rett described his cohort with normal development in the first nine months, and in the two first sets of diagnostic criteria, normal early development was one of the core criteria (The Rett Syndrome Diagnostic Criteria Work Group, 1988; Hagberg et al., 1985; Rett, 1966). Research on early development over several years has however demonstrated that early development in RTT is not always normal, although the abnormalities often are subtle (Bisgaard et al., 2015; Einspieler et al., 2005; Marschik et al., 2013). Many parents have described their children as remarkably placid and with an empty gaze (Einspieler et al., 2019). Research on motor development has revealed an abnormal quality of general movements and finger movements in many infants later diagnosed with RTT (Einspieler et al., 2005), and while stereotypic hand movements

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have been described as evolving in the regression phase, video analyses have revealed stereotypic movements in the pre-regression period as well (Einspieler et al., 2019). A deviant development of early communication skills has also been described. Eye contact and responsive smiling is usually in place in infants later diagnosed with RTT, but the development of both the pre-linguistic vocalizations (cooing, babbling) and gestures are often deviant (Einspieler et al., 2019). We still do not know enough about how early development in RTT differs from early development in other neurodevelopmental disorders. RTT-specific early signs permitting an earlier diagnosis have not yet been identified (Einspieler et al., 2019) and a regression phase is still one of the main reasons for clinicians to suspect RTT (Knight et al., 2016). The regression phase is a core criterion in current RTT diagnostics, and has to be present for both classic and atypical RTT (Neul et al., 2010). The child loses acquired skills, especially hand function and language. The onset of regression is normally between 12 and 19 months, but both earlier and later onset has been described (Fehr et al., 2011). In parallel with the loss of skills, many children go through a period of withdrawal from normal social interaction, which in many cases has resulted in an initial diagnosis of childhood autism (Young et al., 2008). The neuropathological mechanisms of the regression we see in RTT are not yet completely understood (Zoghbi, 2016). The regression may be sudden and dramatic or a more gradual process. Sometimes it has been so subtle and protracted that it may be difficult both to know when it started and in some cases if it has ever been present (Einspieler et al., 2019).

1.4.4 Motor development

As subtle signs of abnormal development are present in many cases from early on, a deviant motor development becomes clearer as months go by. A recent publication found that early gross motor skills like rolling and sitting were acquired by almost all girls with RTT, while motor milestones normally acquired at later age were severely delayed or not reached (Neul et al., 2014). In atypical RTT with mild phenotype, gross motor skills were achieved at a significantly higher level, while those with a more severe phenotype achieved significantly poorer. Differences between classic and atypical RTT

were typically seen in more advanced gross motor skills like crawling and walking. Independently walking was achieved by 53 percent with classic RTT, 78 percent with an atypical mild phenotype and only 7 percent with an atypical severe phenotype (Neul et al., 2014). In the general population, walking independently is a milestone achieved at a mean of 12.1 months (WHO, 2006). In an Australian report of 293 individuals with classic and atypical RTT 46 percent learned to walk independently at a mean age of 19.6 months (Fehr et al., 2011). The development of fine motor skills shows the same differences between classic, atypical mild and atypical severe, but these skills are more often lost in the regression phase than gross motor skills. In the atypical mild group, however, significantly fewer lost fine motor skills than in the two other groups (Neul et al., 2014).

1.4.5 Growth

Even before the first signs of developmental delay, many girls later diagnosed with RTT will display a head growth deceleration. Microcephaly (below -2SD) was found in 81 percent of those diagnosed with RTT in a large American cohort, and the mean head circumference fell below the normative mean already by one month of age (Tarquinio et al., 2012). After this first sign of growth retardation, poor weight gain and height growth follows in the majority. The pathophysiology behind this global growth retardation remains unclear, but some of it may be explained by an increased resting metabolic rate, in combination with more feeding difficulties in girls with RTT compared to controls with equivalent developmental disorders (Isaacs et al., 2003; Platte et al., 2011).

1.4.6 Communication

Communication and language skills are profoundly impaired in RTT; most lose all words in the regression phase and do not get them back. An exception is the females with the preserved speech variant, who are characterized by recovery of some language skills after regression (Neul et al., 2010; Zappella t al., 1998). Some individuals with classic

RTT can speak a few words, but this is rare. However, research has shown that individuals with RTT may use other communication modalities like vocalizations, eye gaze, gestures, body movements, and augmentative communication systems (Bartolotta et al., 2011). The past few years advances in eye tracking technology has made eye tracking devices available for females with RTT in many countries. Parents are shown to be satisfied with the improvement in their daughters' skills when using the devices over time (Townend et al., 2016; Vessoyan et al., 2018). In a small case series of four individuals, all had improvements on communication goals according to their therapists (Vessoyan et al., 2018). In 2018, Ahonniska-Assa and colleagues explored the use of eye tracking technology to assess cognitive functioning and found that eye tracking technology make the communicational signals more easily understood. In addition, they found that the receptive language skills in one third of their sample were at a higher level than expected (Ahonniska-Assa et al., 2018). In spite of these promising results the documented evidence to support eye tracking technology for aided communication is still scarce, and more research is needed (Vessoyan et al., 2018).

1.4.7 Behaviour

A definable behavioural phenotype including hand stereotypies, teeth grinding, anxiety and low/changeable mood, sleep disturbances and respiratory irregularities has evolved in RTT (Cianfaglione et al., 2015). The most common feature is hand stereotypies which are found in almost 100 percent. Repetitive behaviour is found in other severe disorders as well (Goldman et al., 2009; Vidal et al., 2019). Hand stereotypies seem more diagnosis-specific and hand wringing is most common in RTT, with other midline stereotypies also present (Cianfaglione et al., 2015). Sleep problems are another feature common in children with intellectual disabilities, and extremely common in children with RTT. In an international survey by Boban et al. (2018), 93 percent reported problems either with falling asleep or night time wakening, and 44 percent reported that this impacted the family moderately or severely. The sleep problems seem to improve with increasing age in some individuals, but not in all (Wong et al., 2015). An interesting RTT-specific feature is the inappropriate night time laughter, which appears in around

three-quarters of the population (Wong et al., 2015). Internalizing features like anxiety and social withdrawal are highly prevalent. Externalizing behaviour (aggression, self-abuse etc.) is less common, although one feature, inconsolable screaming episodes, is one of the supplementary criteria in RTT and is present in periods in the life of many individuals with RTT (Mount et al., 2001).

1.4.8 Medical issues

Of the main medical comorbidities in RTT, we find respiratory irregularities, gastro-intestinal disorders, epilepsy and scoliosis (Gold et al., 2018). Epilepsy will be described in the next section.

The respiratory irregularities are a part of the autonomic dysfunction in individuals with RTT. Several different types of abnormal respiration are reported, but it can be categorized into two main groups, hyperventilation and breath holding. These breathing disturbances occur mainly when they are awake, but are also seen during sleep (Rohdin et al., 2007), and affect more than 90 percent over the lifespan (Tarquinio et al., 2018). Associated with breath holding is air swallowing and subsequent abdominal bloating, which is prominent in around one third of the population (Mackay et al., 2017; Morton et al., 2000). Parents report an impact on daily life in almost half of individuals with abdominal bloating and in around one third of individuals with hyperventilation and/or breath holding (Mackay et al., 2017). Neither the link between the loss of MeCP2 function and the erratic patterns of breathing, nor the clinical consequences are fully understood (Mackay et al., 2017). There is however a strong association between severe breathing dysfunction and prolonged QT-syndrome in RTT, and the question whether this is associated with the increased risk for sudden death has been raised (Tarquinio et al., 2018).

Several disorders affecting the gastrointestinal system occur more often in individuals with RTT than in the general population. The two most common are gastroesophageal reflux and constipation. Less frequent are biliary tract disorders (Motil et al., 2012). Both gastroesophageal reflux and constipation are conditions of intestinal dysmotility,

and the high prevalence of these disorders in RTT may in part be explained by the autonomic dysfunction (Pini et al., 2016). Constipation is probably caused by several other features as well. Many individuals with RTT drink less than advised, move less than the general population and use medication that have constipation as an adverse effect (Baikie et al., 2014).

Scoliosis affects around three-quarters of individuals with RTT (Ager et al., 2006; Downs et al., 2016b). Non-ambulation is a risk factor for severe scoliosis. Scoliosis may cause pain, deterioration of motor skills and impaired respiratory function (Downs et al., 2016b). The international guidelines on scoliosis in RTT recommend regular follow-up with clinical examination and x-rays, and regular physiotherapy for all girls with scoliosis. They also recommend special care for individuals with specific mutations (R168X, R255X, and R270X) due to increased risk for scoliosis. Surgery is recommended when the cobb angle reaches 40-50 degrees (Downs et al., 2009). There is an increased risk of post-operative complications in RTT, but several publications have shown both care giver satisfaction after the surgery, improved motor function and increased survival (Downs et al., 2016a; Downs et al., 2016c; Larsson et al., 2009).

1.4.9 Epilepsy

Epilepsy is one of the main health problems in RTT and deteriorates the quality of life for both the affected girl or woman and her family (Bahi-Buisson et al., 2008). The lifetime prevalence of epilepsy in RTT is 70-90 percent (Nissenkorn et al., 2015; Pintaudi et al., 2010; Tarquinio et al., 2017). The wide range may be explained by difficulties distinguishing between epileptic and non-epileptic seizures. Many of the common clinical characteristics of RTT may mimic epileptic seizures, like gastroesophageal reflux, breath-holding and hyperventilation, inappropriate laughter or screaming spells, motor dysfunction, freezing of activity and vacant staring episodes (Glaze et al., 1998).

In classic RTT the first seizure rarely occurs before two years of age (Glaze et al., 2010). Median age of onset is reported to be between three and four years, but the range is wide; from birth to into the 40's (Nissenkorn et al., 2010; Pintaudi et al., 2010). The early seizure variant is, as the name indicates, characterized by an early seizure onset

before five months of life (Neul et al., 2010), while the preserved speech variant has later onset and less severe epilepsy (Pintaudi et al., 2010). Multiple seizure types are seen in RTT. Around half of the seizures have a focal onset and half a generalized onset. Specific seizure types like myoclonic jerks, absences, infantile spasms, tonic and atonic seizures are all reported (Nissenkorn et al., 2015; Tarquinio et al., 2017).

The burden of epilepsy varies significantly; 30-40 percent are reported to be drug resistant (Pintaudi et al., 2010; Vignoli et al., 2012), and around 20 percent have weekly or daily seizures (Bao et al., 2013). Again, the early seizure variant stands out with 80 percent of the individuals reported to be drug-resistant (Pintaudi et al., 2010). In 2017, a comprehensive article from the North-American Natural History Study for the first time describes a pattern of remission and relapse of seizures in RTT (Tarquinio et al., 2017). In their cohort a pattern of remissions (six months or more without seizures) and relapses occurred in 41 percent, while only 16 percent had relentless seizures without ever having experienced seizure-free periods. The remissions occurred across the life span, and although the average remission duration was short, some individuals experienced remissions of more than five years.

No definite recommendations for the choice of antiepileptic drug (AED) treatment in RTT are available. Due to the rarity of the disorder, comprehensive studies on the effectiveness of different AEDs are few. Vignoli and colleagues recommend considering age-dependency when treating patients with epilepsy in RTT. In their study valproate was most effective in children, while carbamazepine was more effective in women aged 15 or more (Vignoli et al., 2017). Both ketogenic diet and vagal nerve stimulation have been reported to be effective in single cases and small case series, but the literature is scarce (Liebhaber et al., 2003; Wilfong et al., 2006).

Attempts to find associations between *MECP2* genotype and epilepsy phenotype have not resulted in convincing correlations (Cardoza et al., 2011; Tarquinio et al., 2017). However, a higher prevalence of epilepsy in individuals without *MECP2* mutations has been reported (Glaze et al., 2010; Jian et al., 2006).

While the clinical features of epilepsy among children, adolescents and young adults are described thoroughly, less attention has been given to the course of the seizure disorder in older adults with RTT. Early publications reported fewer seizures in

adult age (Naidu et al., 1986; Steffenburg et al., 2001), but recent studies have been conflicting. Few publications focus on older adults; most of them lump adolescents and adults into one group or all individuals 20 years and older together (Bao et al., 2013; Jian et al., 2007; Pintaudi et al., 2010). Since the burden of epilepsy is high and the population of adults with RTT is growing, knowledge on the development of epilepsy into adult and old age is important for appropriate treatment and care-taking.

1.4.10 Aging

As described in the paragraph on epidemiology and survival the life expectancy in RTT has increased (Tarquinio et al., 2015a). In other words; we have an increasing population of aging adults with RTT. To ensure the best possible treatment and quality of life, knowledge on health in these adults is crucial. We cannot assume that the knowledge from research on children and adolescents can be readily transferred to adults. Many of the articles published on RTT in the 80's have just a few adults included in their cohorts (Hagberg et al., 1983; Naidu et al., 1986), and there is still a clear predominance of children and adolescents in many of the large cohorts (Nissenkorn et al., 2010; Pini et al., 2016; Tarquinio et al., 2018). In addition, in many articles all adults are analysed together in one group, not differentiating on age (Anderson et al., 2014; Cass et al., 2003; Vignoli et al., 2012). There are, however, some exceptions. Halbach et al. followed a group of 37 women aged 21-46 years (at the beginning of the study) over five years. Their main findings indicated an improvement in the general health of these adults, with less epilepsy and autonomic disturbances, but a slight motor deterioration. The prevalence of age-related health issues like diabetes and hypertension was lower than in the general population (Halbach et al., 2013). The North-American National History Study has provided lifespan information about three different medical comorbidities epilepsy, breathing disturbances, and gastrointestinal and nutritional problems. Both epilepsy and breathing disturbances are highly prevalent in adults, although the intensity of these symptoms seems highest in late childhood and adolescence. Gastrointestinal problems were more bimodal, with the prevalence of gastroesophageal symptoms decreasing with advanced age while issues in bone health

and alternative feeding methods were more prevalent (Motil et al., 2012; Tarquinio et al., 2017; Tarquinio et al., 2018).

2. RATIONALE

Summarized, the presented literature describes the following about RTT:

- The current diagnostic criteria in RTT are based on clinical characteristics. A mutation in *MECP2* is neither pathognomonic nor necessary, and mutations in other genes do not exclude RTT.

- The technological development in genomic investigations has increased the number of genes associated with RTT to more than one hundred, and revealed mutations in *MECP2* in individuals with a wide variety of phenotypes.

- Expected longevity in RTT has increased considerably, implying that there is an increasing number of adults in the RTT population, including older adults.

- Epilepsy is highly prevalent in RTT and affects quality of life in both the girl/woman with RTT and her family.

- The knowledge on health issues in adults with RTT is scarce.

Hence, the present project aimed to improve the knowledge on these themes.

3. AIMS OF THE STUDY

PRIMARY OBJECTIVE

The overall aim of this thesis is to describe the genotypic and phenotypic variation in the Norwegian Rett Syndrome population, and the development of clinical features in different phases of life.

SECONDARY OBJECTIVES

- Compare individuals with and without *MECP2* mutations within the groups of classic RTT and atypical RTT to see if there are major clinical differences.

- Describe individuals with a RTT diagnosis and mutation in another gene than *MECP2.*

- Describe the diversity of epilepsy in a population of females with RTT, and address the development of the seizure disorder in adulthood.

- Compare health issues in individuals with RTT of different ages, with a special focus on individuals aged 36 or older.

4. METHODS

The studies presented here are part of a multidisciplinary study of individuals with RTT in Norway.

4.1 STUDY POPULATION

The present study is a national survey including participants from all over Norway. The number of inhabitants in Norway per 01.01.2013 was 5 051 275. In 1997, the prevalence of RTT in three Norwegian counties was described. In Nordland and Østfold the prevalence was 1.05 and 0.77 per 10 000 girls, similar to other countries. In Rogaland the prevalence, for unknown reasons, was 3.77 (Skjeldal et al., 1997). The prevalence of RTT in Norway has not been explored after 1997.

The Norwegian patient register, the Norwegian Directorate of Health
The Norwegian patient register is a register of health information on everyone who has received treatment in the specialist health service. 165 individuals had been registered with the ICD-10 diagnosis of F84.2 Rett syndrome from 2009-2012. These data are not appropriate to use for epidemiological purposes; individuals with the diagnosis of RTT not treated in the specialist health service are not counted, and the ones treated in the specialist health service are only counted if the correct diagnostic code is used at the visit. In addition, individuals where the diagnosis has been changed after the initial visit will still be counted as having RTT. However, these data still give an impression of the number of individuals with RTT in Norway.

4.2 PARTICIPANTS

Recruitment took place from 2013 to 2017. Information on the project and invitation to participate was distributed by the Norwegian Rett syndrome Association, Frambu (a Norwegian Resource centre for rare diseases), some habilitation centres and a few neurologists. In addition, some families contacted the authors directly.

Paper I	Paper II	Paper III	Paper IV
•n = 91	•n = 70	•n = 79	•n = 2
•One participant was excluded due to missing genetic testing	•Individuals fulfilling the diagnostic criteria of classic RTT with *MECP2* mutation or no mutation identified •One individual was excluded due to the amount of missing epilepsy data	•Individuals fulfilling the diagnostic criteria of classic or atypical RTT with *MECP2* mutation or no mutation identified	•Case study of two individuals with classic RTT and mutations in the SCN1A gene

Figure 5. *The samples used in the different papers*

The Norwegian Rett syndrome Association sent emails with the information letter to all their members (n=126), they published information in their magazine several times, and members of the research team were invited to their annual national members' meeting to talk about on the study. Frambu distributed information letters by mail to all individuals listed with a diagnosis of RTT in their medical records (n=116). In addition, they informed families they came across in their work, and members of the research group were invited to talk about the project in their course for families with RTT. The habilitation centres HABU, Stavanger university hospital; Habiliteringstjenesten i Hedmark, Innlandet hospital; Trondsletten habiliteringssenter, St.Olavs hospital; Habiliteringssenteret i Vestfold, Vestfold hospital and Habiliteringssenteret i Østfold, Østfold Hospital informed their patients with a diagnosis of RTT about the project. Many of the habilitation centres arranged special days where their patients with RTT could come and be included in the project. In addition, some females were referred directly from the neurologist Eylert Brodtkorb (St.Olavs hospital) and psychiatrist Sigrun Hope (Oslo University Hospital). Lists of names from the Norwegian Rett Syndrome Association, Frambu and the habilitation centres were not revealed to the study group.

However, it is likely that the rate of overlapping must have been high because the number of individuals with RTT reported by the Norwegian Patient registry by 2012 (N=165) is lower than the number of invitations sent out. Since the number of older females with a diagnosis of RTT was relatively low we contacted Public health physicians in municipalities we knew by experience had older inhabitants with RTT, and asked them to inform families with a member with RTT in their municipalities about the project.

Ninety-three families agreed to participate; one was excluded due to the amount of missing data leaving 92 individuals to be included. The samples used in the different papers are shown in Figure 5.

4.3 DATA COLLECTION

Questionnaire
- A questionnaire covering demographic information, nutrition, and motor skills was sent to parents/caregivers (Appendix I).

Interview with parents/caregivers
- Interviews with parents/caregivers were conducted, either in their homes or in a local hospital. The interviews focused on development and medical history (Appendix II). An additional interview with focus on development, communication and habilitation strategies was performed on 72 participants

Clinical examination
- A focused clinical examination of the girl/woman including growth parameters, level of contact, presence of stereotypies and respiration abnormalities as well as assessment of muscle tone, deep tendon reflexes, coordination and scoliosis were conducted (Appendix II).

Genetic workup
- In individuals without an identified mutation in *MECP2,* a genetic workup was done according to the flow sheet in Figure 7.

Review of medical journals
- In most participants a review of medical records was carried out to complete the data sets.

Figure 6. Flowchart for data collection

Data collection followed the flowchart in Figure 6. Questionnaire, interview guide and description of clinical examination are enclosed in Appendix I and II.

Interviews and clinical examinations were mainly performed by Dr. Mari Wold Henriksen (neurology registrar), with the exception of two participants interviewed and examined by Dr. Ola Skjeldal (neuropaediatrician) and three participants by Dr. Gunhild Vestre (paediatrician). An additional interview not used in the studies presented here was performed by Hilde Breck (master of philosophy in psychology, 64 interviews) or Eivind Byrknes (psychologist, 8 interviews).

At the end of the inclusion period seven participants were referred to the project directly from Dr. Eylert Brodtkorb. These participants were neither seen in person nor examined clinically, but the interview was completed by phone with parents or other caregivers. The questionnaire, genetic workup and review of medical journals were completed as described in Figure 6.

4.4 DATA CATEGORIZATION

Review of the diagnosis of the participants was performed based on the 2010 consensus criteria (Neul et al., 2010).

4.4.1 Disease severity

Disease severity was quantified according to the Rett syndrome Severity Scale (RSSS) (Appendix III) with scoring of seven parameters (seizures, respiratory irregularities, scoliosis, ability to walk, hand use, speech and sleep) from 0 (absent/normal) to 3 (severe), meaning 21 is the highest possible score (most severe) (Kaufmann et al., 2012). When analysing RTT severity versus epilepsy in paper II, the seizure sub-score was subtracted. For correlations between genotypes and phenotypes in paper II and III *MECP2* mutations were classified into two groups according to expected phenotypic severity based on a previous report (Cuddapah et al., 2014). The mutations T158 M,

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R168X, R255X, R270X, and large deletions were expected to give a severe phenotype and R133C, R294X, R306C, other point mutations, and c-terminal truncations a mild phenotype.

4.4.2 Growth and age

Age was mainly used in the analyses as a categorical variable; in paper II categorized into four subgroups (1-10 years, 11-20 years, 21-30 years, and above 30 years) and in paper III into three subgroups (1-20 years, 21-35 years and above 35 years). In addition, age was occasionally used in the analyses as a continuous variable. Growth was measured by weight, height, head circumference and calculation of body mass index. Weight, height and body mass index was categorized according to the Norwegian reference standard (Juliusson et al., 2009). Head circumference was categorized using normative z-scores (Rollins et al., 2010). Microcephaly was defined as having a head circumference more than two standard deviations below the mean for the given age and gender.

4.4.3 Ambulation

Ambulation was categorized in an ordinal fashion ('walking independently', 'walking with support' or 'not walking'), both as present skills and as the best skills so far in life. Decline in walking skills were categorized as change 'from being ambulant to non-ambulant' or 'from walking independently to walking with support'.

4.4.4 Epilepsy

Epileptic seizures in RTT may be difficult to distinguish clinically from non-epileptic events. In this study EEG findings could not be systematically assessed. We therefor did not include equivocal epileptic symptoms with low symptom burden and little or no impact on quality of life as epileptic seizures. Care was taken not to interpret non-

epileptic events as head turning, unspecific twitching, staring, jerking, trembling, laughing and respiratory abnormalities as epileptic seizures (Glaze et al., 1998). Active epilepsy was defined as seizures within the last five years (International League Against Epilepsy, 1997). Seizures were categorized by semiological features and were identified as either focal onset motor seizures or unknown onset tonic-clonic or other motor seizures according to the recently revised ILAE seizure classification (Fisher et al., 2017). Seizure frequency was categorized as ≥daily; <daily ≥weekly; <weekly ≥monthly; <monthly >yearly; or seizure free. Seizure patterns were divided into four categories; group 1: never seizures; group 2: diagnosed with epilepsy, but seizure free for more than five years; group 3: active epilepsy with remissions more than six months within last five years; group 4: persistent seizures without remissions.

4.5 GENETIC WORKUP

The participants were tested genetically according to the flow chart in Figure 7. The genetic analyses used were Sanger sequencing, MLPA and NGS. NGS-analyses conducted prior to 2017 were single patient analyses with a gene panel of 57 genes (Appendix IV). In 2017 the number of genes in the diagnostic gene panel for intellectual disability available from the laboratory increased >1400 and the analytic approach was changed to trio test (proband, mother and father) (Appendix V). Participants with negative results of the single patient analysis were re-examined with a larger panel and trio test if both parents were available. For the analyses conducted through usual clinical assessment prior to this study, the methodology for the analyses may be varying. However, for the analyses done by our study group (n=17) the following descriptions are correct:

4.5.1 Sanger sequencing

Sanger sequencing is the conventional method to determine the nucleotide sequence of DNA (Sanger et al., 1977). It can detect point mutations and small deletions and

duplications, but not deletions or duplications of whole exons. The Sanger sequencing used in this project examined all coding regions in *MECP2* and its flanking intron sequences.

4.5.2 MLPA

MLPA is based on multiplex PCR and determines the relative number of copies of each *MECP2* exon (Erlandson et al., 2003). Deletions or duplications of one or several whole exons, which cannot be detected by sequencing, can be revealed by this technique. MLPA in this project was performed with Salsa MLPA kit P015 from MRC-Holland

4.5.3 NGS

NGS is a set of new technologies which allow us to sequence DNA much quicker and less expensive than Sanger sequencing. This means that whole exomes can be sequenced in one analysis. In this project, whole exome sequencing (WES) using Agilent SureSelect Target Enrichment Kit (Agilent Technologies, Santa Clara, CA) on Illumina HiSeq 2500 with pair-end runs was performed. Alignment, mapping, and variant calling were done by Genome Analysis Tool Kit. Reads were mapped to the reference sequence (GRCh37/hg19). Following bioinformatic filtration, analysis of coding regions and intron/exon boundaries of predefined genes was performed. The first gene panel used included 57 genes (Appendix IV) before the available panel in the laboratory used increased to 1479 genes (Appendix V). When the number of genes in the panel increased due to new knowledge the probands analysis was offered as a trio analysis only. In trio analysis the proband's sequence is compared to DNA from the mother and father. The pathology of the mutation was assessed by the use of Alamut Visual software (Interactive Biosoftware, France) and the guidelines of American College of Medical Genetics and Genomics and the association for Molecular Pathology (ACMG) (Richards et al., 2015).
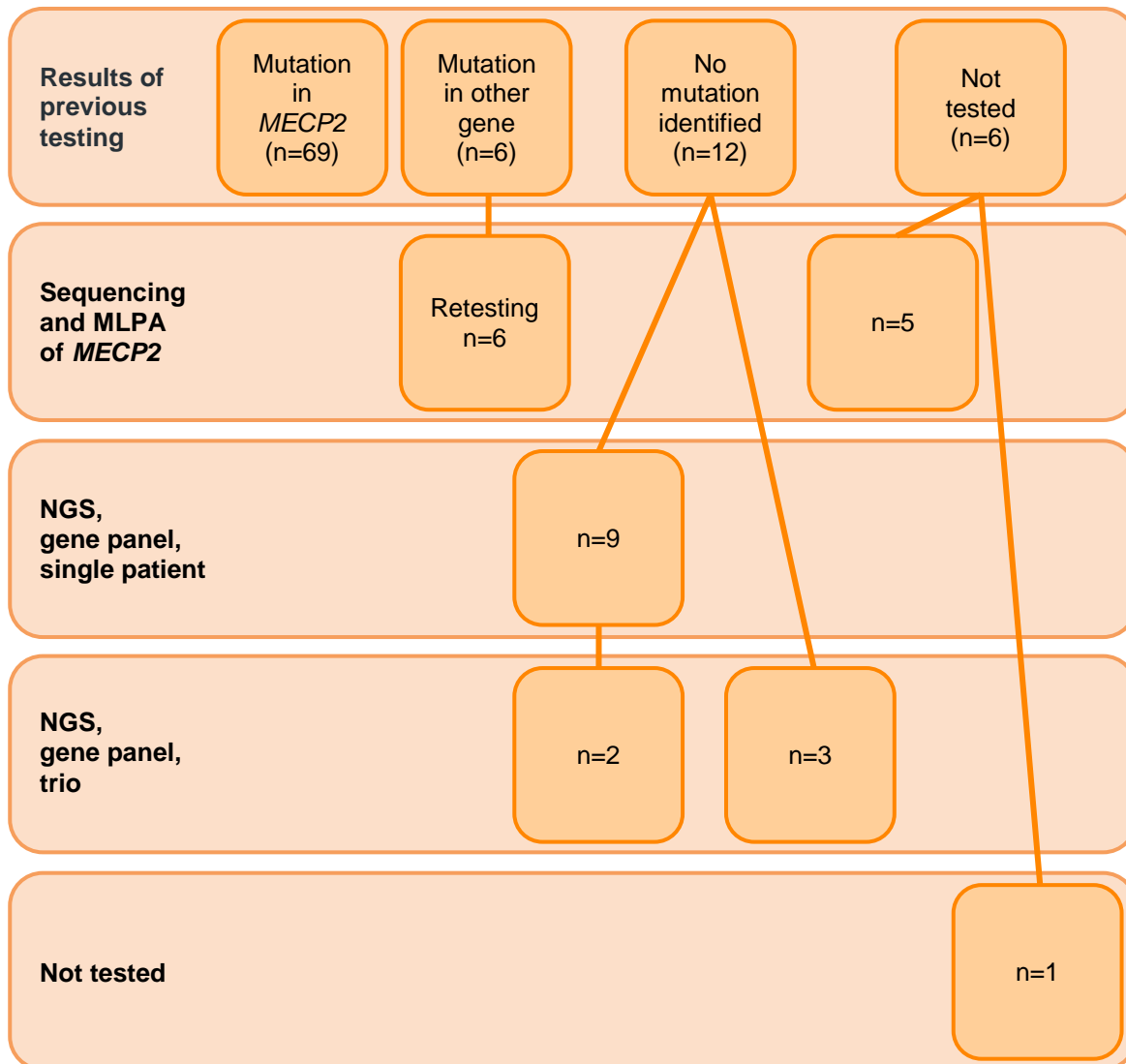
Figure 7. *Flow chart for genetic workup*

4.6 STATISTICS

Statistical analyses were performed using SPSS for windows version 23 in all papers. Significance level was ≤0.05. Missing data was handled by restricting analyses to individuals with complete data on the variables included in the particular analysis.

4.6.1 Paper I

Paper 1 included mean and standard deviations or median and inter quartile range for continuous data, and absolute and relative frequencies for categorical data. Continuous data were compared with independent sample t-test and categorical data with chi square test or fisher exact test if expected cell count was less than five.

4.6.2 Paper II

In paper II, the descriptive analyses included mean and standard deviations or median and inter quartile range for continuous data, and absolute and relative frequencies for categorical data. Independent samples t- test were used to compare groups with continuous variables. Chi Square or Fisher's Exact Test were used for categorical variables. To assess the frequency of seizures, both cross-sectional and retrospective longitudinal data were analysed. A multiple linear regression model was used to explore the relationship between RTT severity and seizure patterns with adjustments for age and mutation groups.

4.6.3 Paper III

In paper III, the descriptive analyses include mean and standard deviations or median and inter quartile range for continuous data, and absolute and relative frequencies for categorical data. Chi square or Fisher's exact test were used to compare groups on categorical measures. One-way ANOVA with post hoc tests were used to compare groups on continuous measures.

4.6.4 Paper IV

Article IV is a case report and no statistics were used in this article.

4.7 ETHICAL ISSUES

Ethics approval was obtained from the Regional Committee for Medical Research Ethics (REK 2012/1572). The design of the study with interviews of parents/other caregivers

made them independent participants in the project. Hence, two letters of information were sent, one directed to the parents/other caregivers and one directed to the individual with RTT, and consent to participate was obtained from both. Since individuals with RTT in general are unable to give informed consent, their consents were given by parents or legal guardians. Consents to participate were obtained prior to inclusion. It was emphasized that participation was voluntary and if they did not want to participate or if they wanted to withdraw their consent during the time of the project it would have no consequences for treatment or follow up. In paper IV "*De novo* mutations in *SCN1A* are associated with classic Rett syndrome: a case report" an additional consent to publish was obtained from parents before publication. In paper I "Genetic and clinical variations in a Norwegian sample diagnosed with Rett syndrome" all parents of the girls presented individually consented and they read the texts about their daughters before publication.

The genetic testing performed in the project was done for diagnostic purposes. According to Norwegian law, genetic counselling prior to such testing is not mandatory (Bioteknologiloven, 2003, §5), and was thus not offered in the project. All results were forwarded in written form to parents/guardians and included in the letter was contact information to the research team in case of questions. In cases where the genetic tests revealed new mutations, the actual female and her parents/other caregivers were offered to be referred to a medical geneticist for counselling.

The information letter contained contact information to all members of the research group and the participants were encouraged to contact us if needed.

5. SUMMARY OF RESULTS

5.1 PAPER I

Henriksen MW, Breck H, Sejersted Y, Diseth T, von Tetzchner S, Paus B, Skjeldal OH. Genetic and clinical variations in a Norwegian sample diagnosed with Rett syndrome. *Manuscript sent to European Journal of Paediatric Neurology June 27, 2019*

In this study we aimed to describe the phenotypic traits of the individuals in the sample according to the 2010 diagnostic criteria, to investigate their genotypes and to compare the phenotypes of individuals with and without *MECP2* mutations. Table 2 shows the mutated genes and their association to the RTT diagnostic subgroups.

Table 2.

		Diagnostic subgroup		
		Classic RTT	**Atypical RTT**	**Non-RTT**
Mutated gene	***MECP2***	69	5	3
	SCN1A	2		
	SYNGAP1		1	
	SMC1A		1	
	CDKL5		1	1
	FOXG1		1	1
	13q deletion			1
	No identified mutation	1	3	1

Significant differences between the individuals with *MECP2* mutations and the ones without were found. Individuals with *MECP2* mutations had higher frequency of loss of hand use and/or loss of language and the RTT characteristic eye gaze. Grossly abnormal development during the first six months and an earlier onset of epilepsy were more frequent in individuals without *MECP2* mutations. Onset of epilepsy before regression was less prevalent in the *MECP2* group. This may reflect that several of the mutated genes in this group are genes previously associated with epileptic encephalopathies.

In summary, these results support recent findings of a more heterogeneous genetic background of RTT than earlier thought, although the differences between the individuals with and without MECP2 mutations indicates that the current diagnostic criteria might include individuals with other disorders in the RTT spectrum.

5.2 PAPER II

Henriksen MW, Breck H, von Tetzchner S, Paus B, Skjeldal OH, Brodtkorb E. Epilepsy in Rett syndrome – course and characteristics in adult age. *Epilepsy research 145(2018)134-139*

The aim of this paper was to describe the diversity of epilepsy in a population of females with RTT, and to address the development of the seizure disorder in adulthood. Only participants with classic RTT, with either a *MECP2*-mutation or no mutation (n=70) were included. The participants were divided into four groups based on age at inclusion (1-10 years, 11-20 years, 21-30 years and >30 years), and the epilepsy features of the participants in the different groups were compared. Active epilepsy (seizures last five years) was present in 60-67 percent in all three age groups above the age of ten. No significant differences in seizure frequency between the groups were found, but weekly seizures or more tended to occur most often in children, with a decrease in adolescents and young adults, and with a slight increase in older adults. The prevalence of tonic-clonic seizure was similar in the three oldest age groups. In the total sample, epilepsy was or had been present in 70 percent, with a median onset age at four years. Unremitting seizures were present in 69 percent of those with active epilepsy whereas 31 percent had experienced remissions lasting six months or more within the last five years. Among the 21 individuals in the oldest group, only three had never had seizures and four had achieved seizure control for more than five years.

In summary, active epilepsy was present in two thirds of adults above the age of 30 years, and both the frequency and the severity of seizures remained high.

5.3 PAPER III

Henriksen MW, Breck H, von Tetzchner S, Paus B, Skjeldal OH. Health issues in adults with Rett syndrome. *Revised manuscript sent to Developmental Neurorehabilitation May 28, 2019.*

The aim of this paper was to describe six of the main health issues in individuals with RTT, and to compare the prevalence of these health issues in different age groups. A special focus was on the participants aged 36 or older. The six health issues described were scoliosis, respiratory irregularities, gastrointestinal dysmobility, growth, ambulation and epilepsy. In addition, the RSSS were assessed. The prevalence of the six health issues and the mean severity scores were compared in three age groups; younger (1-20 years), middle (21-35) years and older (36 years and older). Significant differences in mean severity score between the younger and the middle age group were found. The point prevalence of the six health issues was not significantly different between the age groups. The participants were divided into two groups based on the presumed severity of their *MECP2* mutation. The older age group had a significantly higher proportion of "mild" mutations compared to the two other groups. In addition, everyone in the older group had been able to walk independently at some point in life, compared to only two thirds of the individuals in the two other age groups. Scoliosis affected almost everyone in the two adult age groups, and around half of all adults had been through surgery. Epilepsy, constipation and breath holding affected more than 60 percent of the individuals aged 36 years or older.

In summary, all the six main health issues studied continued to be major concerns in adult age, and the RSSS score did increase from children/adolescents to adults. However, health did not decline with increasing age during adulthood, but this finding might be affected by a healthy survivor bias skewing the results towards better health in adults. All in all, the results indicate a need for regular medical follow up for adults with RTT.

5.4 PAPER IV

Henriksen MW, Ravn K, Paus B, von Tetzchner S, Skjeldal OH. De novo mutations in *SCN1A* are associated with classic Rett syndrome: a case report. *BMC Medical Genetics (2018) 19:184*

The aim of paper IV was to describe the surprising genetic finding of presumed pathological *SCN1A* mutations in two females with classic Rett syndrome. The present females are both adults (19 and 32 years, respectively) and both fulfil the diagnostic criteria of classic RTT. However, they have aggressive epilepsy with earlier seizure onset than expected in RTT. Case 1 presented with her first seizure at five months of age. Her development was normal until 15 months, then it stagnated and subsequently she lost her language and hand function and developed hand stereotypies. Her epilepsy continued to be a major concern in her life with daily seizures, multiple seizure types and several status epilepticus. At age 19, she fulfilled all main criteria of RTT and ten of eleven supportive criteria. Case two had a similar development. Her first seizure occurred at the age of seven months, she lost hand function and language between 12 and 15 months and her epilepsy remained severe. At inclusion she had several bilateral tonic-clonic seizures a week, and fulfilled four main criteria and nine supportive criteria of RTT. Both females had a presumed pathological de novo mutation in *SCN1A.* In addition, the 19 year old had an investigation of mRNA revealing a significantly reduced level of *MECP2* mRNA compared to three healthy controls.

In conclusion, in *MECP2* negative individuals with RTT and early onset epilepsy *SCN1A* should be considered in the molecular routine screening.

6. METHODOLOGICAL CONSIDERATIONS

6.1 STUDY DESIGN AND SAMPLE SIZES

The present study has a cross-sectional design. Cross-sectional studies are relatively fast and easy to conduct, they allow for numerous variables and provide a snap shot of the group studied at a specific point of time. Since all data are collected at once, cross-sectional studies are less prone to drop outs and missing data. There are, however, several limitations with the design: they cannot give information on causality, they only give information on differences between different groups, not development with time and they are susceptible to bias, especially selection bias (Yu et al., 2012).

The sample size of the present project was naturally limited by the number of individuals with RTT in Norway. The relatively low number of participants may affect the results, which have to be confirmed in larger studies.

6.2 SAMPLE REPRESENTABILITY AND EXTERNAL VALIDITY

External validity describes to what extent the results from a study can be generalized to populations outside the study population. One important factor in external validity is whether the sample is exposed to selection bias, meaning that the sample is not properly randomized (Fletcher, 2014). The present project is nationwide and population-based. Population-based projects are less prone to selection bias because they aim to sample from a whole population not from a group that is pre-selected, like in a clinic-based project. But in spite of the population-based design, there are still pitfalls to avoid. Are all individuals with RTT in Norway diagnosed, did we reach all, and who did not respond?

6.2.1 Diagnosis

The health care system in Norway is publicly funded, with free health care for children under the age of 18. The public health care centre in the municipality contacts all families with newborns just a few days after birth and offers 14 regular visits between birth and the child's fifth birthday (https://www.helsedirektoratet.no). If deviant development is suspected, the public health care centre will refer the child to the local paediatric ward. This way the risk for socio-economic differences in who gets diagnosed as a child is small. In adults born before the diagnosis of RTT was internationally known (1983) this may be different. These individuals may have received a diagnosis of unspecified intellectual disability in their youth and were never been re-diagnosed. It is reasonable to assume that the proportion of individuals with unidentified RTT is higher in adults. Whether the clinical characteristics of adults with an RTT diagnosis and adults undiagnosed with RTT differ is not known. However, socio-economic factors and severity of the disease might influence who is diagnosed, since both factors are believed to influence the use of specialist health care service (Halldorsson et al., 2002; Moore et al., 2005). And the diagnosis of RTT is usually made by a specialist (Bisgaard et al., 2015; Tarquinio et al., 2015b). In the present study, the increased use of specialist health care when faced with difficult-to-treat epilepsy may possibly have resulted in a higher proportion of diagnosed individuals with epilepsy than without. Hence, there is a risk of a falsely elevated prevalence of epilepsy in adults.

6.2.2 Recruitment method

Participants in the present study were recruited through Frambu, a Norwegian Resource centre for rare diseases, The Norwegian Rett syndrome Association and some habilitation centres and neurologists.

Frambu is a National Centre for rare disorders. They have a nationwide responsibility for the diagnosis of RTT. The centre is a centre of expertise, which spreads interdisciplinary knowledge to both families and service providers around the family. They do not have individual medical examinations or set diagnoses. Frambu have been

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operating since the 1950's, and the very first meeting for families and professionals in Norway with RTT on the agenda was arranged here in 1987 (https://frambu.no/). Consequently, a large number of individuals of any age with an RTT diagnosis have been to Frambu at some point. All individuals with a diagnosis of RTT in the medical journals of Frambu were invited to the project.

The Norwegian Rett Syndrome Association was founded in the afore-mentioned RTT-meeting at Frambu in 1987, and has since then been an active parent association with members nationwide. Currently they have above 120 members with RTT and a large number of family members (S.R. Larsen, board member, Norwegian Rett Syndrome Association, personal communication, September 10, 2017). All members were invited to participate in this project.

In the start of the recruitment period we planned to contact all habilitation centres in Norway to ask them if they could inform and invite all their patients with RTT to the present project. Due to practical issues this was not feasible. We did however cooperate with five centres, three of which included both children and adults and two with only children.

Information about both Frambu and the parent association is given to all families with a child newly diagnosed with RTT; there is no reason to believe there is any selection bias there. However, language problems may have led to fewer members with other cultural backgrounds. The unfinished recruitment from habilitation centres might have given a skewed bias towards more children and more participants from the parts of the country where we had collaborating habilitation centres.

6.2.3 Non-responder-bias

A limitation in the present study is that due to confidentiality the lists of invited individuals from our collaborators were not revealed to the study group. Hence, we don't know exactly how many were invited to the study, and cannot estimate the exact response rate. Current prevalence data on RTT in Norway does not exist, but 165

individuals with a diagnosis of RTT were reported to the Norwegian Patient Register from the Specialist Health Services from 2008 to 2012. Though this number is not accurate, it gives an idea of the prevalence. With 92 participating families, we estimate that around 55 percent of the available individuals were included. We are unable to say how many of the remaining individuals are non-responders and how many did not get the invitation. Figure 8 compares the number of individuals in the study and in the patient register both in age groups and geographical distribution.

As illustrated in Figure 8a), the proportion of individuals included in the study is higher in children than in adults. One possible reason is that some of the adults registered between 2008 and 2012 may be deceased. Another reason is that some of the included children were not born, or not diagnosed in 2012, giving a falsely high inclusion rate in the youngest age group. Other than these methodological differences, a selection bias
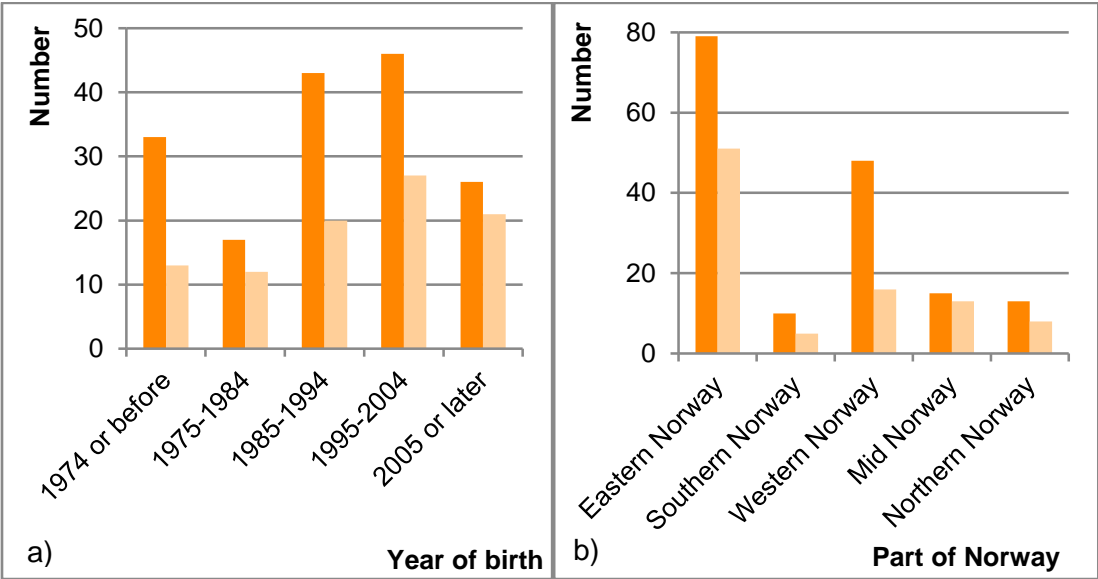
Figure 8. *Number of individuals registered in the Norwegian patient register (dark) and individuals participating in the present project (light) categorized by a) year of birth and b) by residence (Mari Wold Henriksen)*

towards more children is possible; more adults than children live without their parents, and other care givers are probably less prone to participate in this kind of survey. Another possible bias is that families with children with a severe phenotype may be so exhausted they cannot bear to participate in a study. With respect to the geographical distribution (Figure 8b), the proportion of included individuals is lower in the western part of Norway. This is because this particular population has participated in several studies through the last decades and many families did not want to participate in another one. The last non-responder bias discussed is due to language issues. Information about the study was only given in Norwegian, which may have resulted in fewer participants with another native language.

6.2.4 Samples in the different articles

In paper I all participants, except two with missing data, were included, regardless of mutations and whether they met the diagnostic criteria of RTT or not. In paper II and III, however, mutation in another gene than *MECP2* was an exclusion criterion, but individuals without an identified pathological mutation were included. Classic RTT was an inclusion criterion in paper II and classic or atypical RTT in paper III. Paper IV was a case report and included all participants with a mutation in *SCN1A*.

In paper II we chose to only include individuals with classic RTT since the features of epilepsy is different in classic and in the different types of atypical RTT (Pintaudi et al., 2010).Due to the relatively small sample a division into the different groups of atypical RTT was not possible. The two individuals with a classic RTT phenotype and mutations in SCN1A were excluded because SCN1A is a gene associated with epileptic encephalopathy and it is reasonable to assume that these mutations affect the epileptic phenotype of the two girls.

The aim in paper III was to describe several main health issues in adults with RTT, and since these issues generally do not differ that much between the different subgroups of

RTT, we chose to include all individuals fulfilling the diagnostic criteria for classic and atypical RTT. The individuals with mutations in other genes than *MECP2* were excluded due to the possible effect of the mutation on the phenotype.

6.3 ASSESSMENTS, RELIABILITY AND INTERNAL VALIDITY

"Internal validity is the degree to which the results of a study are correct for the sample being studied" (Fletcher et al., 2014, p.11). Internal validity is threatened by different forms of bias. Traditionally bias is categorized into three main forms: selection bias, information bias and confounders (Thelle et al., 2015).

6.3.1 Selection bias

Selection bias was presented in the previous section where the sample representability was discussed, but it is also an important factor of the internal validity. In both paper II and III we have compared the prevalence and characteristics of several health issues in different age groups. A form of selection bias in a design such as this is the healthy survivor bias. Longevity in RTT is associated with severity of the syndrome (Tarquinio et al., 2015a), meaning that those who have survived into adult age probably have had a less severe status from childhood. This bias might have skewed the results towards better health in the older groups.

6.3.2 Information bias

Information bias includes several important sources of bias relevant for the present project: self-reporting bias, misclassifications and confirmation bias.

All research based on self-reporting (questionnaires, surveys and interviews) are at risk of unreliable answers caused by social desirability, reduced memory (recall bias) or other factors (Althubaiti, 2016). In the present project, recall bias is relevant. The

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parents/care givers interviewed in this project were asked historical questions about their child's development and clinical history. Recalling this data may be more difficult for parents with adult children. In addition, more than 80 percent of children lived in parental homes, while less than 20 percent of adults did. Parents may be more updated on their child's health when they live together than after they move out. We have tried to limit the recall bias both by comparing the data from the interviews with medical records and by asking the parents to prepare themselves before the interviews by looking into old diaries and photo albums. Since all participants have been through similar experiences our results will probably not be skewed as much due to recall bias as in case control studies where sick participants are compared to healthy controls.

Misclassification of variables can potentially skew the results of a study significantly, and it is especially serious if it differs between study groups. In the present study, the biggest risk for misclassification is in the prevalence of epilepsy. It is difficult to clinically distinguish between epileptic and non-epileptic seizures in RTT (Glaze et al., 1998), and EEG has not been a part of this study. However, care was taken not to interpret typical episodic RTT behaviour as epileptic seizures. The main aim of Paper I was to compare the prevalence in different age groups, and if we had misclassified seizures, it would be the same in all age groups, implying that the main results are probably not particularly affected by this.

The confirmation/observer bias is the, often unintended, tendency of the researcher to favour information that confirms his/her pre-existing beliefs (Althubaiti, 2016). It can happen both during data collection and interpretation. In the present study, most interviews and interpretations were done by the same researcher, which increases the risk for observer bias, but also increases the reliability. However, the interviews concerning medical issues were structured, which decreases the risk for observer bias. All diagnoses were reassessed according to current diagnostic criteria. If there was any doubt about the diagnosis (of an individual), it was reassessed by two physicians.

6.3.3 Confounders

The last main group of bias is confounders. Confounders are variables other than the ones studied which affect the dependent variable (Thelle et al., 2015). Since the main aims in this thesis were to describe different variables in the sample, and not to conclude on causal relationships, the risk for confounders affecting the results is lower. There are a few exceptions, where associations between different variables have been found. In these cases, statistical methods of stratification or regression analyses where used to adjust for potential confounders.

6.3.4 Assessments

The severity of the syndrome was assessed by the RSSS (Kaufmann et al., 2012). Except for this scale the assessments of variables were not collected by standardized instruments, but by an unstandardized questionnaire and a semi-structured interview (Appendix I and II). The strength of unstandardized data collection is the open structure allowing a broad approach and reflection around interesting topics. A limitation is however that comparison to previous studies becomes less reliable.

Due to factors beyond the control of the research group, the data collection could not follow the planned structure at all times. This might have influenced the reliability of the results. However, since the interviews were open-structured, we tried to ensure that all important variables for studies presented in this thesis were answered.

This thesis aims to describe health issues in adults with RTT. We do, however, acknowledge that our data does not cover all important factors of health. Other medical issues, as well as additional features of a healthy life, such as wellbeing, communication and social life, could have been examined

7. GENERAL DISCUSSION

The overall aim of this thesis was to describe the genotypical and phenotypical variation in the Norwegian Rett Syndrome population, and the development of clinical features in different phases of life. To achieve this, we invited individuals with a diagnosis of RTT in Norway, independent of age and geography. Of the 92 individuals included, 73 had classic RTT, 12 had atypical RTT and seven did not fulfil the 2010 diagnostic criteria. In line with existing literature (Neul et al., 2010), 96 percent of all individuals with classic RTT had mutations in *MECP2*, and as expected the proportion with *MECP2* mutations was significantly lower in atypical RTT. In addition, nine individuals had mutations in other genes and no mutation was identified in five individuals (Table 2). When we compared clinical characteristics in individuals with and without *MECP2* mutations several significant differences were observed. When examining the clinical characteristics and their occurrence in different phases of life our results showed that the main medical issues in childhood remains a concern in adulthood, including epilepsy, which has been thought to improve or even diminish in adult age.

7.1 GENETIC AND CLINICAL VARIATIONS IN RETT SYNDROME

7.1.1 Differences between individuals with and without mutations in *MECP2*

Compared to individuals with mutation in *MECP2* individuals without a mutation in *MECP2* have significantly more abnormal early development, less loss of hand use and language, less presence of «eye pointing» and earlier onset of epilepsy. The differences in onset of epilepsy were also significant when analysed within the diagnostic subgroups of classic and atypical RTT.

The results from the present study were in line with the few articles addressing this issue. Temudo et al. (2011) compared individuals with and without a mutation in *MECP2* in a cohort of 87 individuals with RTT. They found that individuals without a *MECP2* mutation seldom had normal development in the first year of life, they had more growth failure and less eye pointing. Stagnation of development occurred earlier than in the

group with *MECP2* mutations and purposeful hand use and language were seldom acquired. Charman et al. (2005) described 240 individuals with RTT and found significantly earlier onset of regression, earlier onset of first seizure and higher prevalence of an event or illness that may have caused neurological deficit in the group without *MECP2* mutations. Other studies have described differences between individuals with and without *MECP2* mutations in specific health issues. One article on epilepsy found that none of the six individuals with onset of epilepsy during the first year of life had *MECP2* mutations while 87 percent of those with later onset of epilepsy had the mutation (Nissenkorn et al., 2010). Likewise, the absence of a *MECP2* mutation was associated with early onset of epilepsy in an Australian article (Jian et al., 2006). However, no differences in seizure rate were detected (Jian et al., 2007). Motil et al. described no significant differences in most gastrointestinal and nutritional problems, except increased feeding difficulties and less short stature in those without a *MECP2* mutation (Motil et al., 2012).

An important difference in methodology between these articles is the diagnostic criteria used. Most articles addressing this theme are older than the latest diagnostic criteria (Neul et al., 2010). Consequently, many of the individuals included in these other studies had not had experienced regression, as opposed to the present study. In the study of Temudo et al., only 25 percent of the individuals without *MECP2* mutations had experienced regression, and in Charman et al.'s study 21 of the 240 participants had not shown regression. Hence, the inclusion criteria differ in the articles and consequently the samples cannot be compared directly.

In addition, the methods for genetic testing have evolved. MLPA for detecting large deletions was first described in a RTT context in 2003 (Erlandson et al., 2003) and mutations in exon 1 of *MECP2* as a possible cause for RTT were not revealed until 2004 (Mnatzakanian et al., 2004). In addition, NGS is more sensitive than Sanger sequencing (Behjati et al., 2013). These advances indicate that the number of undiscovered *MECP2* mutations in the groups without an identified mutation might be higher in the older studies than in the present.

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In spite of these methodological differences, the results from the present study and the current literature on the field do complement each other. The findings can largely be divided into two main categories: abnormal early development and early onset of epilepsy.

The apparently normal early development in RTT has been considered a central feature of RTT since Andreas Rett first described the syndrome in 1966 (Rett, 1966).Although the evidence for a subtle abnormal development from birth is growing (Cosentino et al., 2019), the findings from both the present study and in the literature of less abnormal early development in individuals with *MECP2* mutations, indicate that the absence of a functioning MeCP2-protein has less consequences in the very first months of life than later. The same pattern of development is seen in MeCP2 mutant mice (Kerr et al., 2008). A possible contributing mechanism to the delayed onset of severe symptoms in RTT is MeCP2's binding of methylated cytosines in the CH context. MeCp2 binds both cytosines followed by guanine (called CG methylation) and cytosines followed by other bases than guanine (called non-CG methylation or CH methylation). Methylation of CH emerges when neurons mature, which in mice is parallel in time to when the symptoms of RTT develops (Lombardi et al., 2015). Even with increasing knowledge on the pathophysiology of RTT, the number of questions on how the absence of a functioning MeCP2-protein results in such a devastating syndrome is high.

Onset of epilepsy during the first year of life is extremely rare in RTT with *MECP2* mutations. The first seizure does not usually appear before 3-5 years of age, after the period of developmental stagnation and regression (Jian et al., 2006). This indicates that epilepsy is not a part of the pathophysiology behind the regression period (Olson et al., 2015). In RTT without *MECP2* mutations, however, the presence of early onset epilepsy is much more prevalent, which also reflects in the number of genes known from developmental and epileptic encephalopathies now associated with RTT (Schonewolf-Greulich et al., 2017a). In many individuals with RTT without *MECP2* mutations, onset of epilepsy occurs before developmental regression, which raises the question of whether the epilepsy is a contributing cause of regression in these individuals, like it is believed to be in epileptic encephalopathies (Scheffer et al., 2017).

7.1.2 Other genes in RTT

In the present sample, nine individuals, six with RTT and three not fulfilling the criteria for RTT, had mutations in a total of six other genes than *MECP2* (Table 2). Four individuals with RTT had mutations in SCN1A, SMC1A or SYNGAP1, all of which have formerly been associated with epileptic encephalopathies (Huisman et al., 2017; Vlaskamp et al., 2019; Zuberi et al., 2011). Four individuals had a mutation in *FOXG1* or *CDKL5*, which are genes known for years to be associated with RTT (Ariani et al., 2008; Tao et al., 2004). In both FOXG1 and CDKL5 one of the individuals fulfilled the diagnostic criteria of RTT and one did not. Finally, one individual without RTT had a large deletion in chromosome 13q.

As described in detail in paragraph 1.3.4 in the "Introduction and background"-chapter mutations in more than a hundred different genes have been described in individuals with RTT or a RTT-like disorder. Almost half of these were identified as the sole pathological mutation in an individual with a classic or atypical RTT phenotype (Ehrhart et al., 2018; Iwama et al., 2019; Nakamura et al., 2018; Percy et al., 2018; Pescucci et al., 2003; Schonewolf-Greulich et al., 2017a; Shimada et al., 2018; Srivastava et al., 2018; Williamson et al., 2015; Yoo et al., 2017). The interpretation of these findings varies between the different authors, and Zaghlula and colleagues emphasized in 2018 the important point that finding a pathological mutation in an individual with RTT-phenotype does not automatically indicate a causal relationship (Zaghlula et al., 2018).

Many of the genes now associated with RTT are genes known to be associated with other syndromes or with epilepsy (Schonewolf-Greulich et al., 2017a).Sometimes the phenotypes of the individuals tested border between RTT and another syndrome. There are several syndromes with intellectual disability, absent speech and seizures which have been shown to have overlapping phenotypes that may be difficult to distinguish (Vrecar et al., 2017). In the present study the two individuals with a classic RTT phenotype and mutations in SCN1A have clinical characteristics associated with both RTT and Dravet syndrome. They had early onset of epilepsy, first seizure was a prolonged febrile seizure, regression developed after onset of epilepsy and they still have drug-resistant aggressive epilepsy with daily to weekly seizures of multiple

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semiology, all features of Dravet syndrome. At the same time, they fulfil all main and eight and ten, respectively, supportive criteria in the 2010 diagnostic criteria for RTT (Neul et al., 2010).

In parallel with the occurrence of these "new RTT-genes", it has been suggested that individuals with mutations in *CDKL5* and *FOXG1* should no longer be diagnosed with atypical RTT, but with *CDKL5* disorder and *FOXG1* syndrome (Fehr et al., 2013; Kortum et al., 2011). This has in part been implemented, but it is still variable whether these new entities are used both in clinical practice and in scientific publications. Among the arguments for defining *FOXG1* syndrome as a separate entity and no longer as part of RTT, are presence of true dyskinesias, brain imaging abnormalities, lack of regression and lack of respiratory irregularities. In addition, individuals with mutations in *FOXG1* often give poor eye contact, contrary to what is known from RTT (Kortum et al., 2011). The two individuals in article I with mutations in *FOXG1* were both suspected to be blind the first year of life and none of them had respiratory irregularities. Otherwise they had many typical RTT features, but only one of them had been through a regression period. Hence, one fulfilled the diagnostic criteria for atypical RTT, the other did not. The same was the case for the two individuals with *CDKL5* mutations; one had atypical RTT and one did not fulfil the diagnostic criteria, and the difference was the regression period. The lack of regression in many individuals with *CDKL5* mutations is one of the main arguments for separating between the early seizure variant of RTT and a *CDKL5* disorder. Regression was present in less than a third of a cohort of 77 individuals with *CDKL5* mutations. In addition, abnormal early development and early onset of epilepsy were almost universal, while hand stereotypies, respiratory irregularities and scoliosis were less prevalent than in individuals with RTT (Fehr et al., 2013).

7.1.3 Clinical implications

The results from the present study with significant differences between individuals with and without a mutation in *MECP2,* indicate that the current diagnostic criteria may include individuals with a different disorder under the RTT umbrella. Critics of the

current clinical diagnostic criteria advocate mutations in *MECP2* to be the primary focus of the definition of RTT. They argue that both clinical trials and basic research will benefit from such an approach (Srivastava et al., 2018). However, to leave the old clinical diagnosis in favour of pure genetic diagnoses will leave a not insignificant number of individuals undiagnosed. In the present sample, five individuals (5.4 percent) are genetically unexplained in spite of thorough genomic examination. Living without a specific formal diagnosis has, unfortunately, been reported to make it more difficult to access care and therapies (Moeschler et al., 2014), and mutation-negative individuals will also lose important factors like condition-specific support groups. In addition, a diagnosis based on genetics alone will in many cases have a wide phenotypic spectrum, in *MECP2* from mild ID to severe RTT. Thus we may lose some of the benefits that having a more homogenous group give for habilitation, clinical research and solidarity between families affected. Even if a molecular diagnosis may not be the answer, the results from the present study with significant differences in clinical characteristic between individuals with RTT with and without mutations in *MECP2* indicate that the current diagnostic criteria should be revised to be more accurate. And individuals without an identified pathological mutation in *MECP2* should go through further genomic investigations. In the present study, six individuals with classic or atypical RTT had mutations in other genes, and all but one had early onset epilepsy. To find the right etiological diagnosis can in some cases be important for treatment of epilepsy.

7.2 HEALTH ISSUES IN ADULTS WITH RETT SYNDROME

7.2.1 Epilepsy

The main findings regarding epilepsy in this thesis are summarized in Table 3. A high prevalence of active epilepsy persists in older age, and also a high seizure frequency, as well as the presence of bilateral tonic-clonic seizures.

***Table 3** Prevalence of active epilepsy, seizure frequency and seizure semiology within the last year*

	N	**Active epilepsy** n (percent of n total)	**>Weekly seizures** n (percent of n active epilepsy)	**Bilateral tonic-clonic seizures** n (percent of n active epilepsy)
1-10 years	17	5 (29)	3 (60)	1 (20)
11-20 years	18	12 (67)	3 (25)	6 (50)
21-30 years	16	10 (63)	4 (40)	6 (60)
>30 years	21	14 (67)	7 (50)	9 (64)
Total	72	41 (57)	17 (41)	22 (54)

There has been a common notion that epilepsy in RTT is less prevalent and less severe in adult age. This was described way back in 1992 by Witt-Engestrom, and later by Steffenburg in 2001 (Steffenburg et al., 2001; Witt Engerstrom, 1992). In the latter article, Steffenburg concludes *"On the whole, epilepsy tended to quieten down after 20 y of age"*. Since then several other articles have more or less supported this statement (Bao et al., 2013; Cass et al., 2003; Glaze et al., 2010; Halbach et al., 2013; Vignoli et al., 2012). In a comprehensive article on epilepsy from the North-American National History Study, the prevalence of seizures was reported to peak in late adolescence and decrease thereafter, although fluctuations in seizure severity continued throughout adulthood (Tarquinio et al., 2017). Only two former articles have clearly stated that epilepsy is a major concern in adulthood; Pintaudi et al. did not find differences in drug resistant epilepsy between adults and children, and Anderson et al. described that the majority of adults had active epilepsy (Anderson et al., 2014; Pintaudi et al., 2010)

The cited articles have several methodological differences that may explain some of the divergent results. First; in article II of the present thesis the sample consists of classic RTT. In most of the others, except Tarquinio et al., the samples contain both classic and atypical RTT, and the results are neither adjusted for subgroups, nor are the proportions of classic RTT versus atypical RTT addressed in the different age groups. The severity and frequency of seizures have been reported to differ in relation to subgroups (Pintaudi et al., 2010; Tarquinio et al., 2017), implying that subgroups might be a confounder when compared to the present sample consisting of classic RTT only.

Another important difference between the cited studies is the organization in age groups. In most articles all adults or even all adolescents and adults are clumped together in one large group (Anderson et al., 2014; Bao et al., 2013; Cass et al., 2003; Pintaudi et al., 2010; Steffenburg et al., 2001; Vignoli et al., 2012). As a consequence, changes occurring in adulthood might not be recognized. In the present study we demonstrated a trend towards an increasing seizure frequency in adults older than 30 years. This finding would not have been identified without a differentiation between adults of different ages.

Moreover, the definition of active epilepsy varies significantly between the articles. The period without seizures necessary for being categorized as seizure free spans from 6 months in the article from Tarquinio et al. to five years in the present study (Tarquinio et al., 2017). In their article Tarquinio and colleagues described a pattern of remissions and relapses of seizures in RTT; almost half of their participants had experienced periods of six months or more with a total remission before the seizures relapsed. Accordingly, differences regarding the duration of the observation periods and the definition of seizure freedom considerably influence the reported seizure patterns in various studies.

To summarize; according to paper II, epilepsy remains a major concern in adults with RTT, contrary to several other studies concluding with less active epilepsy in adulthood. However, direct comparisons are not possible due to methodological differences. The results regarding epilepsy in article II concern classic RTT, and cannot be generalized to atypical RTT. The categorization of adults into older (>30 years) and younger (20-30 years) adults, provides us with important information on changes in the course of epilepsy in adulthood, which is an area that previously has been insufficiently explored.

7.2.2 Other health issues

When analysing the prevalence of other main medical issues, such as scoliosis, ambulation, growth, respiration and gastrointestinal dysmobility in RTT in different age groups, the main findings were not significantly different in children and adolescents (1-

20 years), young adults (21-35 years) and older adults (>35 years). As in epilepsy, these other issues continue to be major concerns in adulthood, but they seem to stabilize and do not deteriorate with further advancing age. However, there was a significant increase in mean RSSS scores from the younger to the middle age group, but the increase did not continue into older age. One third of the women aged 36 or older still walked independently. Nevertheless, half of the women at that age had experienced a decline in walking skills, which most often occurred during adolescence, not in adult age.

These results are in line with other studies reporting stability, or even improvement, in the general health of adults with RTT (Anderson et al., 2014; Halbach et al., 2013; Smeets et al., 2009; Vignoli et al., 2012). Nevertheless, three studies have found a worsening of general severity with increasing age (Colvin et al., 2003; Cuddapah et al., 2014; D. Young et al., 2011). When looking beyond the general aspects and into the details, we find different results in different areas; autonomic disturbances and gastrointestinal issues were found to improve in adult age (Cass et al., 2003; Halbach et al., 2013; Motil et al., 2012; Tarquinio et al., 2018) while musculoskeletal disorders often deteriorate and growth retardation are more prevalent (Cass et al., 2003; Halbach et al., 2013; Motil et al., 2012; Vignoli et al., 2012). Stage IV was defined as the stage of the disease where previously mobile girls with RTT gradually lost walking abilities due to increased spasticity and severe scoliosis (Hagberg et al., 1986). The staging system, and especially stage IV, has later been questioned. Recent research has shown that despite a slight deterioration in gross motor skills with increasing age, a large proportion of adults with RTT remains ambulant (Foley et al., 2011; Halbach et al., 2013; Schonewolf-Greulich et al., 2017b), and the risk for declining ambulation skills is higher in adolescence than in adulthood (Foley et al., 2011; Vignoli et al., 2012). Improvements have even been reported in ambulation skills in adult age (Halbach et al., 2013; Jacobsen et al., 2001).

Differences in methodology need to be addressed when comparing these results. Most important are the different designs of the studies. The majority of articles, including the present one, are cross-sectional, while a few are longitudinal. Cross sectional studies have increased risk of survival bias, especially when different age groups are compared,

such as those cited in this text. In the present project, the proportion of individuals with mutations presumed to give a mild phenotype was significantly higher in the older group compared to the middle and younger age groups. In addition, all individuals in the older group had been ambulant at some point in life in contrast to the two younger groups, where only around two-thirds had been able to walk. Both ambulation and the severity of mutations are associated with survival (Tarquinio et al., 2015a), indicating a healthy survivor bias in the present project. Of the three longitudinal studies addressing general health in adults with RTT, two report an increase in general severity with increasing age (Cuddapah et al., 2014; D. Young et al., 2011).

There are also differences in the variables analysed, in the distribution of age and in the categorization of age groups. Moreover, several different forms of severity scales have been used in the various cited articles. These scales consist of several parameters, some measure developmental traits, in which the scores are the same throughout life , and others measure the current state of clinical characteristics, like epilepsy or sleep (Colvin et al., 2003; Young et al., 2011). Some severity scales consist mainly of the first kind of parameters, others mainly of the latter kind, indicating that direct comparisons will give unreliable results. Only a few of the articles addressing health in adult age differentiate between different age groups within adulthood (Halbach et al., 2013; Vignoli et al., 2012; Young et al., 2011). The other articles comprise all adults, which mean that comparisons give important answers only regarding differences between children and the wide group of adults (Anderson et al., 2014; Cass et al., 2003; Colvin et al., 2003; Cuddapah et al., 2014). Since the latest survival data in RTT shows more than 70 percent survival at 45 years (Tarquinio et al., 2015a), knowledge on health changes within the adult lifespan is increasingly important.

In summary, the findings of the present project of an increase in mean severity scores from children to adults, and stabilization in older adulthood do not differ significantly from the existing literature in the field, although direct comparisons are difficult due to methodological differences. Main health problems continue to be prevalent in adult age, while walking abilities stabilized. However, the present project does contribute with knowledge on how health parameters differ between younger and older adults. This

field has not previously been explored sufficiently. It is possible that the presence of a healthy survivor bias underestimates the severity of health in the oldest participants, a suspicion strengthened by the reported increased severity with increasing age in longitudinal studies.

7.2.3 Clinical implications

Children are usually considered a vulnerable group, both in health care and in society in general and for good reasons, since they cannot take care of themselves and are dependent on others. In most countries strategies are implemented to ensure that all children are taken well care of. However, personally I believe that in groups of individuals with such a high "dependency level" as individuals with RTT, adults are even more vulnerable than children. Most children are taken care of by affectionate parents who speak up for them and advocate their rights. In adult age, at some point, the parents are no longer around, or do not have the capacity to look after them as they did before. In parallel a transition from child-centered multiprofessional health care to the more fragmented adult-oriented specialist health care takes place, which is often less comprehensive in terms of the total handicap burden.

An Australian article reports a decrease in health service use with increasing age in individuals with RTT, in spite of a deteriorating health (Young et al., 2011). The challenges of transition of individuals with intellectual disabilities from paediatric to adult services have been described in several articles (Gauthier-Boudreault et al., 2017; Innes et al., 2012).

The results from the present study with high prevalence of epilepsy with frequent and severe seizures and other general health issues in adults with RTT emphasize the importance of a safe and well-planned transition into adult-oriented health care and a continued specialist health care service for adults with RTT. Seizures in RTT have been proven to have a negative impact on quality of life (Bahi-Buisson et al., 2008), hence, optimal treatment of the seizure disorder is important. Any individual with difficult-to-treat epilepsy should be treated at a high competence level disregarding age and

intellectual performance. Neurologists must be aware of the particular challenges in the management of subjects with RTT, particularly the many other episodic symptoms which may be mistaken for seizures, as well as the characteristic features and the course of the epilepsy which is often difficult to treat.

8. Future Perspectives

The era of next generation sequencing changes the genetic landscape as we know it. In several clinical syndromes believed to be caused by mutations in a specific gene, associations with mutations in a high number of other genes are now published. And vice versa; mutations in genes formerly believed to be associated with specific phenotypes are now found in individuals with totally different phenotypes (Steel et al., 2017; Watson et al., 2001). These findings from clinical studies, including the present, can be hypothesis generating for basic research on gene circuits and may give us knowledge on disease modifying genes, which may explain some of this phenotypic variation. More knowledge on pathophysiological mechanisms can potentially reveal new targets for treatment. To measure the effect of potentially new therapies, accurate diagnostic criteria are important. The results from the present study of significant differences between individuals with and without mutations in MECP2 indicate that the current diagnostic criteria might not be accurate enough, and there is a need for further revisions. These results do however need to be confirmed in larger populations.

With the increased longevity in RTT, we have a growing population of adults with RTT. The present study show that most of the main health issues in childhood remain a major concern in adulthood, but there was no evidence of increased severity with increasing age. However, like in many of the other studies with focus on health in adults with RTT, the present study was cross-sectional, which increases the risk for a healthy survivor bias skewing the results. Longitudinal studies in large populations with focus on older adults and aging are needed. More knowledge on health in this part of the RTT population is crucial for proper care and treatment, and it is important for planning of future structures in health care services to take care of this growing group. This brings us to another important factor, not included in the present study but associated: the use of health care service in adult age. An Australian study showed that visits to a medical specialist were most frequent in children (Moore et al., 2005), and another showed less use of health care services in adult age, in spite of an increased clinical severity (Young et al., 2011). In people with such an extensive care dependency as individuals with RTT, and other similar conditions, there is no reason to think that adults need less help,

support and treatment than children. To my knowledge, the only studies addressing this subject in RTT are the two Australian ones (Moore et al., 2005; Young et al., 2011). The results from these studies cannot be directly transferred to another country, since how the health care is organized differs significantly between countries. Hence, thorough examination of health care service use in individuals with RTT and similar disorders should be carried out in more countries, to ensure an equal service regardless of age.

Finally, the big question in the future is whether scientific progress can provide us with a treatment for RTT that can cure the disorder or improve the symptoms considerably. In mouse models, restoration of the mutated *MECP2* reversed a large number of the mice's symptoms (Guy et al., 2007). This has given a great motivation for the search for a cure.

9. CONCLUSION

Although RTT is mainly caused by a mutation in MECP2, a not insignificant number of individuals with an RTT phenotype have mutations in other genes as well, or they have no identified pathological mutations at all. There are, however, several significant differences between individuals with a MECP2 mutation and those without a MECP2 mutation, both in RTT in total and within the diagnostic subgroups of classic and atypical RTT.

Epilepsy continued to be a major concern into adult life, with a high prevalence of active seizures, more frequent seizures than in adolescence and high prevalence of bilateral tonic-clonic seizures. The mean severity, assessed by the RSSS, increased from children/adolescents to young adults, but then it stabilized in adulthood. In general the main health issues addressed by this thesis showed stability in prevalence, regardless of age.

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APPENDIXES

Medical survey

Personals
ID-number: _____   Examination date:_____
Present at the examination:_____

Heredity
Neurological illness in the family   Yes☐ No☐ Don't know☐
                     Relationship:_____
                     If yes, what kind_____
Rett Syndrome   Yes☐ No☐ Don't know☐
                     Relationship:_____
Epilepsy   Yes☐ No☐ Don't know☐
                     Relationship:_____
Autism   Yes☐ No☐ Don't know☐
                     Relationship:_____
Other PDDs   Yes☐ No☐ Don't know☐
                     Relationship:_____
                     If yes, what kind_____
Are parents blood relatives: Yes☐   No☐   Don't know☐
                     If yes, relation:_____

Pedigree:

Pregnancy and birth
Has the mother had any miscarriages: Yes☐ No☐ Don't know☐
                     If yes, how many:_____
                     In which week of pregnancy:_____
Was mother well during pregnancy: Yes☐ No☐ Don't know☐
Complications during pregnancy or birth: Yes☐ No☐   Don't know☐
                     If yes, what kind of complications:_____
_____
_____
_____

Birthweight   _____g
Length   _____cm
Head circumference   _____cm
Gestational age   _____w

Apgar score: 1min_____   5min_____

Growth parameters

Head growth
- ☐None to minimal deceleration
- ☐Deleceleration of head growth but >10th percentile after 24 months
- ☐2nd-10th percentile after 24 months
- ☐2nd-10th percentile before 24 months
- ☐<2nd percentile by 24 months
- ☐<2nd percentile by 12 months

3m:_____cm 6m:_____cm 9m:_____cm 12m:_____cm 24m:_____cm

Somatic growth
- ☐Normal growth at 24 months
- ☐25th-50th percentile at 24 months
- ☐5th-25th percentile at 24 months
- ☐<5th percentile at 24 months

3m:_____cm 6m:_____cm 9m:_____cm 12m:_____cm 24m:_____cm

Menarche: Yes☐ No☐ Don't know☐ if yes: age_____(year)
Menopause: Yes☐ No☐ Don't know☐ if yes: age_____(year)
Oral contraception: Yes☐ No☐ Not anymore☐   Don't know☐
             If yes: what kind_____
             Age of onset:_____ (year) Age when ended:_____(year)

Onset of symptoms

At what age did the parents start to worry about her development?
_____(month/year)
What did they react to?
_____
_____
_____

When did the health service react to their worries (public health clinic/family
doctor/pediatrician)?_____(month/year)

First diagnose_____Diagnosed by:_____

Age at diagnosis of Rett syndrome_____(month/year)Diagnosed by:_____

       Based on: Clinical findings☐   EEG☐   Genetic testing☐ Don't know☐

Regression: Age of onset

☐No regression

☐>10 years

☐>5 years

☐>30 months

☐18-30 months

☐12-18 months

☐6-18 months

☐<6 months

Which skills disappeared?_____

Feeding and digestion

Nutritional challenges?   Yes☐   No☐   Not anymore☐   Don't know☐

     If yes, age:_____(year/month)

     What kind?_____

Abdominal pain:	Yes☐	No☐	Not anymore☐	Don't know☐
Obstipation:	Yes☐	No☐	Not anymore☐	Don't know☐
Diarrhia:	Yes☐	No☐	Not anymore☐	Don't know☐
Gastroesophageal reflux:	Yes☐	No☐	Not anymore☐	Don't know☐
Vomit or regurgitation:	Yes☐	No☐	Not anymore☐	Don't know☐
Gallbladder disease:	Yes☐	No☐	Not anymore☐	Don't know☐
Pancreatitis:	Yes☐	No☐	Not anymore☐	Don't know☐
Gastritis/ulcus	Yes☐	No☐	Not anymore☐	Don't know☐

Investigations and/or treatment:_____

_____

_____

_____

_____

Feeding difficulties: Yes☐   No☐   Not anymore☐   Don't know☐

    If yes, describe:_____

_____

Gastrostomy button: Yes☐ No☐ Not anymore☐ Don't know☐
    If yes, age:_____(year/month)
    If no, has it been considered?_____
    If removed, why and when:_____

As caregivers, what are your experiences with PEG?
    Insertion:_____
    Use:_____

Epilepsy/seizures

Epilepsy: Yes☐ No☐ Not anymore☐ Don't know☐
    If yes, age of onset: _____(year/month)
    If recovered, last seizure: _____(year/month)

Infantile spasms: Yes☐ No☐ Don't know☐

Seizure presentation:

	GTK	KPA	EPA	Other(describe)	Don't Know
0-6 months					
7-24months					
2-5 years					
6-12 years					
13-17 years					
18-24 years					
25-35 years					
Elderly					

Description of any non-epileptic seizures:_____
_____
_____

Seizure frequency:

	>2/d	1-2/d	2-6/w	1-5/mo	<1/mo	Don't know
0-6 mo						
7-24mo						
2-5 yrs						
6-12 yrs						
13-17 yrs						
18-24 yrs						
25-35 yrs						
Elderly						

Diurnal variation?_____
Seizure provoking factors?_____

History of antiepileptic druguse:

Drug	Age	Effect				Discont. due to side eff.
		Seizurefree	Responder	No effect	Don't know	

Ketogenic diet: Yes☐ No☐ Not anymore☐ Don't know☐
        If yes, effect:_____
Vagus stimulator: Yes☐ No☐ Not anymore☐ Don't know☐
        If yes, effect:_____
Epilepsy surgery: Yes☐ No☐ Don't know☐
        If yes, date of surgery:_____
        Effect:_____

The parents feel that epilepsy is a:
        big problem ☐   medium problem ☐   small problem☐
Describe what they feel is problematic:_____
_____

How do the parents feel the epilepsy has developed through life:
        Better ☐   Same ☐   Worse ☐
When did it change:_____
_____

Respiration

☐No dysfunction

Breathholding Yes☐ No☐ Not anymore☐ Don't know☐
        If yes:
                Age of onset:_____(month/year)
                Intermittent☐ Constant☐
                ☐Diurnal variation:_____

        ☐Provoking factors:_____
    If resolved:
        Age when better:_____(month/year)

Hyperventilation   Yes☐   No☐   Not anymore☐   Don't know☐
    If yes:
        Age of onset:_____(month/year)
        Intermittent☐   Constant☐
        ☐Diurnal variation:_____
        ☐Provoking factors:_____
    If resolved:
        Age when better:_____(month/year)

Air swallowing   Yes☐   No☐   Not anymore☐   Don't know☐
    If yes:
        Age of onset:_____(month/year)
        Intermittent☐   Constant☐
        ☐Diurnal variation:_____
        ☐Provoking factors:_____
    If resolved:
        Age when better:_____(month/year)

Cyanosis with breathholding   Yes☐ No☐ Not anymore☐ Don't know☐
    If yes:
        Age of onset:_____(month/year)
        Intermittent☐   Constant☐
        ☐Diurnal variation:_____
        ☐Provoking factors:_____
    If resolved:
        Age when better:_____(month/year)

The parents feel that the respiratory dysfunction is a:
    Big problem☐   Medium problem☐   Small problem☐
Describe what they feel is problematic:_____
_____

How do the parents feel the respiratory dysfunction has developed through life:
    Better☐   Same☐   Worse☐
When did it change:_____
_____

Autonomic dysfunction

☐No dysfunction
☐Yes

       Debut age:_____(month/year)
       ☐Intermittent cool/pink feet and/or hands
       ☐Intermittent cool/blue feet and/or hands
       ☐Severe cold/blue/sweaty feet and/or hands
       ☐Small feet
          Shoe size:_____

☐Resolved
          Age:_____(month/year)
☐Don't know

Sleep

☐Normal

Night-time screaming   Yes☐   No☐   Not anymore☐   Don't know☐

       If yes:
          ☐<Weekly
          ☐>Weekly
          ☐Nightly
          Age of onset:_____(month/year)
       If resolved, age:_____(month/year)

       The parents feel that the night-time screaming is a:
       Big problem☐   Medium problem☐   Small problem☐
       Describe what they feel is problematic:_____
_____

       How do the parents feel the night-time screaming has developed through life:
       Better☐   Same☐   Worse☐
       When did it change:_____
_____

Night-time laughter   Yes☐   No☐   Not anymore☐   Don't know☐
       If yes:
          ☐<Weekly
          ☐>Weekly
          ☐Nightly
          Age of onset:_____(month/year)
       If resolved, age:_____(month/year)

The parents feel that the night-time laughter is a:
Big problem☐   Medium problem☐   Small problem☐
Describe what they feel is problematic:_____
_____

How do the parents feel the night-time laughter has developed through life:
Better☐   Same☐   Worse☐
When did it change:_____
_____

Other night-time arousals   Yes☐   No☐   Not anymore☐   Don't know☐

    If yes:
        ☐<Weekly
        ☐>Weekly
        ☐Nightly
        Age of onset:_____(month/year)
    If resolved, age:_____(month/year)

The parents feel that the other night-time arousals is a:
Big problem☐   Medium problem☐   Small problem☐
Describe what they feel is problematic:_____
_____

How do the parents feel the other night-time arousals has developed through life:
Better☐   Same☐   Worse☐
When did it change:_____
_____

☐Frequent daytime naps

Scoliosis

☐None
☐<20 degrees
☐20-40 degrees
☐>40 degrees
☐S-shaped curve
☐C-shaped curve

Surgery   Yes☐   No☐   Don't know☐
                if yes: what kind of
        surgery_____Date:_____

        Result:
                ☐Very successful
                ☐Quite successful
                ☐Unchanged
                ☐Exacerbated
        Other orthopedic surgery?_____

Osteoporosis

Bone break or fracture?   Yes☐   No☐   Don't know☐
                If yes, number and localisation:_____

Cause of fracture:
        ☐No trauma/spontanously
        ☐Fall from own height
        ☐Fall from higher than own height
        ☐Other_____
        ☐Don't know
Diagnosed with osteoporosis?   Yes☐   No☐   Don't know☐
DXA-scann performed?   Yes☐   No☐   Don't know☐
                If yes, results:_____

Genetic testing

MECP2-mutation   Yes☐   No☐   Not tested☐   Don't know☐
Other mutations?_____
Which mutation in MECP2? _____

Other abnormalities in biochemical, neuroradiological or neurophysiological
investigations:_____
_____
_____

Other medical conditions that require regular medication: Yes ☐   No ☐ Don't know☐
        If yes, which condition:_____
        Medication:_____

Diagnostic criteria

Regression followed by recovery or stabilization:

Main criteria:
- Partial or complete loss of acquired purposeful hand skills:
- Partial or complete loss of acquired spoken language:
- Gait abnormalities: Impaired (dyspraxic) or absence of ability:
- Stereotypic hand movements such as hand wringling/squeezing, clapping/tapping,   mouthing and washing/rubbing automatisms:

Exclusion criteria for typical RTT:
- Brain injury secondary to trauma (peri- og postnatally), neurometabolic disease, or severe infection that causes neurologiscal problems
- Grossly abnormal psychomotor development in first six month of life

Supportive criteria for atypical RTT:
- Breathing disturbances when awake
- Bruxism when awake
- Impaired sleep pattern
- Abnormal muscle tone
- Peripheral vasomotor disturbances
- Scolioses/kyphosis
- Growth retardation
- Small cold hands and feet
- Inappropiate laughing/screaming spells
- Diminished response to pain
- Intense eye communication – "eye pointing"

Clinical examination

Age:_____(year/months)
Weight:_____kg
Height:_____cm
Headcircumference:_____cm

Contact:
      ☐No contact
      ☐Eye contact
      ☐Smile
      ☐Verbal contact
Stereotypies:
      ☐Yes
      ☐No
Respiration:
      ☐Normal
      ☐Hyperventilation
      ☐Breathholding
      ☐Cyanosis
Ataxia:
      ☐Arms
      ☐Legs
      ☐No
Apraxia/dyspraxia:
      ☐Yes
      ☐No
Muscletone:
      ☐Hypotonic
      ☐Hypertonic
      ☐Normal
Deep tendon reflexes:
      ☐Hyporeflexia
      ☐Hyperreflexia
      ☐Normal
Contractures:
      ☐Yes, where _____
      ☐No
Uses ortoses/corsett:
      ☐Yes, where _____
      ☐No
Scoliosis:
      ☐Yes
      ☐No

Spørreskjema

Rett-syndrom- en populasjonsbasert kartleggingsundersøkelse

Skjemaet tar mellom 30 og 45 minutter å fylle ut. Under intervjuet vil vi komme inn på noen av de samme tema, men spørsmålene i dette skjemaet er lettere å fylle ut når du sitter og ser på det. Hvis du har noen spørsmål eller det er noe du synes er vanskelig, kan du notere disse så kan vi gå gjennom disse under avtalte intervju. Du kan også ringe/sende mail til stipendiat Mari Wold Henriksen, tlf: 92089044, e-post: mari-w-h@hotmail.com

Personalia

Bostedsfylke: _____

Boligområde:
❏ Stor by (Oslo, Bergen, Trondheim, Stavanger)
❏ Mindre by (eks. Hammerfest, Kragerø, Grimstad)
❏ Spredtbygd område

Boligforhold:
❏ Foreldrehjem
❏ Andre slektninger
❏ Bolig/institusjon

Familie

Mors fødselsår og måned: _____

Mors utdanning:
❏ Grunnskole eller videregående
❏ Opptil 3-årig universitet/høyskole
❏ Mer enn 3-årig universitet/høyskole

Mors arbeid/beskjeftigelse: _____

Fars fødselsår og måned: _____

Fars utdanning:
❏ Grunnskole eller videregående
❏ Opptil 3-årig universitet/høyskole
❏ Mer enn 3-årig universitet/høyskole

Fars arbeid/beskjeftigelse: _____

Søsken
Antall "helsøstre" _____   Antall "halvsøstre" _____
Antall "helbrødre" _____   Antall "halvbrødre" _____
Nummer i søskenflokken _____

Har mor eller far opplevd spesielle vanskeligheter i oppveksten og på skolen, f.eks knyttet til lesing, som dere kan huske?

Mor   ❏ Nei   ❏ Ja   Beskriv:

_____
_____
_____
_____

Far   ❑ Nei   ❑Ja   Beskriv:

_____
_____
_____
_____

Har noen av foreldrene hatt noen alvorlige sykdommer?

Mor   ❑ Nei   ❑ Ja   Beskriv:

_____
_____
_____
_____

Far   ❑ Nei   ❑Ja   Beskriv:

_____
_____
_____
_____

Har noen av søskene hatt noen spesielle problemer i oppveksten eller på skolen?

❑ Nei   ❑ Ja   Beskriv:

_____
_____
_____
_____

Ernæring
Denne delen handler om din datters ernæringssituasjon og hennes spiseferdigheter.
Ble hun ammet?
❑ Nei   ❑ Ja   ❑ Vet ikke
Sugde hun normalt?
❑ Nei   ❑ Ja   ❑ Husker ikke   ❑Vet ikke
Hvis nei, var dette et problem?
❑ Nei   ❑ Ja
Beskriv:_____

Sett et kryss under det tallet som passer best med din datters spiseferdigheter. Legg merke til at betydningen av tallene varierer for hvert spørsmål – tallverdiene går ikke i samme retning på alle spørsmål. Les hvert spørsmål nøye.

Spørsmål	Venstre	1	2	3	4	5	6	7	Høyre
Hvordan opplever du måltidene med datteren din?	Svært vanskelige	1	2	3	4	5	6	7	Enkle
Hvor bekymret er du for din datters spisesituasjon?	Ikke bekymret	1	2	3	4	5	6	7	Veldig bekymret
Hvor stor matlyst har datteren din?	Aldri sulten	1	2	3	4	5	6	7	God matlyst
Hvis hun under måltidet avviser maten, når i måltidet begynner hun å avvise maten?	I begynnelsen	1	2	3	4	5	6	7	Mot slutten

Hvor lang tid bruker hun på måltidene? (i minutter)	1-10	11-20	21-30	31-40	42-50	51-60	60+

Spørsmål	Venstre	1	2	3	4	5	6	7	Høyre
Hvordan oppfører hun seg under måltidene?	God oppførsel	1	2	3	4	5	6	7	Utfordrende oppførsel
Har datteren din brekninger, må hun spytte ut, eller kaster hun opp av enkelte matvarer?	Aldri	1	2	3	4	5	6	7	Nesten alltid
Beholder hun maten i munnen uten å svelge den?	Nesten alltid	1	2	3	4	5	6	7	Aldri
Må du løpe etter henne, eller bruke leker og lignende som avledning ved måltidene?	Aldri	1	2	3	4	5	6	7	Nesten alltid
Må du bruke tvang for å få henne til å spise?	Nesten alltid	1	2	3	4	5	6	7	Aldri
Hvordan tygger (eller suger) datteren din maten?	Godt	1	2	3	4	5	6	7	Veldig dårlig
Hvordan synes du datteren din vokser?	Dårlig vekst	1	2	3	4	5	6	7	Vokser fint
Hvordan påvirker hennes spiseferdigheter forholdet ditt til henne?	Veldig negativt	1	2	3	4	5	6	7	Ikke i det hele tatt
Hvordan påvirker hennes spiseferdigheter forholdene i familien?	Ikke i det hele tatt	1	2	3	4	5	6	7	Veldig negativt

Hvilken utsagn beskriver best hvordan datteren din spiser? Kryss av ett alternativ:

Det er ikke trygt for datteren min å spise. Hun kan ikke spise. All ernæring gis gjennom sonde.	
Datteren min får spise litt i munnen, men tilpasninger er nødvendig (mat med tilpasset konsistens, gitt med spesielle hjelpemidler, eller med en tilrettelagt sittestilling). Det meste av ernæringen gis gjennom sonde.	
Datteren min kan spise og ingen tilpasninger er nødvendig. Det meste av ernæringen gis likevel gjennom sonde.	
Datteren min kan spise og ingen tilpasninger er nødvendig. Hun spiser det meste i munnen, men har fortsatt behov for noe mat gjennom sonden.	
Datteren min spiser i munnen, men det er behov for tilpasninger/tilrettelegging. Det er ikke behov for tilleggsernæring gjennom sonde.	
Datteren min spiser alt i munnen. Det er ikke behov for tilleggsernæring gjennom sonde.	

Sett kryss ved alle de matvarene hun vil ta i mot og svelge uten vansker:

Kald mat	
Romtemperert mat	
Varm mat	
Flytende/væske	
Puréer	
Blandet konsistens (suppe med kjøtt, grønnsaker)	
Grovmoset mat (gaffelmoste poteter/grønnsaker)	
Lett tygget mat (kjeks, ostepop, french fries)	
Vanskelig tygget mat (trevlet kjøtt, epler)	

Har hun PEG/gastrostomi/magesonde?
❒ Nei   ❒ Ja

Hvis hun har PEG, hvor mye ernæring vil du anslå at hun får gjennom denne?
❒ All mat
❒ Om lag halvparten
❒ Ekstra væske
❒ Medisin
❒ Annet: _____

Hvilken utsagn beskriver best hvordan datteren din ernæres? Kryss av ett alternativ:

Spiser i munnen	Ernæres gjennom sonde	x
0%	100%	
25%	75%	
50%	50%	
75%	25%	
100%	0%	

Hvis hun spiser mat i munnen, kan hun selv føre maten til munnen?
❏ Ved hjelp av skje etc.
❏ Ved hjelp av skje med assistanse
❏ Ved hjelp av fingrene
❏ Nei

Hvilken kost beskriver best hvordan din datter spiser?
❏ Normalkost
❏ Unngår melk
❏ Ketogen diett
❏ Annen diett: _____

Unngår hun visse næringsmidler?
❏ Nei   ❏ Ja
Hvilke: _____

Bruker hun tilskudd i maten?
❏ Nei
❏ Energipulver
❏ Energidrikk
❏ Ekstra fett i maten
❏ Annet: _____

Bruker hun tilskudd som vitaminer, kalsium etc. ?
❏ Vet ikke   ❏ Nei   ❏ Ja, hva: _____

Tar din datter tran/Omega 3?
❏ Nei   ❏ Ja   ❏ Vet ikke

Er feilsvelging et problem for din datter?
❏ Nei   ❏ Ja   ❏ Vet ikke

Har din datter diabetes?
❏ Vet ikke   ❏ Nei   ❏ Ja, alder: _____(mnd/år)
                              Behandling:_____

Tanngnissing
Denne delen handler om tanngnissing.

Gnisser eller skjærer hun tenner?
❏ Nei   ❏ Ja   ❏ Har gjort, men ikke nå lenger   ❏ Vet ikke

Alder ved debut:_____(mnd/år)   ❏Husker ikke

Alder ved opphør:_____(mnd/år)   ❏Husker ikke

Oppstår gnissingen til bestemte tider av døgnet?

Dagen
- ❏ Aldri
- ❏ Noen gang i uken
- ❏ Daglig
- ❏ Vet ikke

Natten
- ❏ Aldri
- ❏ Noen gang i uken
- ❏ Daglig
- ❏ Vet ikke

Hvis hun gnisser tenner daglig, hvor mye av tiden vil du si hun gjør dette?
- ❏ Gjør mye av tiden
- ❏ Gjør noe av tiden
- ❏ Vet ikke

Er det noen situasjoner hun gnisser mer tenner enn andre?
- ❏ Ved aktivitet
- ❏ Ved stress
- ❏ Ved kjedsomhet
- ❏ Påkalle oppmerksomhet
- ❏ Annet:_____
- ❏ Vet ikke

Kan jenta selv kontrollere gnissingen (f.eks: stopper hun hvis dere ber henne slutte?)
- ❏ Nei   ❏ Ja   ❏ Noen ganger   ❏ Vet ikke

Får hun noen behandling for tanngnissing?
- ❏ Nei   ❏ Vet ikke   ❏ Ja:
    - ❏ Tannlege
    - ❏ Medisiner
    - ❏ Bittskinne
    - ❏ Smokk
    - ❏ Myk klut å bite i
    - ❏ Annet:_____

Hvis hun bruker medisiner mot epilepsi: Ble det endring i gnissingen etter oppstart av medisinen?
- ❏ Bedre   ❏ Verre   ❏ Uendret   ❏ Vet ikke   ❏ Bruker ikke

Hvis hun har felt melketennene sine: avtok gnissingen da hun felte de?
- ❏ Nei   ❏ Ja   ❏ Vet ikke   ❏ Har ikke felt melketenner ennå

Hvor stort problem vurderer foreldrene at tanngissingen er?
- ❏ Stort   ❏ Middels   ❏ Lite

Hva er problemet?_____

Hvordan vil du/dere beskrive at utviklingen har vært over tid?
❏ Bedre   ❏ Uendret   ❏ Verre   ❏ Vet ikke

Når har det vært endringer?_____

Motoriske ferdigheter
Dette avsnittet handler om din datters motoriske ferdigheter.

Kan hun sitte på gulvet uten støtte?
❏ Ja   ❏ Med støtte   ❏ Nei   ❏ Har kunnet tidligere, men kan ikke lenger

Alder da hun lærte: _____(mnd/år)   ❏Husker ikke

Alder da hun eventuelt mistet ferdighet: _____(mnd/år)   ❏Husker ikke

Er dette en ferdighet hun tidligere har mistet, for så å komme tilbake?
❏   Nei
❏   Ja Når mistet hun den? _____(mnd/år)   ❏Husker ikke
        Når kom den tilbake? _____(mnd/år)   ❏Husker ikke

Kan hun sitte på en stol/krakk uten støtte?
❏ Ja   ❏ Med støtte   ❏ Nei   ❏ Har kunnet tidligere, men kan ikke lenger

Alder da hun lærte: _____(mnd/år)   ❏Husker ikke

Alder da hun eventuelt mistet ferdighet: _____(mnd/år)   ❏Husker ikke

Er dette en ferdighet hun tidligere har mistet, for så å komme tilbake?
❏   Nei
❏   Ja Når mistet hun den? _____(mnd/år)   ❏Husker ikke

        Når kom den tilbake? _____(mnd/år)   ❏Husker ikke

Kan hun stå uten støtte?

❏ Ja ❏ Med støtte ❏ Nei ❏ Har kunnet tidligere, men kan ikke lenger

Alder da hun lærte: _____(mnd/år) ❏Husker ikke

Alder da hun eventuelt mistet ferdighet: _____(mnd/år) ❏Husker ikke

Er dette en ferdighet hun tidligere har mistet, for så å komme tilbake?
❏ Nei
❏ Ja Når mistet hun den? _____(mnd/år) ❏Husker ikke

   Når kom den tilbake? _____(mnd/år) ❏Husker ikke

Kan hun reise seg fra en stol?

❏ Ja ❏ Med støtte ❏ Nei ❏ Har kunnet tidligere, men kan ikke lenger

Alder da hun lærte: _____(mnd/år) ❏Husker ikke

Alder da hun eventuelt mistet ferdighet: _____(mnd/år) ❏Husker ikke

Er dette en ferdighet hun tidligere har mistet, for så å komme tilbake?
❏ Nei
❏ Ja Når mistet hun den? _____(mnd/år) ❏Husker ikke

   Når kom den tilbake? _____(mnd/år) ❏Husker ikke

Kan hun reise seg opp fra liggende/sittende på gulvet?

❏ Ja ❏ Med støtte ❏ Nei ❏ Har kunnet tidligere, men kan ikke lenger

Alder da hun lærte: _____(mnd/år) ❏Husker ikke

Alder da hun eventuelt mistet ferdighet: _____(mnd/år) ❏Husker ikke

Er dette en ferdighet hun tidligere har mistet, for så å komme tilbake?
❏ Nei

❑ Ja Når mistet hun den? _____(mnd/år)   ❑Husker ikke

        Når kom den tilbake? _____(mnd/år)   ❑Husker ikke

Kan hun bøye seg ned for å berøre gulvet og så reise seg opp igjen?
❑ Ja   ❑ Med støtte   ❑ Nei   ❑ Har kunnet tidligere, men kan ikke lenger

Alder da hun lærte: _____(mnd/år)   ❑Husker ikke

Alder da hun eventuelt mistet ferdighet: _____(mnd/år)   ❑Husker ikke

Er dette en ferdighet hun tidligere har mistet, for så å komme tilbake?
❑ Nei
❑ Ja Når mistet hun den? _____(mnd/år)   ❑Husker ikke

        Når kom den tilbake? _____(mnd/år)   ❑Husker ikke

Kan hun gå uten støtte?
❑ Ja   ❑ Med støtte   ❑ Nei   ❑ Har kunnet tidligere, men kan ikke lenger

Alder da hun lærte: _____(mnd/år)   ❑Husker ikke

Alder da hun eventuelt mistet ferdighet: _____(mnd/år)   ❑Husker ikke

Er dette en ferdighet hun tidligere har mistet, for så å komme tilbake?
❑ Nei
❑ Ja Når mistet hun den? _____(mnd/år)   ❑Husker ikke

        Når kom den tilbake? _____(mnd/år)   ❑Husker ikke

Hvor langt tror du hun kan gå uten støtte? Antall steg: _____
❑ Mindre enn 10 steg
❑ Mer enn 10 steg
❑ Vet ikke

Hvor langt tror du hun kan gå med støtte? Antall steg: _____

❒ Mindre enn 10 steg
❒ Mer enn 10 steg
❒ Vet ikke

Kan hun gå i ulendt terreng?

❒ Ja   ❒ Med støtte   ❒ Nei   ❒ Har kunnet tidligere, men kan
                                                          ikke lenger

Alder da hun lærte: _____(mnd/år)   ❒Husker
                                                                                                    ikke

Alder da hun eventuelt mistet ferdighet: _____(mnd/år)   ❒Husker
                                                                                                    ikke

Er dette en ferdighet hun tidligere har mistet, for så å komme tilbake?
❒ Nei
❒ Ja Når mistet hun den? _____(mnd/år)   ❒Husker
                                                                                                    ikke

          Når kom den tilbake? _____(mnd/år)   ❒Husker
                                                                                                    ikke

Kan hun gå i trapper?

Opp trappen   *Ned trappen*
❒ Ja   Når: ____(mnd/år)   ❒ Ja   Når: ____(mnd/år)
❒ Med støtte   ❒ Med støtte
❒ Aldri lært   ❒ Aldri lært
❒ Mistet   Når: ____(mnd/år)   ❒ Mistet   Når: ____(mnd/år)
❒ Gjenopptatt ferdighet   ❒ Gjenopptatt ferdighet
     Når mistet: ____(mnd/år)   Når mistet: ____(mnd/år)
     Når kom tilbake:____(mnd/år)   Når kom
                                                                      tilbake:____(mnd/år)

Kan hun løpe?
❒ Ja   ❒ Med støtte   ❒ Nei   ❒ Har kunnet tidligere, men kan
                                                          ikke lenger

Alder da hun lærte: _____(mnd/år)   ❒Husker
                                                                                                    ikke

Alder da hun eventuelt mistet ferdighet: _____(mnd/år)   ❒Husker
                                                                                                    ikke

Er dette en ferdighet hun tidligere har mistet, for så å komme tilbake?
❒ Nei

❏   Ja Når mistet hun den? _____(mnd/år)   ❏Husker ikke

        Når kom den tilbake? _____(mnd/år)   ❏Husker ikke

Bruker hun noen form for gåhjelpemiddel?
❏ Nei   ❏ Ja Hvilke: _____

Er hun rullestolbundet?
❏ Nei   ❏ Ja   Hvis ja, alder: _____ (år/mnd)

Klarer hun å bruke hendene sine?
❏ Ja   ❏ Delvis   ❏ Aldri kunnet

❏ Kunne bruke hendene sine, mistet ferdigheten før den igjen kom tilbake
Når mistet hun den? _____(mnd/år)   ❏Husker ikke

Når kom den tilbake? _____(mnd/år)   ❏Husker ikke

❏ Har kunnet tidligere, men kan ikke lenger
Alder:_____(mnd/år)

Har hun en hånd som ser ut til å være dominant?
❏ Høyrehendt
❏ Venstrehendt
❏ Kapphendt (begge)
❏ Vet ikke

Har hun håndstereotypier? (vaskebevegelse, hånd til munnen osv.)?
❏ Nei   ❏ Ja   ❏ Vet ikke

Hvis ja, hvor mye av tiden vil du si at hun utfører disse bevegelsene?
❏ Gjør mye av tiden
❏ Gjør noe av tiden
❏ Vet ikke

Kan du huske ved hvilken alder disse håndbevegelsene startet?
❏ Før 18 mnd
❏ 18-36 mnd
❏ Etter 36 mnd
❏ Senere enn 10 års alder
❏ Husker ikke

Vi takker for at du tok deg tid til å fylle ut skjemaet!

Rett Syndrome Severity Scale

Frequency and manageability of seizures
      0 = No seizures
      1 = Easily managed with medications
      2 = Managed with medications but breakthroughs occur
      3 = Recalcitrant seizures requiring multiple medications for control

Respiratory irregularities
      0 = Not present
      1 = Consist of minimal breath-holding spells
      2 = Breath-holding and hyperventilation for less than half the period
      3 = Hyperventilation and breath-holding, for more than half the wake period, with or without cyanotic episodes

Scoliosis
      0 = Not present
      1 = Less than 20 degrees
      2 = Less than 30 degrees
      3 = Greater than 30 degrees or if surgical correction had taken place

Ability to walk
      0 = Normal gait
      1 = Mildly apraxic
      2 = Severely apraxic or requiring to be held when patient walked independently
      3 = Requiring support to stand and/or wheelchair bound

Hand use
      0 = Normal
      1 = Purposeful grasping
      2 = Tapping for needs
      3 = No hand use

Speech
      0 = Normal
      1 = Sentences/phrases
      2 = Single words
      3 = Non-verbal

Sleep
      0 = Normal
      1 = Awakens but falls back to sleep
      2 = Fragmented night time sleep with day time sleepiness
      3 = Unable to sleep through the night

Vedlegg til rapport ved high throughput sequencing (HTS) analyse

Genpanel: Epileptisk encefalopati og psykisk utviklingshemming

Genliste med dekningsgrad:

Gen	NCBI transkript	Sykdom	Arvegang (1)	Antall kodende bp + 4 bp (1)	Dekningsgrad (% bp) (2)
ARHGEF9	NM_015185.2	Epileptisk encefalopati type 8 (EIEE8)	X-bundet	1591	100.0%
ARID1A	NM_006015.4	Mental retardasjon type 14 (MRD14)	AD	6938	100.0%
ARID1B	NM_020732.3	Mental retardasjon type 12 (MRD12)	AD	6830	100.0%
ARX	NM_139058.2	Epileptisk encefalopati type 1 (EIEE1, West syndrom), X-bundet mental retardasjon type 29	X-bundet	1709	99.6%
ATRX	NM_000489.4	Alfa-thassemi/mental retardasjon syndrom (ATRX), X-bundet mental retardasjon- og ansiktshypotoni-syndrom type 1 (MRXHF1)	X-bundet	7619	100.0%
CASK	NM_003688.3	FG-syndrom type 4 (FGS4) / Mental retardasjon og mikrocefali med pontin og cerebellar hypoplasi (MICPCH)	X-bundet	2874	100.0%
CDH15	NM_004933.2	Mental retardasjon 3 (MRD3)	AD	2501	100.0%
CDKL5	NM_003159.2	Epileptisk encefalopati type 2 (EIEE2), Angelman-like syndrom	X-bundet	3173	100.0%
CNTNAP2	NM_014141.5	Kortikal dysplasi-fokal epilepsi syndrom, Pitt-Hopkins like syndrom type 1 (PTHSL1)	AR	4092	100.0%
CTCF	NM_006565.3	Mental retardasjon type 21 (MRD21)	AD	2224	100.0%
CTNNB1	NM_001904.3	Mental retardasjon type 19 (MRD19)	AD	2402	100.0%
CUL4B	NM_003588.3	Mental retardasjon med kortvoksthet, hypogonadisme og atypisk gange	X-bundet	2826	100.0%
DYNC1H1	NM_001376.4	Mental retardasjon type 13 (MRD13)	AD	14253	100.0%
DYRK1A	NM_001396.3	Mental retardasjon type 7 (MRD7)	AD	2336	100.0%
EHMT1	NM_024757.4	Kleefstra syndrom (9q-syndrom)	AD	4005	99.4%
FOXG1	NM_005249.4	Kongenitalt Rett syndrom	AD	1474	97.1%
GABRG2	NM_000816.3	Epilepsi med feberkramper type 3 (GEFSP3), Familiær feberkrampe type 8 (FEB8)	AD	1440	100.0%
GATAD2B	NM_020699.2	Mental retardasjon type 18 (MRD18)	AD	1822	100.0%
GNAO1	NM_020988.2	Epileptisk encefalopati type 17 (EIEE17)	AD	1097	100.0%
GRIN1	NM_007327.3	Mental retardasjon type 8 (MRD8)	AD	2897	100.0%
GRIN2A	NM_000833.4	Fokal epilepsi med talevansker og med eller uten mental retardasjon (FESD)	AD	4443	100.0%
GRIN2B	NM_000834.3	Mental retardasjon type 6 (MRD6)	AD	4503	100.0%
KANSL1	NM_001193466.1	Koolen-De Vries syndrom (KDVS)	AD	3374	100.0%
KCNQ2	NM_172107.2	Epileptisk encefalopati type 7 (EIEE7)	AD	2687	100.0%
KIRREL3	NM_032531.3	Mental retardasjon type 4 (MRD4)	AD	2405	100.0%
MBD5	NM_018328.4	Mental retardasjon type 1 (MRD1)	AD	4525	100.0%
MECP2	NM_004992.3	Rett syndrom (RTT)	X-bundet	1473	100.0%
MEF2C	NM_002397.4	Mental retardasjon type 20 (MRD20)	AD	1462	100.0%
NRXN1	NM_001135659.1	Pitt-Hopkins-like syndrom 2 (PTHSL2)	AR	4736	100.0%
OPHN1	NM_002547.2	X-bundet mental retardasjon med cerebellar hypoplasi	X-bundet	2501	100.0%
PACS1	NM_018026.3	Mental retardasjon type 17 (MRD17)	AD	2988	100.0%
PCDH19	NM_001184880.1	Epileptisk encefalopati type 9 (EIEE9, Epilepsi og mental retardasjon som rammer kvinner)	X-bundet (male sparing)	3471	100.0%
PLCB1	NM_015192.3	Epileptisk encefalopati type 12 (EIEE12)	AR	3779	100.0%
PNKP	NM_007254.3	Epileptisk encefalopati type 10 (EIEE10)	AR	1630	100.0%
PNPO	NM_018129.3	Pyridoxamin 5´-fosfat oksidase mangel	AR	814	100.0%
POLG	NM_002693.2	Alpers syndrom, Mitokondrielt recessivt ataxi-syndrom, Progressiv ekstern oftalmoplegi	AR *	3808	100.0%
RAI1	NM_030665.3	Smith-Magenis syndrom (SMS)	AD	5737	100.0%
SCN1A	NM_001165963.1	Epileptisk encefalopati type 6 (EIEE6), Dravet syndrom, GEFS+ type 2	AD	6134	100.0%
SCN2A	NM_021007.2	Epileptisk encefalopati type 11 (EIEE11)	AD	6122	100.0%
SCN8A	NM_014191.3	Epileptisk encefalopati type 13 (EIEE13)	AD	6047	100.0%
SLC19A3	NM_025243.3	Tiamin metabolisme dysfunksjon syndrom type 2 (THMD2)	AR	1511	100.0%
SLC25A22	NM_024698.5	Epileptisk encefalopati type 3 (EIEE3)	AR	1008	100.0%
SLC2A1	NM_006516.2	GLUT1-mangel syndrom (GLUT1DS1, GLUT1DS2)	AD	1519	100.0%
SLC6A8	NM_005629.3	Cerebral kreatinmangel type 1 (CCDS1)	X-bundet	1960	100.0%
SLC9A6	NM_001042537.1	X-bundet mental retardasjon, type Christianson syndrom (MRXSCH)	X-bundet	2170	100.0%
SMARCA2	NM_003070.4	Nicolaides-Baraitser syndrom (NCBRS)	AD	4905	99.5%
SMARCA4	NM_001128849.1	Mental retardasjon type 16 (MRD16)	AD	5180	100.0%
SMARCB1	NM_003073.3	Mental retardasjon type 15 (MRD15)	AD	1194	100.0%

SPTAN1	NM_001130438.2	Epileptisk encefalopati type 5 (EIEE5)	AD	7658	100.0%
ST3GAL3	NM_006279.3	Epileptisk encefalopati type 15 (EIEE15), Autosomal recessiv mental retardasjon, type 12 (MRT12)	AR	1172	100.0%
STXBP1	NM_003165.3	Epileptisk encefalopati type 4 (EIEE4)	AD	1888	100.0%
SYNGAP1	NM_006772.2	Mental retardasjon type 5 (MRD5)	AD	4108	98.3%
SZT2	NM_015284.3	Epileptisk encefalopati type 18 (EIEE 18)	AR	10412	100.0%
TBC1D24	NM_001199107.1	Epileptisk encefalopati type 16 (EIEE16), Familiær infantil myoklon epilepsi (FIME), DOOR syndrom	AR	1708	100.0%
TCF4	NM_001083962.1	Pitt-Hopkins syndrom (PTHS)	AD	2088	100.0%
UBE3A	NM_130838.1	Angelman syndrom (AS)	AD (imprinted)	2599	100.0%
ZEB2	NM_014795.3	Mowat-Wilson syndrom (MOWS)	AD	3681	100.0%

* En fenotype, "autosomal dominant progressive external ophtalmoplegia" (adPEO), er dominant.

Sekvensområder lest mindre enn 10 ganger ved HTS:

Kromosom	Startposisjon	Stopposisjon	Gen__mRNA RefSeq	x dekning
X	25031745	25031747	ARX__NM_139058.2	9
X	25031774	25031778	ARX__NM_139058.2	9
9	140513478	140513503	EHMT1__NM_024757.4	9
14	29236700	29236702	FOXG1__NM_005249.4	9
14	29236705	29236746	FOXG1__NM_005249.4	7
9	2047262	2047287	SMARCA2__NM_003070.4	9
6	33388039	33388110	SYNGAP1__NM_006772.2	0

Utfyllende analyser:

Den utførte analysen påviser ikke større strukturelle avvik som insersjoner, delesjoner og duplikasjoner. Slike genforandringer kan påvises med MLPA (Multiplex Ligation-dependent Probe Amplification). Ved mistanke om enkelte spesifikke diagnoser er det viktig også å utføre MLPA av ett eller flere gener. Dersom ikke annet er nevnt, er MLPA ikke utført i forbindelse med denne analysen. Eventuell MLPA må rekvireres separat på ny rekvisisjon. Ny blodprøve er ikke nødvendig.

Laboratoriet har tilbud om MLPA for følgende av panelets gener (se for øvrig www.genetikkportalen.no):

RAI1: Smith-Mageni syndrom, MLPA detekterer ca. 70% av tilfellene* (P369 Smith-Magenis)

UBE3A: Angelman syndrom, metyleringssensitiv MLPA detekterer ca. 75% av tilfellene* (P336 Prader Willi/Angelman), sekvensering vil detektere kun 10%

EHMT1: Kleefstra syndrom, MLPA detekterer ca. 70% av tilfellene* (P340 EHMT1)

SLC2A1: GLUT1-mangel syndrom, MLPA detekterer 11-14% av tilfellene* (P138 SLC2A1)

MECP2: Rett syndrom, MLPA detekterer opp til 8% av tilfellene* (P015 MECP2)

SCN1A: Dravet syndrom, nytteverdi av MLPA er antatt lav (P137 SCN1A)

ZEB2: Mowat-Wilson syndrom, MLPA detekterer ca. 2% av tilfellene (P169 Hirschsprung-1)

ARX: X-bundet PU og lissencefali, MLPA detekterer en liten andel tilfellene (P189 CDKL5)

CDKL5: Rett syndrom/West syndrom, MLPA detekterer en meget liten andel av tilfellene (P189 CDKL5)

FOXG1: Rett syndrom, MLPA detekterer en meget liten andel av tilfellene (P395 MEF2C-FOXG1)

MEF2C: 5q14 delesjonssyndrom, MLPA detekterer en meget liten andel av tilfellene (P395 MEF2C-FOXG1)

* www.genereviews.org

#Gen	HGNC ID	Transkript	Dekning	Omim gen
AAAS	13666	NM_015665.5	100	605378
AARS	20	NM_001605.2	100	601065
AASS	17366	NM_005763.3	100	605113
ABCB11	42	NM_003742.2	100	603201
ABCB7	48	NM_004299.5	100	300135
ABCC6	57	NM_001171.5	93	603234
ABCC9	60	NM_005691.3	100	601439
ABCD1	61	NM_000033.3	77	300371
ABCD4	68	NM_005050.3	100	603214
ABHD5	21396	NM_016006.4	100	604780
ACAD9	21497	NM_014049.4	99	611103
ACADM	89	NM_000016.5	100	607008
ACADS	90	NM_000017.3	100	606885
ACADVL	92	NM_000018.3	100	609575
ACAN	319	NM_013227.3	84	155760
ACAT1	93	NM_000019.3	100	607809
ACO2	118	NM_001098.2	97	100850
ACOX1	119	NM_004035.6	100	609751
ACP5	124	NM_001111035.2	100	171640
ACSL4	3571	NM_004458.2	99	300157
ACTA1	129	NM_001100.3	100	102610
ACTA2	130	NM_001613.2	100	102620
ACTB	132	NM_001101.3	99	102630
ACTG1	144	NM_001614.3	100	102560
ACVR1	171	NM_001105.4	100	102576
ACVR2B	174	NM_001106.3	100	602730
ACY1	177	NM_000666.2	100	104620
ADA	186	NM_000022.3	100	608958
ADAR	225	NM_001111.4	100	146920
ADCK3	16812	NM_020247.4	100	606980
ADK	257	NM_001123.3	100	102750
ADNP	15766	NM_015339.4	100	611386
ADRA2B	282	NM_000682.6	100	104260
ADSL	291	NM_000026.3	100	608222
AFF2	3776	NM_002025.3	99	300806
AFF3	6473	NM_002285.2	98	
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AFG3L2	315	NM_006796.2	96	604581
AGA	318	NM_000027.3	100	613228
AGK	21869	NM_018238.3	100	610345
AGL	321	NM_000642.2	100	610860
AGPS	327	NM_003659.3	99	603051
AGXT	341	NM_000030.2	100	604285
AHDC1	25230	NM_001029882.3	99	615790
AHI1	21575	NM_017651.4	100	608894
AIFM1	8768	NM_004208.3	100	300169
AIMP1	10648	NM_004757.3	100	603605
AIPL1	359	NM_014336.4	100	604392
AIRE	360	NM_000383.3	100	607358
AK2	362	NM_001625.3	100	103020
AKR1D1	388	NM_005989.3	99	604741
AKT1	391	NM_005163.2	99	164730
AKT3	393	NM_005465.4	99	611223
ALAD	395	NM_000031.5	100	125270
ALDH18A1	9722	NM_002860.3	100	138250
ALDH1A3	409	NM_000693.3	100	600463
ALDH3A2	403	NM_000382.2	100	609523
ALDH4A1	406	NM_003748.3	100	606811
ALDH5A1	408	NM_001080.3	99	610045
ALDH7A1	877	NM_001182.4	99	107323
ALDOA	414	NM_000034.3	100	103850
ALDOB	417	NM_000035.3	100	612724
ALG1	18294	NM_019109.4	55	605907
ALG11	32456	NM_001004127.2	100	613666
ALG12	19358	NM_024105.3	100	607144
ALG13	30881	NM_001099922.2	99	300776
ALG2	23159	NM_033087.3	100	607905
ALG3	23056	NM_005787.5	100	608750
ALG6	23157	NM_013339.3	99	604566
ALG8	23161	NM_024079.4	100	608103
ALG9	15672	NM_024740.2	99	606941
ALMS1	428	NM_015120.4	99	606844
ALPL	438	NM_000478.5	100	171760
ALS2	443	NM_020919.3	100	606352
ALX1	1494	NM_006982.2	100	601527
ALX3	449	NM_006492.2	92	606014
ALX4	450	NM_021926.3	99	605420
AMER1	26837	NM_152424.3	99	300647
AMPD2	469	NM_001257360.1	100	102771
AMT	473	NM_000481.3	100	238310
ANKH	15492	NM_054027.4	100	605145
ANKRD11	21316	NM_013275.5	97	611192
ANKRD26	29186	NM_014915.2	98	610855
ANO5	27337	NM_213599.2	100	608662
ANTXR1	21014	NM_032208.2	98	606410
AP1S2	560	NM_003916.4	91	300629
AP3B2	567	NM_004644.4	99	602166
AP4B1	572	NM_006594.4	100	607245
AP4E1	573	NM_007347.4	100	607244
AP4M1	574	NM_004722.3	100	602296
AP4S1	575	NM_007077.4	100	607243
APOA1BP	18453	NM_144772.2	100	608862
APOPT1	20492	NM_032374.4	100	616003
APTX	15984	NM_175073.2	94	606350
AR	644	NM_000044.4	98	313700
ARCN1	649	NM_001655.4	100	600820
ARFGEF2	15853	NM_006420.2	99	605371
ARG1	663	NM_000045.3	100	608313
ARHGAP31	29216	NM_020754.3	99	610911
ARHGEF6	685	NM_004840.2	100	300267
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ARID1A	11110	NM_006015.4	98	603024
ARID1B	18040	NM_020732.3	99	614556
ARID2	18037	NM_152641.3	99	609539
ARL6	13210	NM_177976.3	100	608845
ARMC4	25583	NM_018076.4	94	615408
ARSA	713	NM_000487.5	100	607574
ARSB	714	NM_000046.3	100	611542
ARSE	719	NM_000047.2	99	300180
ARX	18060	NM_139058.2	87	300382
ASAH1	735	NM_177924.4	100	613468
ASL	746	NM_000048.3	99	608310
ASPA	756	NM_000049.2	100	608034
ASPH	757	NM_004318.3	100	600582
ASPM	19048	NM_018136.4	99	605481
ASS1	758	NM_000050.4	98	603470
ASXL1	18318	NM_015338.5	100	612990
ASXL2	23805	NM_018263.5	99	612991
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ATIC	794	NM_004044.6	99	601731
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ATP13A2	30213	NM_022089.3	99	610513
ATP1A3	801	NM_152296.4	100	182350
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ATP8B1	3706	NM_005603.4	96	602397
ATR	882	NM_001184.3	99	601215
ATRX	886	NM_000489.4	99	300032
AUH	890	NM_001698.2	100	600529
AUTS2	14262	NM_015570.3	98	
B3GALNT2	28596	NM_152490.4	100	610194
B3GALT6	17978	NM_080605.3	84	615291
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BBS1	966	NM_024649.4	100	209901
BBS10	26291	NM_024685.3	100	610148
BBS12	26648	NM_152618.2	100	610683

BBS2	967	NM_031885.3	100	606151	CDC6	1744	NM_001254.3	100	602627
BBS4	969	NM_033028.4	100	600374	CDH15	1754	NM_004933.2	100	114019
BBS5	970	NM_152384.2	99	603650	CDH23	13733	NM_022124.5	100	605516
BBS7	18758	NM_176824.2	99	607590	CDH3	1762	NM_001793.5	100	114021
BBS9	30000	NM_198428.2	99	607968	CDK13	1733	NM_031267.3	99	603309
BCAP31	16695	NM_001139441.1	98	300398	CDK5RAP2	18672	NM_018249.5	100	608201
BCKDHA	986	NM_000709.3	100	608348	CDKL5	11411	NM_003159.2	100	300203
BCKDHB	987	NM_183050.3	99	248611	CDKN1C	1786	NM_000076.2	84	600856
BCL11A	13221	NM_022893.3	100	606557	CDON	17104	NM_016952.4	100	608707
BCOR	20893	NM_017745.5	99	300485	CDT1	24576	NM_030928.3	99	605525
BCS1L	1020	NM_004328.4	100	603647	CENPJ	17272	NM_018451.4	100	609279
BFSP2	1041	NM_003571.3	100	603212	CEP135	29086	NM_025009.4	99	611423
BGN	1044	NM_001711.5	100	301870	CEP152	29298	NM_014985.3	99	613529
BHLHA9	35126	NM_001164405.1	85	615416	CEP290	29021	NM_025114.3	98	610142
BICD2	17208	NM_001003800.1	100	609797	CEP41	12370	NM_018718.2	99	610523
BIN1	1052	NM_139343.2	100	601248	CEP57	30794	NM_014679.4	100	607951
BLM	1058	NM_000057.3	100	604610	CEP63	25815	NM_025180.3	100	614724
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BMP2	1069	NM_001200.3	100	112261	CHAMP1	20311	NM_001164144.2	100	616327
BMP4	1071	NM_001202.5	100	112262	CHD2	1917	NM_001271.3	100	602119
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BMPR1B	1077	NM_001203.2	100	603248	CHD7	20626	NM_017780.3	100	608892
BOLA3	24415	NM_212552.2	92	613183	CHD8	20153	NM_001170629.1	100	610528
BRAF	1097	NM_004333.4	98	164757	CHM	1940	NM_000390.3	99	300390
BRAT1	21701	NM_152743.3	99	614506	CHMP1A	8740	NM_002768.4	100	164010
BRCA2	1101	NM_000059.3	99	600185	CHRDL1	29861	NM_001143981.1	100	300350
BRIP1	20473	NM_032043.2	100	605882	CHRNA1	1955	NM_000079.3	100	100690
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BRWD3	17342	NM_153252.4	99	300553	CHRNB2	1962	NM_000748.2	100	118507
BSND	16512	NM_057176.2	100	606412	CHRNG	1967	NM_005199.4	100	100730
BTD	1122	NM_000060.4	100	608306	CHST14	24464	NM_130468.3	99	608429
BUB1B	1149	NM_001211.5	99	602860	CHST3	1971	NM_004273.4	100	603799
C12orf57	29521	NM_138425.3	100	615140	CHSY1	17198	NM_014918.4	99	608183
C12orf65	26784	NM_152269.4	99	613541	CHUK	1974	NM_001278.4	100	600664
C1QTNF5	14344	NM_015645.4	97	608752	CIB2	24579	NM_006383.3	100	605564
C21orf2	1260	NM_004928.2	100	603191	CISD2	24212	NM_001008388.4	77	611507
C21orf59	1301	NM_021254.3	100	615494	CIT	1985	NM_001206999.1	100	605629
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C2orf71	34383	NM_001029883.2	99	613425	CLCN4	2022	NM_001830.3	100	302910
C4orf26	26300	NM_178497.3	100	614829	CLCN7	2025	NM_001287.5	99	602727
C5orf42	25801	NM_023073.3	99	614571	CLCNKB	2027	NM_000085.4	99	602023
C8orf37	27232	NM_177965.3	100	614477	CLDN19	2040	NM_148960.2	99	610036
CA2	1373	NM_000067.2	100	611492	CLMP	24039	NM_024769.3	100	611693
CA5A	1377	NM_001739.1	99	114761	CLN3	2074	NM_001042432.1	100	607042
CA8	1382	NM_004056.5	99	114815	CLN5	2076	NM_006493.2	100	608102
CACNA1C	1390	NM_000719.6	100	114205	CLN6	2077	NM_017882.2	99	606725
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CAMTA1	18806	NM_015215.3	100	611501	CLP1	16999	NM_006831.2	100	607621
CARS2	25695	NM_024537.3	100	612800	CLPB	30664	NM_001258394.2	100	616254
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CBL	1541	NM_005188.3	99	165360	CNKSR2	19701	NM_001168647.2	99	300724
CBS	1550	NM_000071.2	99	613381	CNOT3	7879	NM_014516.3	100	604910
CC2D1A	30237	NM_017721.4	100	610055	CNTNAP1	8011	NM_003632.2	99	602346
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CCBE1	29426	NM_133459.3	100	612753	COASY	29932	NM_025233.6	100	609855
CCDC103	32700	NM_213607.2	100	614677	COG1	6545	NM_018714.2	100	606973
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CCDC39	25244	NM_181426.1	99	613798	COL10A1	2185	NM_000493.3	100	120110
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CCDC41	17966	NM_016122.2	99	615847	COL11A2	2187	NM_080680.2	100	120290
CCDC65	29937	NM_033124.4	100	611088	COL13A1	2190	NM_001130103.1	100	120350
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CCDC8	25367	NM_032040.4	100	614145	COL1A1	2197	NM_000088.3	99	120150
CCDC88C	19967	NM_001080414.3	100	611204	COL25A1	18603	NM_198721.3	99	610004
CCND2	1583	NM_001759.3	100	123833	COL2A1	2200	NM_001844.4	100	120140
CCNO	18576	NM_021147.4	99	607752	COL4A1	2202	NM_001845.5	99	120130
CD96	16892	NM_198196.2	100	606037	COL4A2	2203	NM_001846.3	100	120090
CDC45	1739	NM_001178010.2	100		COL4A3	2204	NM_000091.4	98	120070

COL4A3BP	2205	NM_001130105.1	100	604677	DENND5A	19344	NM_015213.3	100	617278
COL4A4	2206	NM_000092.4	99	120131	DEPDC5	18423	NM_001242896.1	100	614191
COL6A1	2211	NM_001848.2	100	120220	DHCR24	2859	NM_014762.3	100	606418
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COL9A1	2217	NM_001851.4	100	120210	DHFR	2861	NM_000791.3	99	126060
COL9A2	2218	NM_001852.3	99	120260	DHODH	2867	NM_001361.4	100	126064
COL9A3	2219	NM_001853.3	99	120270	DHTKD1	23537	NM_018706.6	100	614984
COLEC11	17213	NM_024027.4	100	612502	DIS3L2	28648	NM_152383.4	100	614184
COMP	2227	NM_000095.2	97	600310	DKC1	2890	NM_001363.4	100	300126
COQ2	25223	NM_015697.7	99	609825	DLAT	2896	NM_001931.4	100	608770
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COQ9	25302	NM_020312.3	100	612837	DLG3	2902	NM_021120.3	99	300189
COX10	2260	NM_001303.3	100	602125	DLL3	2909	NM_016941.3	96	602768
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COX6B1	2280	NM_001863.4	100	124089	DMD	2928	NM_004006.2	99	300377
COX7B	2291	NM_001866.2	88	300885	DMP1	2932	NM_004407.3	100	600980
CPAMD8	23228	NM_015692.3	97	608841	DMPK	2933	NM_004409.4	100	605377
CPS1	2323	NM_001875.4	100	608307	DNA2	2939	NM_001080449.2	100	601810
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CRB1	2343	NM_201253.2	100	604210	DNAH5	2950	NM_001369.2	99	603335
CRB2	18688	NM_173689.6	99	609720	DNAJC12	28908	NM_021800.2	100	606060
CRBN	30185	NM_016302.3	100	609262	DNM1	2972	NM_004408.3	97	602377
CREBBP	2348	NM_004380.2	99	600140	DNMT3A	2978	NM_175629.2	99	602769
CRELD1	14630	NM_015513.4	100	607170	DNMT3B	2979	NM_006892.3	100	602900
CRX	2383	NM_000554.5	100	602225	DOCK6	19189	NM_020812.3	99	614194
CRYAA	2388	NM_000394.3	100	123580	DOCK7	19190	NM_001271999.1	99	615730
CRYBA1	2394	NM_005208.4	100	123610	DOCK8	19191	NM_203447.3	100	611432
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CRYGC	2410	NM_020989.3	100	123680	DRC1	24245	NM_145038.4	100	615288
CRYGD	2411	NM_006891.3	100	123690	DSG1	3048	NM_001942.3	99	125670
CSNK2A1	2457	NM_001895.3	100	115440	DSPP	3054	NM_014208.3	98	125485
CSPP1	26193	NM_024790.6	100	611654	DSTYK	29043	NM_015375.2	100	612666
CSTA	2481	NM_005213.3	100	184600	DVL1	3084	NM_004421.2	100	601365
CSTB	2482	NM_000100.3	100	601145	DVL3	3087	NM_004423.3	100	601368
CTC1	26169	NM_025099.5	100	613129	DYM	21317	NM_017653.3	100	607461
CTCF	13723	NM_006565.3	99	604167	DYNC1H1	2961	NM_001376.4	100	600112
CTDP1	2498	NM_004715.4	96	604927	DYNC2H1	2962	NM_001080463.1	99	603297
CTNNB1	2514	NM_001904.3	100	116806	DYRK1A	3091	NM_001396.4	100	600855
CTNND1	2515	NM_001206885.1	100	601045	DYX1C1	21493	NM_130810.3	100	608706
CTNS	2518	NM_004937.2	100	606272	EBF3	19087	NM_001005463.2	99	605788
CTSA	9251	NM_000308.3	99	613111	EBP	3133	NM_006579.2	99	300205
CTSD	2529	NM_001909.4	100	116840	ECEL1	3147	NM_004826.3	98	605896
CTSK	2536	NM_000396.3	100	601105	EDA	3157	NM_001399.4	99	300451
CUL4B	2555	NM_003588.3	99	300304	EDN1	3176	NM_001955.4	100	131240
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CYB5R3	2873	NM_000398.6	98	613213	EDNRB	3180	NM_000115.4	100	131244
CYC1	2579	NM_001916.4	100	123980	EEF1A2	3192	NM_001958.3	100	602959
CYP1B1	2597	NM_000104.3	100	601771	EFNB1	3226	NM_004429.4	100	300035
CYP2U1	20582	NM_183075.2	98	610670	EFTUD2	30858	NM_004247.3	100	603892
DAG1	2666	NM_004393.5	100	128239	EGR2	3239	NM_000399.4	100	129010
DARS	2678	NM_001349.3	100	603084	EHMT1	24650	NM_024757.4	99	607001
DARS2	25538	NM_018122.4	100	610956	EIF2AK3	3255	NM_004836.6	98	604032
DBT	2698	NM_001918.3	100	248610	EIF2S3	3267	NM_001415.3	99	300161
DCAF17	25784	NM_025000.3	100	612515	EIF4A3	18683	NM_014740.3	100	608546
DCDC2	18141	NM_016356.4	100	605755	ELAC2	14198	NM_018127.6	100	605367
DCHS1	13681	NM_003737.3	99	603057	ELMO2	17233	NM_182764.2	100	606421
DCX	2714	NM_178153.2	100	300121	ELN	3327	NM_001278939.1	100	130160
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DDC	2719	NM_000790.3	100	107930	EMC1	28957	NM_015047.2	100	616846
DDHD1	19714	NM_001160147.1	98	614603	EMG1	16912	NM_006331.7	100	611531
DDHD2	29106	NM_015214.2	100	615003	ENPP1	3356	NM_006208.2	96	173335
DDOST	2728	NM_005216.4	100	602202	EOGT	28526	NM_173654.2	100	614789
DDR2	2731	NM_006182.2	100	191311	EP300	3373	NM_001429.3	100	602700
DDX11	2736	NM_030653.3	89	601150	EPG5	29331	NM_020964.2	99	615068
DDX3X	2745	NM_001193416.2	99	300160	ERCC1	3433	NM_202001.2	100	126380
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DEAF1	14677	NM_021008.3	95	602635	ERCC3	3435	NM_000122.1	100	133510
DECR1	2753	NM_001359.1	100	222745	ERCC4	3436	NM_005236.2	100	133520

Gene	ID	RefSeq	%	OMIM	Gene	ID	RefSeq	%	OMIM
ERCC5	3437	NM_000123.3	100	133530	FOXE1	3806	NM_004473.3	99	602617
ERCC6	3438	NM_000124.3	100	609413	FOXE3	3808	NM_012186.2	82	601094
ERCC6L2	26922	NM_001010895.2	100	615667	FOXF1	3809	NM_001451.2	100	601089
ERCC8	3439	NM_000082.3	100	609412	FOXG1	3811	NM_005249.4	94	164874
ERF	3444	NM_006494.3	100	611888	FOXL2	1092	NM_023067.3	99	605597
ERLIN2	1356	NM_007175.6	100	611605	FOXN1	12765	NM_003593.2	100	600838
ERMARD	21056	NM_018341.2	100	615532	FOXP1	3823	NM_032682.5	100	605515
ESCO2	27230	NM_001017420.2	99	609353	FOXP2	13875	NM_014491.3	100	605317
ETFA	3481	NM_000126.3	100	608053	FOXP3	6106	NM_014009.3	99	300292
ETFB	3482	NM_001985.2	100	130410	FOXRED1	26927	NM_017547.3	100	613622
ETFDH	3483	NM_004453.3	100	231675	FRAS1	19185	NM_025074.6	100	607830
ETHE1	23287	NM_014297.4	100	608451	FREM1	23399	NM_144966.5	100	608944
EVC	3497	NM_153717.2	96	604831	FREM2	25396	NM_207361.5	100	608945
EVC2	19747	NM_147127.4	98	607261	FRMD7	8079	NM_194277.2	100	300628
EXOSC3	17944	NM_016042.3	99	606489	FRMPD4	29007	NM_014728.3	100	300838
EXPH5	30578	NM_015065.2	100	612878	FRRS1L	1362	NM_014334.3	82	604574
EXT1	3512	NM_000127.2	99	608177	FTCD	3974	NM_006657.2	97	606806
EXT2	3513	NM_207122.1	100	608210	FTL	3999	NM_000146.3	100	134790
EYA1	3519	NM_000503.5	100	601653	FTO	24678	NM_001080432.2	100	610966
EZH2	3527	NM_004456.4	100	601573	FTSJ1	13254	NM_012280.3	99	300499
FAH	3579	NM_000137.2	100	613871	FUCA1	4006	NM_000147.4	100	612280
FAM105B	25118	NM_138348.5	98	615712	FYCO1	14673	NM_024513.3	100	607182
FAM111A	24725	NM_022074.3	100	615292	FZD5	4043	NM_003468.3	100	601723
FAM126A	24587	NM_032581.3	99	610531	FZD6	4044	NM_003506.3	100	603409
FAM134B	25964	NM_001034850.2	99	613114	GAA	4065	NM_000152.4	100	606800
FAM161A	25808	NM_032180.2	100	613596	GABRA1	4075	NM_000806.5	100	137160
FAM20A	23015	NM_017565.3	99	611062	GABRB3	4083	NM_000814.5	99	137192
FAM20C	22140	NM_020223.3	100	611061	GABRG2	4087	NM_000816.3	100	137164
FAM58A	28434	NM_152274.4	83	300708	GAD1	4092	NM_000817.2	100	605363
FANCA	3582	NM_000135.3	100	607139	GALC	4115	NM_000153.3	99	606890
FANCB	3583	NM_001018113.2	99	300515	GALE	4116	NM_000403.3	100	606953
FANCC	3584	NM_000136.2	100	613899	GALK1	4118	NM_000154.1	100	604313
FANCD2	3585	NM_033084.4	100	613984	GALNS	4122	NM_000512.4	99	612222
FANCE	3586	NM_021922.2	95	613976	GALT	4135	NM_000155.3	100	606999
FANCF	3587	NM_022725.3	100	613897	GAMT	4136	NM_000156.5	100	601240
FANCG	3588	NM_004629.1	100	602956	GAS8	4166	NM_001286209.1	99	605178
FANCI	25568	NM_001113378.1	99	611360	GATA2	4171	NM_032638.4	100	137295
FANCL	20748	NM_018062.3	100	608111	GATA4	4173	NM_002052.4	89	600576
FANCM	23168	NM_020937.3	100	609644	GATA6	4174	NM_005257.5	92	601656
FAR1	26222	NM_032228.5	98	616107	GATAD2B	30778	NM_020699.3	100	614998
FAT4	23109	NM_024582.4	100	612411	GATM	4175	NM_001482.2	100	602360
FBN1	3603	NM_000138.4	100	134797	GBA	4177	NM_001005741.2	100	606463
FBN2	3604	NM_001999.3	100	612570	GBA2	18986	NM_020944.2	100	609471
FBP1	3606	NM_000507.3	100	611570	GCDH	4189	NM_000159.3	100	608801
FBXL4	13601	NM_012160.4	100	605654	GCH1	4193	NM_000161.2	100	600225
FEZF1	22788	NM_001160264.2	100	613301	GCSH	4208	NM_004483.4	85	238330
FGD1	3663	NM_004463.2	98	300546	GDF5	4220	NM_000557.4	100	601146
FGF10	3666	NM_004465.1	100	602115	GDF6	4221	NM_001001557.3	100	601147
FGF12	3668	NM_004113.5	100	601513	GDI1	4226	NM_001493.2	100	300104
FGF3	3681	NM_005247.2	100	164950	GFAP	4235	NM_002055.4	99	137780
FGF9	3687	NM_002010.2	100	600921	GFER	4236	NM_005262.2	100	600924
FGFR1	3688	NM_023110.2	100	136350	GFM1	13780	NM_024996.5	100	606639
FGFR2	3689	NM_000141.4	100	176943	GHR	4263	NM_000163.4	99	600946
FGFR3	3690	NM_000142.4	100	134934	GJA1	4274	NM_000165.4	100	121014
FH	3700	NM_000143.3	95	606945	GJA3	4277	NM_021954.3	100	121015
FHL1	3702	NM_001449.4	99	300163	GJA8	4281	NM_005267.4	100	600897
FIG4	16873	NM_014845.5	100	609390	GJB2	4284	NM_004004.5	100	121011
FKBP14	18625	NM_017946.3	100	614505	GJB3	4285	NM_024009.2	100	603324
FKRP	17997	NM_024301.4	100	606596	GJC2	17494	NM_020435.3	96	608803
FKTN	3622	NM_001079802.1	99	607440	GK	4289	NM_000167.5	93	300474
FLAD1	24671	NM_025207.4	100	610595	GLB1	4298	NM_000404.3	99	611458
FLNA	3754	NM_001456.3	100	300017	GLDC	4313	NM_000170.2	95	238300
FLNB	3755	NM_001457.3	100	603381	GLDN	29514	NM_181789.3	100	608603
FLT4	3767	NM_002020.4	99	136352	GLE1	4315	NM_001003722.1	100	603371
FLVCR1	24682	NM_014053.3	99	609144	GLI2	4318	NM_005270.4	99	165230
FLVCR2	20105	NM_017791.2	100	610865	GLI3	4319	NM_000168.5	100	165240
FMN2	14074	NM_020066.4	88	606373	GLIS2	29450	NM_032575.2	100	608539
FMR1	3775	NM_002024.5	99	309550	GLIS3	28510	NM_152629.3	100	610192
FOLR1	3791	NM_016725.2	100	136430	GLUD1	4335	NM_005271.4	99	138130
FOXC1	3800	NM_001453.2	99	601090	GLUL	4341	NM_002065.6	100	138290
FOXC2	3801	NM_005251.2	100	602402	GM2A	4367	NM_000405.4	100	613109

Gene	ID	Transcript	%	OMIM	Gene	ID	Transcript	%	OMIM
GMNN	17493	NM_001251989.1	99	602842	HPGD	5154	NM_000860.5	100	601688
GMPPA	22923	NM_205847.2	100	615495	HPRT1	5157	NM_000194.2	98	308000
GMPPB	22932	NM_021971.2	100	615320	HPS1	5163	NM_000195.4	100	604982
GNA11	4379	NM_002067.4	100	139313	HPSE2	18374	NM_021828.4	100	613469
GNAI3	4387	NM_006496.3	100	139370	HR	5172	NM_005144.4	99	602302
GNAO1	4389	NM_020988.2	100	139311	HRAS	5173	NM_005343.3	100	190020
GNAQ	4390	NM_002072.4	94	600998	HSD17B10	4800	NM_004493.2	99	300256
GNAS	4392	NM_000516.5	100	139320	HSD17B4	5213	NM_000414.3	99	601860
GNB1	4396	NM_002074.4	100	139380	HSD3B7	18324	NM_025193.3	100	607764
GNB5	4401	NM_016194.3	100	604447	HSF4	5227	NM_001538.3	99	602438
GNPAT	4416	NM_014236.3	100	602744	HSPD1	5261	NM_002156.4	99	118190
GNPTAB	29670	NM_024312.4	100	607840	HSPG2	5273	NM_005529.6	99	142461
GNPTG	23026	NM_032520.4	99		HUWE1	30892	NM_031407.6	99	300697
GNS	4422	NM_002076.3	98	607664	HYAL1	5320	NM_153281.1	100	607071
GORAB	25676	NM_152281.2	100	607983	HYLS1	26558	NM_145014.2	100	610693
GPC3	4451	NM_004484.3	99	300037	IARS	5330	NM_002161.5	100	600709
GPC6	4454	NM_005708.4	100	604404	IDS	5389	NM_000202.7	100	300823
GPR126	13841	NM_020455.5	100		IDUA	5391	NM_000203.4	98	252800
GPR179	31371	NM_001004334.3	100	614515	IFIH1	18873	NM_022168.3	99	606951
GPR56	4512	NM_005682.6	100	604110	IFITM5	16644	NM_001025295.2	99	614757
GPSM2	29501	NM_013296.4	100	609245	IFT122	13556	NM_052985.3	100	606045
GPX4	4556	NM_001039847.2	90	138322	IFT140	29077	NM_014714.3	99	614620
GRHL2	2799	NM_024915.3	100	608576	IFT172	30391	NM_015662.2	99	607386
GRHL3	25839	NM_198174.2	100	608317	IFT43	29669	NM_052873.2	100	614068
GRIA3	4573	NM_000828.4	99	305915	IFT80	29262	NM_020800.2	99	611177
GRIK2	4580	NM_021956.4	100	138244	IGBP1	5461	NM_001551.2	100	300139
GRIN1	4584	NM_007327.3	100	138249	IGF1	5464	NM_000618.4	100	147440
GRIN2A	4585	NM_000833.4	100	138253	IGF1R	5465	NM_000875.4	100	147370
GRIN2B	4586	NM_000834.3	100	138252	IGF2	5466	NM_000612.5	100	147470
GRIN2D	4588	NM_000836.2	84	602717	IGFBP7	5476	NM_001553.2	99	602867
GRM1	4593	NM_001278066.1	100	604473	IGHMBP2	5542	NM_002180.2	99	600502
GRM6	4598	NM_000843.3	98	604096	IGSF1	5948	NM_001170961.1	99	300137
GSPT2	4622	NM_018094.4	100	300418	IHH	5956	NM_002181.3	100	600726
GTF2E2	4651	NM_002095.5	99	189964	IL11RA	5967	NM_001142784.2	100	600939
GTF2H5	21157	NM_207118.2	100	608780	IL1RAPL1	5996	NM_014271.3	100	300206
GTPBP3	14880	NM_133644.3	100	608536	IMPAD1	26019	NM_017813.4	100	614010
GUCY2C	4688	NM_004963.3	100	601330	INPP5E	21474	NM_019892.5	99	613037
GUSB	4696	NM_000181.3	92	611499	INPPL1	6080	NM_001567.3	99	600829
HACE1	21033	NM_020771.3	100	610876	IQSEC2	29059	NM_001111125.2	97	300522
HADH	4799	NM_005327.4	100		IRF6	6121	NM_006147.3	100	607199
HADHA	4801	NM_000182.4	99	600890	IRX5	14361	NM_005853.5	99	606195
HAX1	16915	NM_006118.3	100	605998	ISPD	37276	NM_001101426.3	99	614631
HCCS	4837	NM_005333.4	100	300056	ITCH	13890	NM_031483.6	100	606409
HCFC1	4839	NM_005334.2	99	300019	ITGA3	6139	NM_002204.3	99	605025
HCN1	4845	NM_021072.3	100	602780	ITGA7	6143	NM_002206.2	100	600536
HDAC4	14063	NM_006037.3	99	605314	ITGA8	6144	NM_003638.2	99	604063
HDAC8	13315	NM_018486.2	100	300269	ITPR1	6180	NM_002222.5	100	147265
HEATR2	26013	NM_017802.3	95	614864	IVD	6186	NM_002225.3	100	607036
HECW2	29853	NM_020760.3	99	617245	JAG1	6188	NM_000214.2	99	601920
HESX1	4877	NM_003865.2	100	601802	JAGN1	26926	NM_032492.3	100	616012
HEXA	4878	NM_000520.5	100	606869	JAK3	6193	NM_000215.3	99	600173
HEXB	4879	NM_000521.3	100	606873	JAM3	15532	NM_032801.4	100	606871
HGSNAT	26527	NM_152419.2	95	610453	KANSL1	24565	NM_001193466.1	99	612452
HIBCH	4908	NM_014362.3	99	610690	KARS	6215	NM_001130089.1	100	601421
HINT1	4912	NM_005340.6	100	601314	KAT6A	13013	NM_006766.4	100	601408
HIST1H1E	4718	NM_005321.2	100	142220	KAT6B	17582	NM_012330.3	100	605880
HIVEP2	4921	NM_006734.3	100	143054	KBTBD13	37227	NM_001101362.2	100	613727
HLCS	4976	NM_000411.6	100	609018	KCNA2	6220	NM_001204269.1	100	176262
HMGCL	5005	NM_000191.2	100	613898	KCNB1	6231	NM_004975.3	100	600397
HMGCS2	5008	NM_005518.3	100	600234	KCNC1	6233	NM_001112741.1	100	176258
HMX1	5017	NM_018942.2	85	142992	KCNC3	6235	NM_004977.2	89	176264
HNF1B	11630	NM_000458.3	99	189907	KCNE1	6240	NM_000219.5	100	176261
HNF4A	5024	NM_175914.4	99	600281	KCNH1	6250	NM_172362.2	100	603305
HNRNPH2	5042	NM_001199974.1	100	300610	KCNJ10	6256	NM_002241.4	100	602208
HNRNPU	5048	NM_031844.2	100	602869	KCNJ11	6257	NM_000525.3	100	600937
HOXA1	5099	NM_005522.4	100	142955	KCNJ6	6267	NM_002240.4	100	600877
HOXA13	5102	NM_000522.4	88	142959	KCNMA1	6284	NM_002247.3	100	600150
HOXB1	5111	NM_002144.3	100	142968	KCNQ1	6294	NM_000218.2	97	607542
HOXC13	5125	NM_017410.2	100	142976	KCNQ2	6296	NM_172107.3	100	602235
HOXD13	5136	NM_000523.3	100	142989	KCNQ3	6297	NM_004519.3	100	602232
HPD	5147	NM_002150.2	100	609695	KCNT1	18865	NM_020822.2	99	608167

Gene	ID	RefSeq	%	OMIM	Gene	ID	RefSeq	%	OMIM
KCTD1	18249	NM_001258221.1	100	613420	MAN2B1	6826	NM_000528.3	99	609458
KCTD7	21957	NM_153033.4	100	611725	MANBA	6831	NM_005908.3	100	609489
KDM5B	18039	NM_006618.4	100	605393	MAOA	6833	NM_000240.3	100	309850
KDM5C	11114	NM_004187.3	99	314690	MAP2K1	6840	NM_002755.3	99	176872
KDM6A	12637	NM_021140.3	98	300128	MAP2K2	6842	NM_030662.3	99	601263
KIAA0226	28991	NM_001145642.4	100	613516	MAP3K1	6848	NM_005921.1	99	600982
KIAA0586	19960	NM_001244189.1	99	610178	MAP3K7	6859	NM_003188.3	99	602614
KIAA1109	26953	NM_015312.3	99	611565	MAPRE2	6891	NM_001143826.2	100	605789
KIAA1279	23419	NM_015634.3	100	609367	MASP1	6901	NM_139125.3	100	600521
KIAA2022	29433	NM_001008537.2	100	300524	MAT1A	6903	NM_000429.2	99	610550
KIDINS220	29508	NM_020738.3	100	615759	MATN3	6909	NM_002381.4	84	602109
KIF11	6388	NM_004523.3	98	148760	MBD5	20444	NM_018328.4	100	611472
KIF1A	888	NM_004321.7	99	601255	MBOAT7	15505	NM_001146083.2	99	606048
KIF22	6391	NM_007317.2	100	603213	MC2R	6930	NM_000529.2	100	607397
KIF2A	6318	NM_001098511.2	100	602591	MCCC1	6936	NM_020166.4	100	609010
KIF4A	13339	NM_012310.4	99	300521	MCCC2	6937	NM_022132.4	100	609014
KIF5C	6325	NM_004522.2	100	604593	MCEE	16732	NM_032601.3	100	608419
KIF7	30497	NM_198525.2	97	611254	MCOLN1	13356	NM_020533.2	99	605248
KIRREL3	23204	NM_032531.3	99	607761	MCPH1	6954	NM_024596.4	100	607117
KIT	6342	NM_000222.2	100	164920	MDH2	6971	NM_005918.3	99	154100
KLF1	6345	NM_006563.4	100	600599	MECOM	3498	NM_004991.3	100	165215
KLHL40	30372	NM_152393.3	100	615340	MECP2	6990	NM_004992.3	100	300005
KLHL7	15646	NM_001031710.2	100	611119	MECR	19691	NM_001024732.3	100	608205
KMT2A	7132	NM_001197104.1	100	159555	MED12	11957	NM_005120.2	99	300188
KMT2D	7133	NM_003482.3	100	602113	MED17	2375	NM_004268.4	100	603810
KPTN	6404	NM_007059.3	100	615620	MED23	2372	NM_015979.3	99	605042
KRAS	6407	NM_004985.4	100	190070	MEF2C	6996	NM_002397.4	99	600662
KRIT1	1573	NM_194456.1	100	604214	MEGF10	29634	NM_032446.2	100	612453
KRT74	28929	NM_175053.3	100	608248	MEGF8	3233	NM_001410.2	100	604267
L1CAM	6470	NM_000425.4	99	308840	MEOX1	7013	NM_004527.3	100	600147
L2HGDH	20499	NM_024884.2	99	609584	MESP2	29659	NM_001039958.1	96	605195
LAMA1	6481	NM_005559.3	100	150320	MFSD2A	25897	NM_001136493.2	100	614397
LAMA2	6482	NM_000426.3	100	156225	MFSD8	28486	NM_152778.2	100	611124
LAMB1	6486	NM_002291.2	100	150240	MGAT2	7045	NM_002408.3	100	602616
LAMC3	6494	NM_006059.3	99	604349	MGP	7060	NM_000900.4	99	154870
LAMP2	6501	NM_002294.2	96	309060	MICU1	1530	NM_006077.3	99	605084
LARGE	6511	NM_004737.5	99	603590	MID1	7095	NM_000381.3	100	300552
LARP7	24912	NM_016648.3	96	612026	MITF	7105	NM_000248.3	100	156845
LARS2	17095	NM_015340.3	100	604544	MKKS	7108	NM_018848.3	100	604896
LBR	6518	NM_002296.3	99	600024	MKS1	7121	NM_017777.3	100	609883
LDB3	15710	NM_001080116.1	100	605906	MLC1	17082	NM_015166.3	100	605908
LEMD3	28887	NM_014319.4	99	607844	MLYCD	7150	NM_012213.2	99	606761
LEPRE1	19316	NM_022356.3	100	610339	MMAA	18871	NM_172250.2	100	607481
LFNG	6560	NM_001040167.1	85	602576	MMAB	19331	NM_052845.3	100	607568
LHX3	6595	NM_014564.4	100	600577	MMACHC	24525	NM_015506.2	100	609831
LHX4	21734	NM_033343.3	100	602146	MMADHC	25221	NM_015702.2	98	611935
LIG4	6601	NM_002312.3	100	601837	MMP13	7159	NM_002427.3	100	600108
LINS	30922	NM_001040616.2	100		MMP21	14357	NM_147191.1	99	608416
LIPN	23452	NM_001102469.1	100	613924	MNX1	4979	NM_005515.3	75	142994
LMBRD1	23038	NM_018368.3	97	612625	MOCS1	7190	NM_005943.5	100	603707
LMNA	6636	NM_170707.3	98	150330	MOCS2	7193	NM_176806.3	100	603708
LMX1B	6654	NM_002316.3	100	602575	MOGS	24862	NM_006302.2	99	601336
LONP1	9479	NM_001276480.1	100	605490	MORC2	23573	NM_014941.3	100	616661
LRAT	6685	NM_004744.4	100	604863	MPDU1	7207	NM_004870.3	100	604041
LRBA	1742	NM_006726.4	100	606453	MPI	7216	NM_002435.2	100	154550
LRIG2	20889	NM_014813.2	100	608869	MPLKIP	16002	NM_138701.3	100	609188
LRIT3	24783	NM_198506.4	94	615004	MPV17	7224	NM_002437.4	100	137960
LRP2	6694	NM_004525.2	100	600073	MRE11A	7230	NM_005591.3	99	600814
LRP4	6696	NM_002334.3	99	604270	MRPS22	14508	NM_020191.2	100	605810
LRP5	6697	NM_002335.3	99	603506	MSL3	7370	NM_078629.3	98	
LRPPRC	15714	NM_133259.3	99	607544	MSX1	7391	NM_002448.3	100	142983
LRRC6	16725	NM_012472.5	99	614930	MSX2	7392	NM_002449.4	100	123101
LTBP2	6715	NM_000428.2	100		MTHFR	7436	NM_005957.4	100	607093
LTBP3	6716	NM_001130144.2	99		MTM1	7448	NM_000252.2	100	300415
LYST	1968	NM_000081.3	99	606897	MTO1	19261	NM_012123.3	99	614667
MAB21L2	6758	NM_006439.4	100	604357	MTR	7468	NM_000254.2	100	156570
MAF	6776	NM_005360.4	88	177075	MTRR	7473	NM_002454.2	100	602568
MAFB	6408	NM_005461.4	100	608968	MUT	7526	NM_000255.3	100	609058
MAGEL2	6814	NM_019066.4	96	605283	MYCN	7559	NM_005378.5	100	164840
MAMLD1	2568	NM_005491.4	100	300120	MYH3	7573	NM_002470.3	100	160720
MAN1B1	6823	NM_016219.4	99	604346	MYH6	7576	NM_002471.3	99	160710

Gene	ID	Transcript	%	OMIM	Gene	ID	Transcript	%	OMIM
MYH8	7578	NM_002472.2	100	160741	NYX	8082	NM_022567.2	99	300278
MYH9	7579	NM_002473.5	99	160775	OBSL1	29092	NM_015311.2	99	610991
MYO5A	7602	NM_000259.3	99	160777	OCRL	8108	NM_000276.3	99	300535
MYO5B	7603	NM_001080467.2	99	606540	OFD1	2567	NM_003611.2	94	300170
MYO7A	7606	NM_000260.3	99	276903	OPHN1	8148	NM_002547.2	100	300127
MYT1L	7623	NM_015025.3	100	613084	ORC1	8487	NM_004153.3	100	601902
NAA10	18704	NM_003491.3	99	300013	ORC4	8490	NM_002552.4	99	603056
NAA15	30782	NM_057175.4	99	608000	ORC6	17151	NM_014321.3	100	607213
NAGA	7631	NM_000262.2	100	104170	OTC	8512	NM_000531.5	100	300461
NAGLU	7632	NM_000263.3	96	609701	OTOGL	26901	NM_173591.3	99	614925
NAGS	17996	NM_153006.2	100	608300	OTX2	8522	NM_001270524.1	100	600037
NALCN	19082	NM_052867.3	100	611549	OXCT1	8527	NM_000436.3	100	601424
NANS	19237	NM_018946.3	100	605202	P4HB	8548	NM_000918.3	100	176790
NBAS	15625	NM_015909.3	100	608025	PACS1	30032	NM_018026.3	99	607492
NBN	7652	NM_002485.4	100	602667	PAFAH1B1	8574	NM_000430.3	95	601545
NDE1	17619	NM_001143979.1	100	609449	PAH	8582	NM_000277.1	100	612349
NDP	7678	NM_000266.3	100	300658	PAK3	8592	NM_002578.4	99	300142
NDST1	7680	NM_001543.4	100	600853	PALB2	26144	NM_024675.3	100	610355
NDUFA1	7683	NM_004541.3	100	300078	PAPSS2	8604	NM_001015880.1	99	603005
NDUFA10	7684	NM_004544.3	98	603835	PARN	8609	NM_002582.3	100	604212
NDUFAF2	28086	NM_174889.4	94	609653	PAX2	8616	NM_003987.4	100	167409
NDUFB11	20372	NM_001135998.2	96	300403	PAX3	8617	NM_181457.3	100	606597
NDUFS1	7707	NM_005006.6	100	157655	PAX6	8620	NM_000280.4	100	607108
NDUFS4	7711	NM_002495.3	100	602694	PAX8	8622	NM_003466.3	100	167415
NDUFS7	7714	NM_024407.4	100	601825	PAX9	8623	NM_006194.3	99	167416
NDUFS8	7715	NM_002496.3	100	602141	PC	8636	NM_000920.3	100	608786
NDUFV1	7716	NM_007103.3	100	161015	PCBD1	8646	NM_000281.3	99	126090
NEK1	7744	NM_012224.2	100	604588	PCCA	8653	NM_000282.3	99	232000
NEK8	13387	NM_178170.2	100	609799	PCCB	8654	NM_000532.4	100	232050
NEU1	7758	NM_000434.3	99	608272	PCDH19	14270	NM_001184880.1	100	300460
NF1	7765	NM_000267.3	95	613113	PCGF2	12929	NM_007144.2	100	
NFIX	7788	NM_002501.3	100	164005	PCNT	16068	NM_006031.5	99	605925
NFU1	16287	NM_001002755.2	100	608100	PCYT1A	8754	NM_005017.3	99	123695
NGLY1	17646	NM_018297.3	100	610661	PDE10A	8772	NM_001130690.2	100	610652
NHP2	14377	NM_017838.3	100	606470	PDE4D	8783	NM_001104631.1	100	600129
NHS	7820	NM_198270.3	98	300457	PDE6G	8789	NM_002602.3	100	180073
NIPBL	28862	NM_133433.3	98	608667	PDE6H	8790	NM_006205.2	100	601190
NKX2-1	11825	NM_001079668.2	100		PDGFRB	8804	NM_002609.3	99	173410
NKX2-5	2488	NM_004387.3	100		PDHA1	8806	NM_000284.3	99	300502
NKX3-2	951	NM_001189.3	99		PDHX	21350	NM_003477.2	100	
NLGN3	14289	NM_018977.3	100	300336	PDSS1	17759	NM_014317.4	98	607429
NME1	7849	NM_000269.2	100	156490	PDSS2	23041	NM_020381.3	99	610564
NMNAT1	17877	NM_022787.3	100	608700	PEPD	8840	NM_000285.3	99	613230
NODAL	7865	NM_018055.4	100	601265	PET100	40038	NM_001171155.1	100	614770
NOG	7866	NM_005450.4	100	602991	PEX1	8850	NM_000466.2	100	602136
NONO	7871	NM_001145410.1	100	300084	PEX10	8851	NM_153818.1	99	602859
NOP10	14378	NM_018648.3	100	606471	PEX12	8854	NM_000286.2	100	601758
NOTCH1	7881	NM_017617.4	99	190198	PEX13	8855	NM_002618.3	100	601789
NOTCH2	7882	NM_024408.3	99	600275	PEX14	8856	NM_004565.2	99	601791
NPC1	7897	NM_000271.4	99	607623	PEX16	8857	NM_004813.2	100	603360
NPC2	14537	NM_006432.3	100	601015	PEX19	9713	NM_002857.3	100	600279
NPHP1	7905	NM_000272.3	100	607100	PEX2	9717	NM_000318.2	100	170993
NPHP3	7907	NM_153240.4	99	608002	PEX26	22965	NM_017929.5	100	608666
NPHP4	19104	NM_015102.4	99	607215	PEX3	8858	NM_003630.2	100	603164
NPHS1	7908	NM_004646.3	99	602716	PEX5	9719	NM_001131025.1	100	600414
NPHS2	13394	NM_014625.3	100	604766	PEX6	8859	NM_000287.3	97	601498
NPR2	7944	NM_003995.3	100	108961	PEX7	8860	NM_000288.3	99	601757
NR2F1	7975	NM_005654.5	100	132890	PGAP2	17893	NM_001256240.1	100	615187
NR2F2	7976	NM_021005.3	100	107773	PGAP3	23719	NM_033419.4	99	611801
NR5A1	7983	NM_004959.4	100	184757	PGK1	8896	NM_000291.3	98	311800
NRAS	7989	NM_002524.4	100	164790	PGM1	8905	NM_002633.2	100	171900
NRXN1	8008	NM_001135659.2	100	600565	PGM3	8907	NM_001199917.1	100	172100
NRXN2	8009	NM_138732.2	99	600566	PHC1	3182	NM_004426.2	98	602978
NSD1	14234	NM_022455.4	100	606681	PHF21A	24156	NM_001101802.1	100	608325
NSDHL	13398	NM_015922.2	100	300275	PHF6	18145	NM_032458.2	98	300414
NSUN2	25994	NM_017755.5	96	610916	PHF8	20672	NM_015107.2	99	300560
NT5C3A	17820	NM_016489.12	99	606224	PHGDH	8923	NM_006623.3	100	606879
NTRK1	8031	NM_001012331.1	99	191315	PHOX2B	9143	NM_003924.3	100	603851
NUBPL	20278	NM_025152.2	99	613621	PIEZO1	28993	NM_001142864.3	99	611184
NUP107	29914	NM_020401.3	100	607617	PIEZO2	26270	NM_022068.3	99	613629
NUP62	8066	NM_001193357.1	100	605815	PIGA	8957	NM_002641.3	99	311770

PIGG	25985 NM_017733.4	100	616918	PTEN	9588 NM_000314.6	100	601728
PIGL	8966 NM_004278.3	100	605947	PTF1A	23734 NM_178161.2	98	607194
PIGN	8967 NM_176787.4	100	606097	PTH	9606 NM_000315.3	100	168450
PIGO	23215 NM_032634.3	100	614730	PTH1R	9608 NM_000316.2	100	
PIGT	14938 NM_015937.5	100	610272	PTHLH	9607 NM_198965.1	100	168470
PIGV	26031 NM_017837.3	100	610274	PTPN11	9644 NM_002834.4	99	176876
PIK3CA	8975 NM_006218.3	100	171834	PTPN14	9647 NM_005401.4	99	603155
PIK3R1	8979 NM_181523.2	100	171833	PTS	9689 NM_000317.2	100	612719
PIK3R2	8980 NM_005027.3	93	603157	PUF60	17042 NM_078480.2	100	604819
PITX1	9004 NM_002653.4	98	602149	PURA	9701 NM_005859.4	99	600473
PITX2	9005 NM_153427.2	100	601542	PVRL4	19688 NM_030916.2	100	609607
PITX3	9006 NM_005029.3	100	602669	PXDN	14966 NM_012293.2	99	605158
PKD1L1	18053 NM_138295.4	100	609721	PYCR1	9721 NM_006907.3	100	179035
PKHD1	9016 NM_138694.3	100	606702	PYCR2	30262 NM_013328.3	100	616406
PLA2G6	9039 NM_003560.3	100	603604	PYGL	9725 NM_002863.4	100	613741
PLCB1	15917 NM_015192.3	100	607120	PYROXD1	26162 NM_024854.4	98	617220
PLCB4	9059 NM_000933.3	100	600810	QARS	9751 NM_005051.2	100	603727
PLCE1	17175 NM_016341.3	99	608414	QDPR	9752 NM_000320.2	100	612676
PLK4	11397 NM_014264.4	99	605031	QRICH1	24713 NM_198880.2	100	617387
PLOD1	9081 NM_000302.3	100	153454	RAB18	14244 NM_021252.4	100	602207
PLOD2	9082 NM_182943.2	99	601865	RAB23	14263 NM_183227.2	100	606144
PLOD3	9083 NM_001084.4	100	603066	RAB39B	16499 NM_171998.3	100	300774
PLP1	9086 NM_000533.4	100	300401	RAB3GAP1	17063 NM_012233.2	100	602536
PMM2	9115 NM_000303.2	100	601785	RAB3GAP2	17168 NM_012414.3	99	609275
PNKP	9154 NM_007254.3	100	605610	RAD21	9811 NM_006265.2	99	606462
PNPLA1	21246 NM_001145717.1	100	612121	RAD50	9816 NM_005732.3	99	604040
PNPLA2	30802 NM_020376.3	99	609059	RAD51C	9820 NM_058216.2	100	602774
PNPO	30260 NM_018129.3	100	603287	RAF1	9829 NM_002880.3	100	164760
PNPT1	23166 NM_033109.4	99	610316	RAI1	9834 NM_030665.3	100	607642
POC1A	24488 NM_015426.4	100	614783	RAPSN	9863 NM_005055.4	99	601592
POC1B	30836 NM_172240.2	99	614784	RARB	9865 NM_000965.4	100	180220
POGZ	18801 NM_015100.3	99	614787	RARS2	21406 NM_020320.4	100	611524
POLD1	9175 NM_002691.3	96	174761	RASA1	9871 NM_002890.2	98	139150
POLG	9179 NM_002693.2	100	174763	RAX	18662 NM_013435.2	99	601881
POLR1A	17264 NM_015425.4	99	616404	RBM10	9896 NM_005676.4	100	300080
POLR1C	20194 NM_203290.3	100	610060	RBM28	21863 NM_018077.2	100	612074
POLR1D	20422 NM_015972.3	100	613715	RBM8A	9905 NM_005105.4	100	605313
POLR3A	30074 NM_007055.3	100	614258	RBPJ	5724 NM_005349.3	99	147183
POLR3B	30348 NM_018082.5	100	614366	RECQL4	9949 NM_004260.3	99	603780
POMGNT1	19139 NM_017739.3	100	606822	RELN	9957 NM_005045.3	100	600514
POMGNT2	25902 NM_032806.5	100	614828	RERE	9965 NM_012102.3	96	605226
POMT1	9202 NM_007171.3	99	607423	RET	9967 NM_020975.4	99	164761
POMT2	19743 NM_013382.5	99	607439	RFT1	30220 NM_052859.3	100	611908
PORCN	17652 NM_203475.2	100	300651	RFX6	21478 NM_173560.3	100	612659
POU1F1	9210 NM_000306.3	100	173110	RIN2	18750 NM_018993.3	100	610222
PPA2	28883 NM_176869.2	99	609988	RIPK4	496 NM_020639.2	100	605706
PPM1D	9277 NM_003620.3	100	605100	RIT1	10023 NM_006912.5	100	609591
PPP1CB	9282 NM_206876.1	100	600590	RLIM	13429 NM_016120.3	100	300379
PPP2R1A	9302 NM_014225.5	100	605983	RMND1	21176 NM_017909.3	100	614917
PPP2R5D	9312 NM_006245.3	100	601646	RNASEH2A	18518 NM_006397.2	100	606034
PPT1	9325 NM_000310.3	100	600722	RNASEH2B	25671 NM_024570.3	100	610326
PQBP1	9330 NM_005710.2	100	300463	RNASEH2C	24116 NM_032193.3	100	610330
PRDM12	13997 NM_021619.2	91	616458	RNASET2	21686 NM_003730.4	99	612944
PREPL	30228 NM_006036.4	100	609557	RNF135	21158 NM_032322.3	99	611358
PRKAR1A	9388 NM_002734.4	99	188830	RNF168	26661 NM_152617.3	100	612688
PRKD1	9407 NM_002742.2	100	605435	ROBO3	13433 NM_022370.3	99	608630
PRMT7	25557 NM_019023.3	100	610087	ROGDI	29478 NM_024589.2	99	614574
PROP1	9455 NM_006261.4	98	601538	ROR2	10257 NM_004560.3	100	602337
PROSC	9457 NM_007198.3	100	604436	RPE65	10294 NM_000329.2	100	180069
PRPS1	9462 NM_002764.3	100	311850	RPGRIP1	13436 NM_020366.3	100	605446
PRRT2	30500 NM_145239.2	100	614386	RPGRIP1L	29168 NM_015272.4	96	610937
PRRX1	9142 NM_022716.3	100	167420	RPS19	10402 NM_001022.3	100	603474
PRSS12	9477 NM_003619.3	100	606709	RPS6KA3	10432 NM_004586.2	99	300075
PRSS56	39433 NM_001195129.1	99	613858	RRAS	10447 NM_006270.4	99	165090
PRUNE	13420 NM_021222.2	100	617413	RRM2B	17296 NM_015713.4	100	604712
PSAP	9498 NM_002778.3	100	176801	RSPH1	12371 NM_080860.3	100	609314
PSAT1	19129 NM_058179.3	99	610936	RSPH3	21054 NM_031924.5	100	615876
PSMB8	9545 NM_148919.3	100	177046	RSPO4	16175 NM_001029871.3	100	610573
PSPH	9577 NM_004577.3	100	172480	RSPRY1	29420 NM_133368.2	100	616585
PTCH1	9585 NM_000264.3	99	601309	RTEL1	15888 NM_032957.4	99	608833
PTDSS1	9587 NM_014754.2	100	612792	RTN4IP1	18647 NM_032730.5	100	610502

Gene	ID	Transcript	%	OMIM	Gene	ID	Transcript	%	OMIM
RTTN	18654	NM_173630.3	99	610436	SLC35C1	20197	NM_018389.4	100	605881
RUNX2	10472	NM_001024630.3	100	600211	SLC35D1	20800	NM_015139.2	99	610804
RYR1	10483	NM_000540.2	99	180901	SLC39A13	20859	NM_152264.4	100	608735
SACS	10519	NM_014363.5	100	604490	SLC39A8	20862	NM_001135147.1	100	608732
SALL1	10524	NM_002968.2	99	602218	SLC46A1	30521	NM_080669.5	99	611672
SALL4	15924	NM_020436.4	99	607343	SLC4A1	11027	NM_000342.3	100	109270
SAMHD1	15925	NM_015474.3	100	606754	SLC4A11	16438	NM_032034.3	100	610206
SATB2	21637	NM_015265.3	99	608148	SLC4A4	11030	NM_003759.3	100	603345
SBDS	19440	NM_016038.3	100	607444	SLC52A3	16187	NM_033409.3	100	613350
SC5D	10547	NM_006918.4	100		SLC5A5	11040	NM_000453.2	100	601843
SCARF2	19869	NM_153334.6	99	613619	SLC5A7	14025	NM_021815.4	100	608761
SCN11A	10583	NM_014139.2	99	604385	SLC6A1	11042	NM_003042.3	100	137165
SCN1A	10585	NM_001165963.1	100	182389	SLC6A17	31399	NM_001010898.3	100	610299
SCN1B	10586	NM_001037.4	98	600235	SLC6A3	11049	NM_001044.4	100	126455
SCN2A	10588	NM_021007.2	99	182390	SLC6A5	11051	NM_004211.4	100	604159
SCN3A	10590	NM_006922.3	100	182391	SLC6A8	11055	NM_005629.3	98	300036
SCN4A	10591	NM_000334.4	100	603967	SLC6A9	11056	NM_001024845.2	100	601019
SCN8A	10596	NM_014191.3	99	600702	SLC9A6	11079	NM_006359.2	100	300231
SCO1	10603	NM_004589.3	99	603644	SLX4	23845	NM_032444.3	100	613278
SCO2	10604	NM_005138.2	100	604272	SMAD3	6769	NM_005902.3	99	603109
SCYL1	14372	NM_020680.3	100	607982	SMAD4	6770	NM_005359.5	100	600993
SDCCAG8	10671	NM_006642.4	100	613524	SMARCA2	11098	NM_003070.4	98	600014
SDHA	10680	NM_004168.3	88	600857	SMARCA4	11100	NM_001128849.1	99	603254
SDHAF1	33867	NM_001042631.2	100	612848	SMARCAL1	11102	NM_014140.3	100	606622
SEC23B	10702	NM_006363.4	99	610512	SMARCB1	11103	NM_003073.4	100	601607
SEC24D	10706	NM_014822.3	100	607186	SMARCE1	11109	NM_003079.4	99	603111
SECISBP2	30972	NM_024077.4	100	607693	SMC1A	11111	NM_006306.3	100	300040
SET	10760	NM_001122821.1	97	600960	SMC3	2468	NM_005445.3	97	606062
SETBP1	15573	NM_015559.2	98	611060	SMCHD1	29090	NM_015295.2	99	614982
SETD1A	29010	NM_014712.2	99	611052	SMG9	25763	NM_019108.3	100	613176
SETD5	25566	NM_001080517.2	100	615743	SMO	11119	NM_005631.4	99	615854
SF3B4	10771	NM_005850.4	100	605593	SMOC1	20318	NM_001034852.2	100	608488
SGSH	10818	NM_000199.3	97	605270	SMOC2	20323	NM_022138.2	99	607223
SH3PXD2B	29242	NM_001017995.2	100	613293	SMPD1	11120	NM_000543.4	99	607608
SHANK1	15474	NM_016148.3	97	604999	SMS	11123	NM_004595.4	95	300105
SHANK3	14294	NM_033517.1	93	606230	SNAP29	11133	NM_004782.3	100	604202
SHH	10848	NM_000193.3	99	600725	SNIP1	30587	NM_024700.3	100	608241
SHOC2	15454	NM_007373.3	100	602775	SNRPB	11153	NM_003091.3	100	182282
SHOX	10853	NM_000451.3	98	312865	SNRPE	11161	NM_003094.3	100	128260
SHROOM3	30422	NM_020859.3	99	604570	SNX14	14977	NM_020468.5	99	616105
SIK1	11142	NM_173354.4	99	605705	SOBP	29256	NM_018013.3	99	613667
SIL1	24624	NM_022464.4	100	608005	SON	11183	NM_032195.2	99	182465
SIN3A	19353	NM_001145357.1	100	607776	SOS1	11187	NM_005633.3	99	182530
SIX1	10887	NM_005982.3	99	601205	SOX10	11190	NM_006941.3	100	602229
SIX3	10889	NM_005413.3	100	603714	SOX11	11191	NM_003108.3	100	600898
SIX5	10891	NM_175875.4	98	600963	SOX17	18122	NM_022454.3	100	610928
SKI	10896	NM_003036.3	99	164780	SOX2	11195	NM_003106.3	100	184429
SKIV2L	10898	NM_006929.4	100	600478	SOX3	11199	NM_005634.2	98	313430
SLC12A6	10914	NM_133647.1	100	604878	SOX5	11201	NM_006940.5	100	604975
SLC13A5	23089	NM_177550.4	100	608305	SOX9	11204	NM_000346.3	100	608160
SLC16A2	10923	NM_006517.4	98	300095	SPAG1	11212	NM_172218.2	99	603395
SLC17A5	10933	NM_012434.4	100	604322	SPARC	11219	NM_003118.3	100	182120
SLC19A3	16266	NM_025243.3	100	606152	SPATA5	18119	NM_145207.2	100	613940
SLC1A2	10940	NM_004171.3	100	600300	SPECC1L	29022	NM_015330.4	100	614140
SLC22A5	10969	NM_003060.3	100	603377	SPEG	16901	NM_005876.4	99	615950
SLC24A4	10978	NM_153646.3	99	609840	SPG11	11226	NM_025137.3	99	610844
SLC25A15	10985	NM_014252.3	99	603861	SPR	11257	NM_003124.4	99	182125
SLC25A19	14409	NM_021734.4	100	606521	SPRED1	20249	NM_152594.2	100	609291
SLC25A20	1421	NM_000387.5	100	613698	SPTAN1	11273	NM_001130438.2	100	182810
SLC25A22	19954	NM_024698.5	100	609302	SPTLC2	11278	NM_004863.3	100	605713
SLC25A26	20661	NM_173471.3	100	611037	SRCAP	16974	NM_006662.2	99	611421
SLC25A38	26054	NM_017875.2	100	610819	SRD5A3	25812	NM_024592.4	100	611715
SLC25A4	10990	NM_001151.3	100	103220	SRPX2	30668	NM_014467.2	100	300642
SLC26A2	10994	NM_000112.3	100	606718	SRY	11311	NM_003140.2	40	480000
SLC27A4	10998	NM_005094.3	100	604194	ST14	11344	NM_021978.3	100	606797
SLC2A1	11005	NM_006516.2	100	138140	ST3GAL3	10866	NM_006279.4	100	606494
SLC2A10	13444	NM_030777.3	100	606145	ST3GAL5	10872	NM_003896.3	96	
SLC2A2	11006	NM_000340.1	100	138160	STAG1	11354	NM_005862.2	99	
SLC33A1	95	NM_004733.3	99	603690	STAMBP	16950	NM_006463.4	100	606247
SLC35A1	11021	NM_006416.4	100	605634	STAR	11359	NM_000349.2	100	300708
SLC35A2	11022	NM_001042498.2	99	314375	STAT1	11362	NM_007315.3	99	600555

Gene	ID	Transcript	%	OMIM	Gene	ID	Transcript	%	OMIM
STAT5B	11367	NM_012448.3	96	604260	TKT	11834	NM_001135055.2	99	606781
STIL	10879	NM_003035.2	100	181590	TM4SF20	26230	NM_024795.4	100	615404
STRA6	30650	NM_022369.3	100	610745	TMCO1	18188	NM_019026.4	100	614123
STS	11425	NM_000351.5	99	300747	TMEM126B	30883	NM_018480.5	100	615533
STT3A	6172	NM_001278503.1	100	601134	TMEM165	30760	NM_018475.4	100	614726
STT3B	30611	NM_178862.2	100	608605	TMEM216	25018	NM_001173990.2	100	613277
STX1B	18539	NM_052874.4	100	601485	TMEM237	14432	NM_001044385.2	100	614423
STXBP1	11444	NM_003165.3	100	602926	TMEM5	13530	NM_014254.2	99	605862
SUCLG1	11449	NM_003849.3	100	611224	TMEM67	28396	NM_153704.5	99	609884
SUMF1	20376	NM_182760.3	99	607939	TMEM70	26050	NM_017866.5	99	612418
SURF1	11474	NM_003172.3	91	185620	TMPRSS6	16517	NM_153609.3	100	609862
SUV420H1	24283	NM_017635.4	100	610881	TMTC3	26899	NM_181783.3	100	617218
SYN1	11494	NM_133499.2	94	313440	TNFRSF13B	18153	NM_012452.2	100	604907
SYNE1	17089	NM_033071.3	100	608441	TP63	15979	NM_003722.4	100	603273
SYNGAP1	11497	NM_006772.2	98	603384	TPM2	12011	NM_003289.3	100	190990
SYP	11506	NM_003179.2	100	313475	TPP1	2073	NM_000391.3	100	607998
SZT2	29040	NM_015284.3	100	615463	TRAIP	30764	NM_005879.2	100	605958
TAB2	17075	NM_015093.5	100	605101	TRAPPC11	25751	NM_021942.5	100	614138
TAC3	11521	NM_013251.3	100	162330	TRAPPC2	23068	NM_001011658.3	98	300202
TACO1	24316	NM_016360.3	99	612958	TRAPPC9	30832	NM_031466.7	100	611966
TACR3	11528	NM_001059.2	100	162332	TREX1	12269	NM_033629.4	100	606609
TAF1	11535	NM_004606.4	99	313650	TRIM32	16380	NM_012210.3	100	602290
TAF2	11536	NM_003184.3	99	604912	TRIM37	7523	NM_015294.4	100	605073
TANGO2	25439	NM_001283186.2	100	616830	TRIO	12303	NM_007118.3	99	601893
TAPT1	26887	NM_153365.2	94	612758	TRIP11	12305	NM_004239.4	99	604505
TAT	11573	NM_000353.2	100	613018	TRIP12	12306	NM_004238.2	99	604506
TAZ	11577	NM_000116.4	100	300394	TRIP4	12310	NM_016213.4	100	604501
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TBCD	11581	NM_005993.4	99	604649	TRPM1	7146	NM_002420.5	100	603576
TBCE	11582	NM_003193.4	100	604934	TRPS1	12340	NM_014112.4	100	604386
TBCK	28261	NM_001163436.2	99	616899	TRPV3	18084	NM_145068.3	100	607066
TBL1XR1	29529	NM_024665.5	99	608628	TRPV4	18083	NM_021625.4	100	605427
TBR1	11590	NM_006593.3	100	604616	TSC1	12362	NM_000368.4	99	605284
TBX1	11592	NM_080647.1	86	602054	TSC2	12363	NM_000548.4	100	191092
TBX15	11594	NM_152380.2	100	604127	TSEN15	16791	NM_052965.3	100	608756
TBX18	11595	NM_001080508.2	99	604613	TSEN2	28422	NM_025265.3	100	608753
TBX20	11598	NM_001077653.2	100	606061	TSEN34	15506	NM_024075.4	98	608754
TBX22	11600	NM_001109878.1	100	300307	TSEN54	27561	NM_207346.2	97	608755
TBX3	11602	NM_005996.3	99	601621	TSHB	12372	NM_000549.4	100	188540
TBX4	11603	NM_018488.3	99	601719	TSHR	12373	NM_000369.2	100	603372
TBX5	11604	NM_000192.3	100	601620	TSPAN7	11854	NM_004615.3	100	300096
TBXAS1	11609	NM_001061.4	100	274180	TTC19	26006	NM_017775.3	96	613814
TCF12	11623	NM_207036.1	100	600480	TTC37	23639	NM_014639.3	100	614589
TCF20	11631	NM_005650.3	100	603107	TTC7A	19750	NM_020458.3	99	609332
TCF4	11634	NM_001083962.1	100	602272	TTC8	20087	NM_198309.3	100	608132
TCN2	11653	NM_000355.3	100	613441	TTI2	26262	NM_001102401.2	100	614426
TCOF1	11654	NM_001135243.1	99	606847	TUBA1A	20766	NM_006009.3	100	602529
TCTN1	26113	NM_001082538.2	99		TUBA8	12410	NM_018943.2	100	605742
TCTN2	25774	NM_024809.4	100	613846	TUBB	20778	NM_178014.3	99	191130
TCTN3	24519	NM_015631.5	100	613847	TUBB4A	20774	NM_006087.3	100	602662
TECPR2	19957	NM_014844.4	100	615000	TUBGCP4	16691	NM_014444.4	99	609610
TEK	11724	NM_000459.4	100	600221	TUBGCP6	18127	NM_020461.3	100	610053
TELO2	29099	NM_016111.3	99	611140	TUFM	12420	NM_003321.4	100	602389
TERT	11730	NM_198253.2	99	187270	TUSC3	30242	NM_006765.3	100	601385
TFAP2A	11742	NM_003220.2	100	107580	TWIST1	12428	NM_000474.3	99	601622
TFAP2B	11743	NM_003221.3	100	601601	TWIST2	20670	NM_057179.2	100	607556
TGDS	20324	NM_014305.3	100	616146	TXNL4A	30551	NM_006701.4	100	611595
TGFB1	11766	NM_000660.6	99	190180	TYR	12442	NM_000372.4	100	606933
TGFB2	11768	NM_003238.4	100	190220	TYRP1	12450	NM_000550.2	100	115501
TGFB3	11769	NM_003239.4	100	190230	UBA5	23230	NM_198329.3	99	610552
TGFBR1	11772	NM_004612.3	95	190181	UBE2A	12472	NM_003336.3	100	312180
TGFBR2	11773	NM_003242.5	100	190182	UBE2T	25009	NM_014176.3	100	610538
TGIF1	11776	NM_173208.2	100	602630	UBE3A	12496	NM_130838.1	99	601623
TH	11782	NM_199292.2	99	191290	UBE3B	13478	NM_130466.3	100	608047
THAP1	20856	NM_018105.2	100	609520	UBR1	16808	NM_174916.2	100	605981
THOC2	19073	NM_001081550.1	99	300395	UGT1A1	12530	NM_000463.2	100	191740
THOC6	28369	NM_024339.4	100	615403	UMPS	12563	NM_000373.3	100	613891
THRA	11796	NM_199334.3	100	190120	UNC80	26582	NM_032504.1	100	612636
TIMM8A	11817	NM_004085.3	97	300356	UPF3B	20439	NM_080632.2	97	300298
TINF2	11824	NM_001099274.1	100	604319	UQCRB	12582	NM_006294.4	100	191330
TK2	11831	NM_004614.4	98	188250	UQCRQ	29594	NM_014402.4	100	612080

UROC1	26444	NM_144639.2	100	613012
UROS	12592	NM_000375.2	100	606938
USB1	25792	NM_024598.3	100	613276
USP18	12616	NM_017414.3	95	607057
USP27X	13486	NM_001145073.2	100	300975
USP9X	12632	NM_001039590.2	99	300072
UVSSA	29304	NM_020894.3	100	614632
VDR	12679	NM_001017535.1	100	601769
VIPAS39	20347	NM_022067.3	100	613401
VLDLR	12698	NM_003383.4	100	192977
VPS13B	2183	NM_017890.4	100	607817
VPS33B	12712	NM_018668.4	100	608552
VRK1	12718	NM_003384.2	100	602168
VSX2	1975	NM_182894.2	100	
WAC	17327	NM_016628.4	100	615049
WDPCP	28027	NM_015910.5	100	613580
WDR11	13831	NM_018117.11	98	606417
WDR19	18340	NM_025132.3	100	608151
WDR34	28296	NM_052844.3	100	613363
WDR35	29250	NM_001006657.1	99	613602
WDR45	28912	NM_007075.3	99	300526
WDR60	21862	NM_018051.4	100	615462
WDR62	24502	NM_001083961.1	99	613583
WDR73	25928	NM_032856.3	100	616144
WNT1	12774	NM_005430.3	100	164820
WNT10B	12775	NM_003394.3	100	601906
WNT3	12782	NM_030753.4	100	165330
WNT4	12783	NM_030761.4	96	603490
WNT5A	12784	NM_003392.4	100	164975
WNT7A	12786	NM_004625.3	100	601570
WRAP53	25522	NM_018081.2	100	612661
WT1	12796	NM_024426.4	99	607102
WWOX	12799	NM_016373.3	100	605131
XPA	12814	NM_000380.3	99	611153
XPC	12816	NM_004628.4	100	613208
XPNPEP3	28052	NM_022098.3	100	613553
XRCC4	12831	NM_022406.3	100	194363
XYLT1	15516	NM_022166.3	99	608124
XYLT2	15517	NM_022167.3	100	608125
YAP1	16262	NM_001130145.2	97	606608
YY1	12856	NM_003403.4	100	600013
ZBTB16	12930	NM_006006.4	100	176797
ZBTB18	13030	NM_205768.2	99	608433
ZBTB20	13503	NM_001164342.2	100	606025
ZC4H2	24931	NM_018684.3	100	300897
ZDHHC15	20342	NM_001146256.1	99	300576
ZDHHC9	18475	NM_016032.3	100	300646
ZEB2	14881	NM_014795.3	100	605802
ZFP57	18791	NM_001109809.2	100	612192
ZFYVE26	20761	NM_015346.3	100	612012
ZIC1	12872	NM_003412.3	100	600470
ZIC2	12873	NM_007129.4	95	603073
ZIC3	12874	NM_003413.3	100	300265
ZMPSTE24	12877	NM_005857.4	100	606480
ZMYND10	19412	NM_015896.3	100	607070
ZMYND11	16966	NM_006624.5	100	608668
ZNF711	13128	NM_021998.4	99	314990
ZNF750	25843	NM_024702.2	100	610226
ZNHIT3	12309	NM_001281432.1	64	604500
ZSWIM6	29316	NM_020928.1	96	615951

PAPER I-IV

Genetic and clinical variations in a Norwegian sample diagnosed with Rett syndrome

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ABSTRACT

Background and purpose: Rett syndrome (RTT) is a neurodevelopmental disorder mainly caused by mutations in *MECP2*. The diagnostic criteria of RTT are clinical; mutations in *MECP2* are neither diagnostic nor necessary, and a mutation in another gene does not exclude RTT. We attempted to correlate genotype and phenotype to see if there are significant clinical differences.

Methods: All available females diagnosed with RTT in Norway were invited to the study. Parents were interviewed, the girl or woman with RTT examined and medical records reviewed. All diagnoses were revisited according to the current diagnostic criteria and exome-based sequencing analyses were performed in individuals without an identified causative mutation. Participants were categorized according to genotypes and RTT diagnosis. Individuals with RTT with and without mutations in *MECP2* were compared.

Results: 91 individuals were included. A presumed causative mutation was identified in 86 individuals, of these, mutations in *MECP2* in 77 individuals and mutations in *SMC1A, SYNGAP1, SCN1A, CDKL5, FOXG1* or chromosome 13q in nine. Seventy-two individuals fulfilled the diagnostic criteria for classic and 12 for atypical RTT. Significant differences in early development, loss of hand use and language, intense eye gaze and the presence of early onset epilepsy were revealed in individuals with RTT according to their MECP2 genotypic status.

Conclusion: Using the current diagnostic criteria, genetic and clinical variation in RTT is considerable. Significant differences between individuals with RTT with and without *MECP2* mutations indicate that *MECP2* is a major determinant for the clinical phenotype in individuals with RTT.

HIGHLIGHTS

- Clinical features differ significantly in RTT with and without *MECP2* mutations
- Epilepsy has later onset in individuals with RTT with *MECP2* mutations
- Deviant early development is less common in individuals with RTT with *MECP2* mutations
- Six individuals with RTT had mutations in *SMC1A, SYNGAP1, SCN1A, CDKL5* or *FOXG1*

KEYWORDS

Rett syndrome, MECP2, Genetic variation, Clinical phenotype, Exome sequencing, Epilepsy

1. INTRODUCTION

For many years the neurodevelopmental disorder Rett syndrome (RTT, OMIM 312750) has been known as a clinical entity mainly caused by mutations in the *MECP2* gene [1]. The disorder almost exclusively affects females, and in its classic form, it is characterized by apparently normal development in the first 6-18 months of life before a regression occurs and acquired skills disappear [2].

The phenotypic spectrum of RTT has evolved since the first description of 22 girls with a homogenous phenotype by Andreas Rett in 1966 [3]. As the number of individuals diagnosed with RTT increased, the phenotype widened, and in 1994 the diagnosis included both classic and atypical RTT [4]. The current diagnostic criteria were published in 2010 [2]. In the last decade, the term RTT-like disorders have been used for individuals sharing many clinical characteristics with RTT, but not fulfilling the diagnostic criteria. In contrast to classic and atypical RTT, the term RTT-like disorder is not clearly defined [5].

Also the genotypic spectrum has extended in RTT. In 2004 and 2008, strong associations were found between atypical RTT and mutations in *CDKL5* and *FOXG1*, respectively [6, 7]. In the last decade, Next Generation Sequencing (NGS) has contributed to the identification of mutations in more than 100 genes other than *MECP2, CDKL5* and *FOXG1* in individuals with RTT or a RTT-like phenotype. Almost half of these as the only identified pathological mutation in individuals fulfilling the diagnostic criteria of classic or atypical RTT [5, 8-16]. The strong association between *MECP2* and RTT is however undisputable, with mutations in *MECP2* found in more than 95% of individuals with classic and 70-90% of individuals with atypical RTT [2].

A large number of studies have addressed the genotype in *MECP2* negative individuals within the RTT spectrum. There are, however, fewer studies comparing the phenotypes of these individuals to the phenotypes of individuals with *MECP2* mutations. Differences in phenotype between individuals with RTT with and without *MECP2* mutations have been reported, especially in early development and in epilepsy [17, 18]. In addition, differences between individuals with and without *MECP2* mutations have been explored in cohorts not based on RTT phenotypes [19]. With the increased number of genes associated with RTT and the increased number of individuals without RTT with a mutation in *MECP2*, more knowledge on phenotype-genotype correlations on the genetic level is important for the accuracy in diagnostics.

The present study investigates a population of females diagnosed with RTT through the last three decades. It examines all participants for the phenotypic traits contained in the 2010 diagnostic criteria for RTT, revisits their diagnoses and performs genomic investigations in individuals without an identified causative mutation. In addition, it compares the phenotypes of

individuals with and without a *MECP2* mutation in the entire RTT group as well as within the RTT diagnostic subgroups of classic and atypical RTT.

2. METHODS

2.1 Participants

Recruitment took place from 2013 to 2017. Invitation to participate was distributed to families or guardians of females with RTT or a RTT-like disorder through the Norwegian Rett Syndrome Association (n=126) and Frambu, the Norwegian Resource Centre for Rare Disorders (n=116). The rate of overlapping between the two search groups was high, as only 165 subjects with RTT had been reported to the Norwegian Patient Registry from the Specialist Health Services in 2013. Lists of names from these sources were not revealed to the study group. In addition, some families with a member with RTT were referred from habilitation clinics and neurologists and some families contacted the authors directly. Review of the diagnosis was based on the latest consensus criteria [2]. Individuals sharing some clinical features with RTT, but not fulfilling the diagnostic criteria were described as non-RTT.

2.2 Procedures

Parents/caregivers were first asked to complete a questionnaire. A meeting with the family at the local hospital or in the home was arranged where a clinical examination was performed together with a semi-structured interview with parents/caregivers. A review of the participants' medical records was carried out to complete the data sets.

2.3 Measures

The clinical examination included growth parameters, level of contact, presence of stereotypies and respiration abnormalities, as well as assessment of muscle tone, deep tendon reflexes, coordination and scoliosis. The interview addressed pregnancy and birth, development of communication and language skills, clinical symptoms and results of previous genetic workup, to the best knowledge of the family. The questionnaire comprised information about demographic background and development of motor skills. Head circumference was categorized using normative z-scores [20]. Disease severity was quantified according to the Rett Syndrome Severity Scale (RSSS) which consists of seven parameters from 0 (absent/normal) to 3 (severe), and a maximum score of 21 (most severe) [21].

2.4 Molecular analysis

In participants with an identified pathogenic mutation in *MECP2*, no further genetic testing was performed. In participants with identified mutations in other genes than *MECP2*, retesting of

MECP2 with Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) was carried out. Participants with no prior testing were tested with Sanger sequencing and MLPA of *MECP2*. Participants with negative result on earlier analyses were tested with exome-based Next Generation Sequencing (NGS) analysis with bioinformatic filtering of a panel of genes known to cause intellectual disability and/or epileptic encephalopathies. From the spring of 2015 sequence variants were classified according to the ACMG criteria [22]. During the diagnostic period, the number of genes in the diagnostic gene panel for intellectual disability available from the laboratory increased from 57 to above 1400. When the number of genes increased the approach changed from a single patient analysis to a trio analysis, which includes proband, father and mother.

2.5 Statistical analysis

The descriptive analyses included mean and standard deviations or median and inter quartile range for continuous data, and absolute and relative frequencies for categorical data. Continuous data were compared with independent sample t-test and categorical data with chi square test or fisher exact test. Significance level was set to ≤0.05. Statistical analyses were performed using SPSS for Windows version 23.

2.6 Ethics

Ethics approval was obtained from the Regional Committee for Medical Research Ethics, South East Norway (No. 2012/1572). Parental or guardian consent was obtained prior to inclusion.

3. RESULTS

Consent to participate was given on behalf of 93 individuals. Two were excluded due to missing clinical or genetic data, leaving 91 individuals available for analyses. The participants were from 1 to 66 years old, with a median age of 19 (interquartile range 8-30). All geographical parts of Norway were represented, and both rural and urban areas. Half of the participants (53%) lived in the parental home and half (47%) in residential facilities.

3.1 Genetic and clinical investigations

Of the 91 eligible participants 77 had a mutation in *MECP2* and nine had mutations in other genes (Figure 1). Eighty-four individuals fulfilled the diagnostic criteria of RTT. Identified mutations and RTT phenotypic traits as contained in the 2010 diagnostic criteria are presented in Table 1. Four individuals had two mutations in *MECP2* (Supplementary Table 1). The distribution of mutations in *MECP2* is shown in Figure 2. Novel mutations in *MECP2* were reported in 12 individuals and their clinical features are described in Table 2. Global severity

was assessed with the Rett Syndrome Severity Scale, and showed considerable variation (Figure 3).

3.2 Phenotype versus *MECP2* genotype in individuals with RTT

Table 3 shows the characteristics of the individuals with RTT and *MECP2* mutations (n=74) and of the individuals with RTT without an identified *MECP2* mutation (n=10). Classic RTT and loss of both hand skills and language skills were significantly more frequent in individuals with *MECP2* mutations. Grossly abnormal development in the first six months of life was present in six of ten (60.0%) individuals in the non-*MECP2* group, and in three of 74 (4.1%) in the *MECP2* group. Both groups presented with a large number of supplementary criteria, but "eye pointing" was significantly more prevalent in individuals with *MECP2* mutations. In addition, fewer individuals with *MECP2* mutations had early onset of the first seizure and onset of epilepsy before developmental regression.

3.3 Phenotype versus *MECP2* genotype in RTT diagnostic subgroups

Of the 72 individuals with classic RTT 69 (95.8%) had a mutation in *MECP2*. In this subgroup, onset of epilepsy was the only significant difference between the individuals with and without *MECP2* mutations (Table 3). Two of three (66.7%) individuals without an identified mutation in *MECP2* had early onset of epilepsy. In comparison, only one of the 69 (1.4%) individuals with *MECP2* mutations had onset of epilepsy during the first year of life, and three (4.3%) had onset of epilepsy before regression.

Of the twelve individuals with atypical RTT, five (41.7%) had a mutation in *MECP2* (Figure 1). There was a significantly higher prevalence of epilepsy and more often onset of epilepsy before regression in the non-*MECP2* group. Six of seven individuals (85.7%) without *MECP2* mutations presented with epilepsy in the first year of life, compared to one of five individuals (20.0%) with *MECP2* mutations, but this difference did not reach statistical significance (Table 3).

3.4 Phenotype in individuals with RTT with mutations in genes other than *MECP2*

Six of the individuals with RTT had mutations in other genes than *MECP2* (Table 1). Two had a classic RTT phenotype and mutations in *SCN1A*; these are described in a previous publication [23].

A novel and de-novo mutation in *SYNGAP1* was present in one participant. Its pathogenicity was not confirmed, but other missense-mutations in the same triplet are reported as pathogenic [24]. She first presented with seizures at the age of three months, and had daily seizures with multiple seizure types throughout childhood.

One girl had a mutation in *SMC1A*. She had early onset epilepsy with both generalized and focal seizures. During the first years of life she had regular seizures, but from school age her seizures clustered with approximately one week a month with frequent seizures and then some weeks without seizures.

One participant had mutations in *CDKL5*. She experienced her first epileptic seizure at seven weeks of age. After a while she responded well to medications and was seizure-free until 12 months of age. In her seizure-free period, she developed normally but lost many acquired skills and developed hand stereotypies when the seizures returned.

Mutations in *FOXG1* were identified in one participant. Her parents had worried about her development and lack of eye contact from birth. She went through a regression phase at three to four years of age.

4. DISCUSSION

In this cohort with presumed RTT, the use of Next Generation Sequencing to supplement the targeted approach enabled the identification of mutations in six different genes as well as a copy number variant. The genetic heterogeneity in this cohort is in line with other studies [25-27]. The clinical diagnosis of RTT was confirmed in 92% of study participants. The presence of individuals with other conditions in the cohort may be explained by differential diagnostic challenges due to the presence of RTT phenocopies in individuals with intellectual disability or epileptic encephalopathy, and possibly by use of former diagnostic criteria, as many of the individuals had been diagnosed with RTT long before the current diagnostic criteria were published. The finding of a presumed pathogenic mutation in *MECP2* in 88% of individuals with confirmed RTT is in agreement with current knowledge [2]. However, mutations in *MECP2* as well as in *FOXG1* and *CDKL5* were identified both in individuals with confirmed RTT and individuals without, illustrating the impact of the clinical diagnostic criteria.

Comparisons of clinical characteristics in individuals with RTT with and without *MECP2* mutations revealed significant differences in the prevalence of features representing two main inclusion criteria and in one exclusion criterion. In addition, there were significant differences in presence of intense eye gaze and onset of epilepsy. Similar findings have been reported by Charman et al. who found a significantly lower frequency of early onset of both regression and epilepsy in individuals with *MECP2* mutations [18]. Temudo et al. described higher frequency of a regressive period with loss of hand use and language and growth retardation in individuals with *MECP2* mutations, and less intense eye gaze and earlier signs of deviant development and autistic traits in individuals without *MECP2* mutations [17].

The studies of Charman and Temudo did not differentiate between classic and atypical RTT. In classic RTT fulfilling all main and no exclusion criteria are required. Hence, the differences in the features representing these criteria between individuals with and without *MECP2* in the total cohort were not seen in classic RTT. However, such differences were neither found in atypical RTT. The only significant differences between individuals with *MECP2* mutations and others in both subgroups were the lower frequency and a later onset of epilepsy in the individuals with *MECP2* mutations. Two of the three individuals with classic RTT without a *MECP2* mutation had early onset epilepsy, which was almost not seen in classic RTT with *MECP2* mutations. Scientific reports on RTT include descriptions of 18 individuals who fulfill the diagnostic criteria of classic RTT and have mutations in other genes than *MECP2* [5, 9, 16, 27-35]. Onset of epilepsy was described for nine of the 18 individuals, five individuals had an early onset (before one year of age) and six individuals presented with the first seizure before regression [5, 27-29, 31, 32]. This is considerably higher than in the individuals with classic RTT and *MECP2* mutation in the present cohort. Similar results were reported by Nissenkorn and colleagues, none of their participants with early onset of epilepsy had a mutation in *MECP2,* while mutations were found in 87% of those with onset after one year of age [36]. Onset of epilepsy before regression might indicate an influence of epilepsy on the development, like in individuals with developmental and epileptic encephalopathies [37] and contrary to classic RTT with *MECP2* mutations, where seizures seldom precede regression and thus is not likely to contribute to the developmental regression [26].

The three individuals in the present sample with *MECP2* mutation but without RTT apparently had no regression and an overall mild phenotype. For two of these three the absence of a clear regression was the only clinical feature lacking for fulfilling the diagnostic criteria for RTT. With introduction of the 2010 diagnostic criteria, regression became required for diagnosing both classic and atypical RTT [2]. However, this requirement can be questioned for several reasons: in some individuals the regression phase may be so subtle and protracted that it is difficult to register [38], and the regression phase may occur so early in life that it is difficult to observe and recognize. If the early development is deviant, the skills normally lost in regression may not yet have been acquired when the phase of neurophysiological regression occurs [39]. Because regression in the first years of life is a rather inaccurate feature, one may consider revising the criteria and omit developmental regression as a requirement.

Many neurodevelopmental disorders have overlapping phenotypes [5]. Evaluation of the nine individuals in the present cohort with mutations in other genes than *MECP2* revealed that they all had clinical features overlapping with both RTT and other syndromes. Two individuals with a classic RTT phenotype had mutations in *SCN1A,* which are associated with the epileptic encephalopathy of Dravet syndrome. Dravet syndrome is characterized by early onset of severe

epilepsy. In the second year of life, a developmental disorder becomes apparent, and developmental regression may occur [40]. In the present sample, the epilepsy of the two with *SCN1A* mutations was Dravet-like.

One girl with atypical RTT had a mutation in *SMC1A. SMC1A* is one of five genes associated with Cornelia de Lange syndrome, but lately several individuals with *SMC1A* mutations and epileptic encephalopathy have been described, some remarkably RTT-like [5, 33, 41]. The distinct feature of seizure clustering seen in the present girl is also described in other individuals with SMC1A mutations [42].

Another participant with atypical RTT had a mutation in *SYNGAP1*. To our knowledge, an atypical RTT phenotype in individuals with mutations in *SYNGAP1* has not been reported before, although Vidal and associates (2017) point to the similarity between girls with this mutation and Rett syndrome [25]. *SYNGAP1*-associated encephalopathy is categorized as a developmental and epileptic encephalopathy with four main comorbid conditions; intellectual disability, behavioural problems, a high pain threshold and ataxia [24]. In addition, developmental regression is not unusual. The present participant shares these characteristics, except for the behavioural problems [24].

The two individuals with mutations in *CDKL5* shared many clinical characteristics but only one of them had regression, which separated them in terms of diagnosis. However, both participants have several characteristics typical of individuals with the suggested *CDKL5* disorder, such as abnormal early development, early onset of epilepsy and mouthing [43].

Finally, mutations in *FOXG1* were found in two participants. Kortum et al. argues that the early abnormal development, lack of regression and lack of respiratory irregularities in combination with brain imaging features are sufficiently distinct to allow clinical recognition of a FOXG1 syndrome [44]. Both participants had poor eye contact from early infancy, normal breathing patterns and abnormal early development. One showed regression. Unfortunately, the present study did not include MRI scanning.

To sum up, six of the nine individuals with mutations in other genes than *MECP2* fulfilled the diagnostic criteria for RTT. Three individuals did not fulfil the criteria but shared many clinical features with RTT. In addition to RTT, these nine presented with features found in other individuals without RTT but with mutations in the same genes. The current diagnostic criteria for RTT are based on clinical characteristics, a mutation in *MECP2* is neither necessary nor diagnostic, and mutations in other genes do not exclude RTT [2]. Some researchers have suggested replacing the clinical features currently used for diagnosing RTT with a molecular diagnosis [13, 45]. At present, it is not clear what such a change would imply, but it seems evident that it would include a wider phenotypic spectrum than the current criteria. The phenotypes will range from severe RTT to mild intellectual disability, and include the non-Rett

variation among males. Hence, it will lose the benefits a diagnosis based on developmental clinical characteristics features for habilitation and clinical research, as well as for solidarity and support between families. At the same time, the findings from the present study suggest important differences between individuals with and without a mutation in *MECP2.* This may suggest that the current diagnostic criteria include individuals with other disorders under the RTT umbrella.

A limitation in the present article is the relatively small number of participants, the results from this study has to be confirmed by future research involving larger populations. The present sample is however population-based and has a wide distribution in age and geographical location, indicating that it is representative for the population of RTT in Norway, strengthening the external validity in spite of the low number.

In conclusion, both the genotypic and the phenotypic variation within RTT are considerable. The clinical severity are ranging from mild phenotypes with basic language skills, ability to walk and only a few RTT characteristics, to severe phenotypes without ability to speak or to walk independently, and with severe epilepsy. Most individuals had a pathologic mutation in MECP2, but in addition mutations in five other genes were revealed. Compared to individuals with RTT without *MECP2* mutations, individuals with RTT with *MECP2* mutations more often had apparently normal development in the first six months of life, had lost functional use of hands and language, and showed a characteristic intense eye gaze. The prevalence of early onset epilepsy was lower in individuals with a *MECP2* mutation than in individuals without a MECP2 mutation, regardless of which RTT subgroup they belonged to.

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Table 1. Presence of RTT phenotypic manifestations in individuals with mutations in different genes (number/number in total)

	MECP2	*SCN1A*	*SYNGAP1*	*SMC1A*	*CDKL5*	*FOXG1*	*13q*del	No mut. id.
Number	77	2	1	1	2	2	1	5
Diagnosis								
Classic	69/77	2/2	0/1	0/1	0/2	0/2	0/1	1/5
Atypical	5/77	0/2	1/1	1/1	1/2	1/2	0/1	3/5
Non-RTT	3/77	0/2	0/1	0/1	1/2	1/2	1/1	1/5
Absolute criteria								
Regression	74/77	2/2	1/1	1/1	1/2	1/2	0/1	4/5
Main criteria								
Loss of hand skills	73/77	2/2	0/1	1/1	0/2	1/2	0/1	4/5
Loss of language	73/77	2/2	1/1	0/1	1/2	1/2	uk	2/5
Gait abnormalities	76/77	2/2	1/1	1/1	2/2	2/2	1/1	5/5
Stereotypies	77/77	2/2	1/1	1/1	2/2	2/2	1/1	5/5
Exclusion criteria								
Brain injury	0/77	0/2	0/1	0/1	0/2	0/2	0/1	0/5
Grossly abn. developm.	0/77	0/2	1/1	1/1	2/2	2/2	1/1	3/5
Supplementary criteria								
Breathing disturbances	60/76	1/2	1/1	1/1	0/2	0/2	0/1	4/5
Bruxism	60/75	2/2	1/1	1/1	1/2	1/2	1/1	4/5
Impaired sleep	61/77	2/2	1/1	1/1	2/2	2/2	1/1	5/5
Abnormal muscle tone	62/76	2/2	1/1	1/1	2/2	2/2	1/1	5/5
Peripheral vasomotor disturbances	36/73	1/2	1/1	1/1	1/2	2/2	1/1	1/5
Scoliosis/kyphosis	65/77	2/2	0/1	1/1	1/2	2/2	1/1	3/5
Growth retardation	39/75	2/2	0/1	0/1	2/2	2/2	1/1	4/5
Small cold hands/feet	66/75	2/2	1/1	1/1	2/2	2/2	1/1	3/5
Laughter/screaming spells	65/68	2/2	1/1	1/1	2/2	1/2	1/1	5/5
Diminished response to pain	39/43	1/2	1/1	0/1	1/2	2/2	1/1	2/2
Eye pointing	62/63	2/2	1/1	1/1	1/2	1/2	0/1	3/4
Other RTT characteristics								
Microcephaly	37/74	0/2	1/1	1/1	1/2	1/2	1/1	2/5
Verbal language	9/77	0/2	0/1	0/1	0/2	0/2	0/1	0/5
Indep. ambulation	45/77	2/2	1/1	1/1	0/2	0/2	0/1	2/5
Reflux	43/76	1/2	1/1	1/1	1/2	2/2	1/1	3/5
Constipation	70/77	1/2	1/1	1/1	1/2	2/2	1/1	5/5
Epilepsy	50/77	2/2	1/1	1/1	2/2	1/2	1/1	5/5
Onset of epilepsy <1y	2/76	2/2	1/1	1/1	2/2	0/2	1/1	4/5
Onset of epilepsy before regression	4/76	2/2	1/1	1/1	1/2	1/2	na	3/5

No ut. id.: No mutation identified, na: not applicable, uk: unknown

Table 2. RTT phenotypic manifestations of individuals with novel mutations in *MECP2*

Mutation in *MECP2*	Single nucleotide variation		Indels									
	c.872C>T	c.1453C>A	c.211_1150del	c.816_1027del	c.817_832dup	c.902_1141del	c.1064_1196del	c.1098_1201del & c.1276_1277dupAG	c.1098_1201del & c.1276_1277dupAG	c.1127_1197del	c.1161_1188del	c.1173_1197del
VUS		Y				Y						
Diagnosis	Non-RTT	Cl.	Cl.	Cl.	Cl.	Cl.	Cl.	Cl.	Cl.	Cl.	Cl.	Cl.
Absolute criteria												
Regression	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Main criteria												
Loss of hand skills	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Loss of language	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Gait abnormalities	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Stereotypies	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Exclusion criteria												
Brain injury	N	N	N	N	N	N	N	N	N	N	N	N
Grossly abn development	N	N	N	N	N	N	N	N	N	N	N	N
Supplementary criteria												
Breathing disturbances	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y	Y
Bruxism	Y	N	Y	Y	N	Y	Y	N	N	Y	N	N
Impaired sleep	Y	Y	Y	Y	N	Y	N	Y	N	Y	Y	Y
Abnormal muscle tone	N	N	Y	Y	N	Y	Y	N	N	Y	Y	Y
Periph. vasomotor disturbances	N	Y	Y	Y	N	N	Y	N	N	N	N	Y
Scoliosis/kyphosis	N	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y
Growth retardation	Y	N	Y	Y	Y	Y	N	N	N	N	N	Y
Small cold hands/feet	Y	Y	Y	Y	Y	N	Y	N	N	Y	Y	Y
Laughter/screaming spells	uk	Y	Y	Y	Y	uk	Y	Y	Y	Y	Y	Y
Diminished response to pain	uk	Y	uk	uk	N	uk	Y	Y	Y	N	uk	Y
«Eye pointing»	Y	Y	uk	Y	Y	Y	Y	Y	Y	Y	Y	Y
Other RTT characteristics												
Microcephaly	Y	N	Y	Y	N	N	Y	N	N	N	N	Y
Verbal language	Y	N	N	N	N	Y	N	Y	Y	N	N	N
Independent ambulation	Y	Y	N	N	N	Y	N	Y	Y	Y	N	N
Reflux	N	Y	N	Y	N	N	N	N	N	Y	Y	Y
Constipation	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y
Epilepsy	N	Y	Y	N	Y	N	N	Y	N	N	Y	Y
Onset of epilepsy (months)	na	6	36	na	144	na	na	60	na	na	108	72
Rett Syndrome Severity score	5	12	17	13	13	10	12	8	6	7	18	13

VUS: variant of unknown significance, Y: yes, N: no, Cl: classic RTT, na: not applicable, uk: unknown

Table 3. Presence of RTT phenotypic manifestations in RTT with and without *MECP2* mutations

	Classic RTT			Atypical RTT			All RTT		
	W. *MECP2*	No *MECP2*	p	W. *MECP2*	No *MECP2*	p	W. *MECP2*	No *MECP2*	p
Number	69	3	-	5	7		74	10	-
Age, mean	23.1	30.0	0.448	21.8	17.3	0.491	23.0	21.1	0.697
Classic RTT	-	-	-	-	-	-	69/74	3/10	<0.001*
Absolute criteria, n/ntotal									
Regression	69/69	3/3	-	5/5	7/7	-	74/74	10/10	-
Main criteria, n/ntotal									
Loss of hand skills	69/69	3/3	-	4/5	4/7	0.576	73/74	7/10	0.005*
Loss of language	69/69	3/3	-	4/5	4/7	0.576	73/74	7/10	0.005*
Gait abnormalities	69/69	3/3	-	4/5	7/7	0.417	73/74	10/10	1.000
Stereotypies	69/69	3/3	-	5/5	7/7	-	74/74	10/10	-
Exclusion criteria, n/ntotal									
Brain injury	0/69	0/3	-	0/5	0/7	-	0/74	0/10	-
Grossly abn. development	0/69	0/3	-	3/5	6/7	0.523	3/74	6/10	<0.001*
Supplementary criteria, n/ntotal									
Breathing disturbances	56/68	1/3	0.097	2/5	5/7	0.558	58/73	6/10	0.226
Bruxism	54/67	3/3	1.000	4/5	4/7	0.576	58/72	7/10	0.425
Impaired sleep	56/69	3/3	1.000	4/5	7/7	0.417	60/74	10/10	0.201
Abnormal muscle tone	56/68	3/3	1.000	5/5	7/7	-	61/73	10/10	0.344
Periph. vasomotor disturbances	33/65	1/3	0.555	2/5	5/7	0.558	35/70	6/10	0.738
Scoliosis/kyphosis	60/69	2/3	0.366	5/5	5/7	0.470	65/74	7/10	0.150
Growth retardation	36/67	3/3	0.599	2/5	5/7	0.558	38/72	4/10	0.514
Small cold hands/feet	58/67	2/3	0.375	5/5	7/7	-	63/72	9/10	1.000
Laughter/screaming spells	59/61	3/3	1.000	5/5	6/7	1.000	64/66	9/10	0.349
Diminished response to pain	35/39	1/2	0.232	3/3	5/6	1.000	38/42	6/8	0.242
"Eye pointing"	54/55	3/3	1.000	5/5	4/6	0.455	59/60	7/9	0.043*
Other RTT characteristics, n/ntotal									
Microcephaly	33/66	0	0.240	3/5	7/7	0.152	36/71	7/10	0.322
Verbal language	4/69	0/3	1.000	2/5	1/7	0.523	6/74	1/10	1.000
Indep. Ambulation	40/69	3/3	0.268	2/5	4/7	1.000	42/74	7/10	0.511
Reflux	39/68	1/3	0.577	4/5	5/7	1.000	43/73	6/10	1.000
Constipation	62/69	2/3	0.301	5/5	6/7	1.000	67/74	8/10	0.290
Epilepsy	48/69	3/3	0.551	2/5	7/7	0.045*	50/74	10/10	0.056
Onset of epilepsy <1y	1/68	2/3	0.004*	1/5	6/7	0.072	2/73	8/10	<0.001*
Onset of epilepsy before regression	3/68	2/3	0.012*	1/5	7/7	0.010*	4/73	9/10	<0.001*
Rett Syndrome Severity Score (mean)	13.2[a]	11.3	0.376	12.8	13.3	0.851	13.2[a]	12.7	0.680

Significant, a: data from four individuals are missing in this analysis
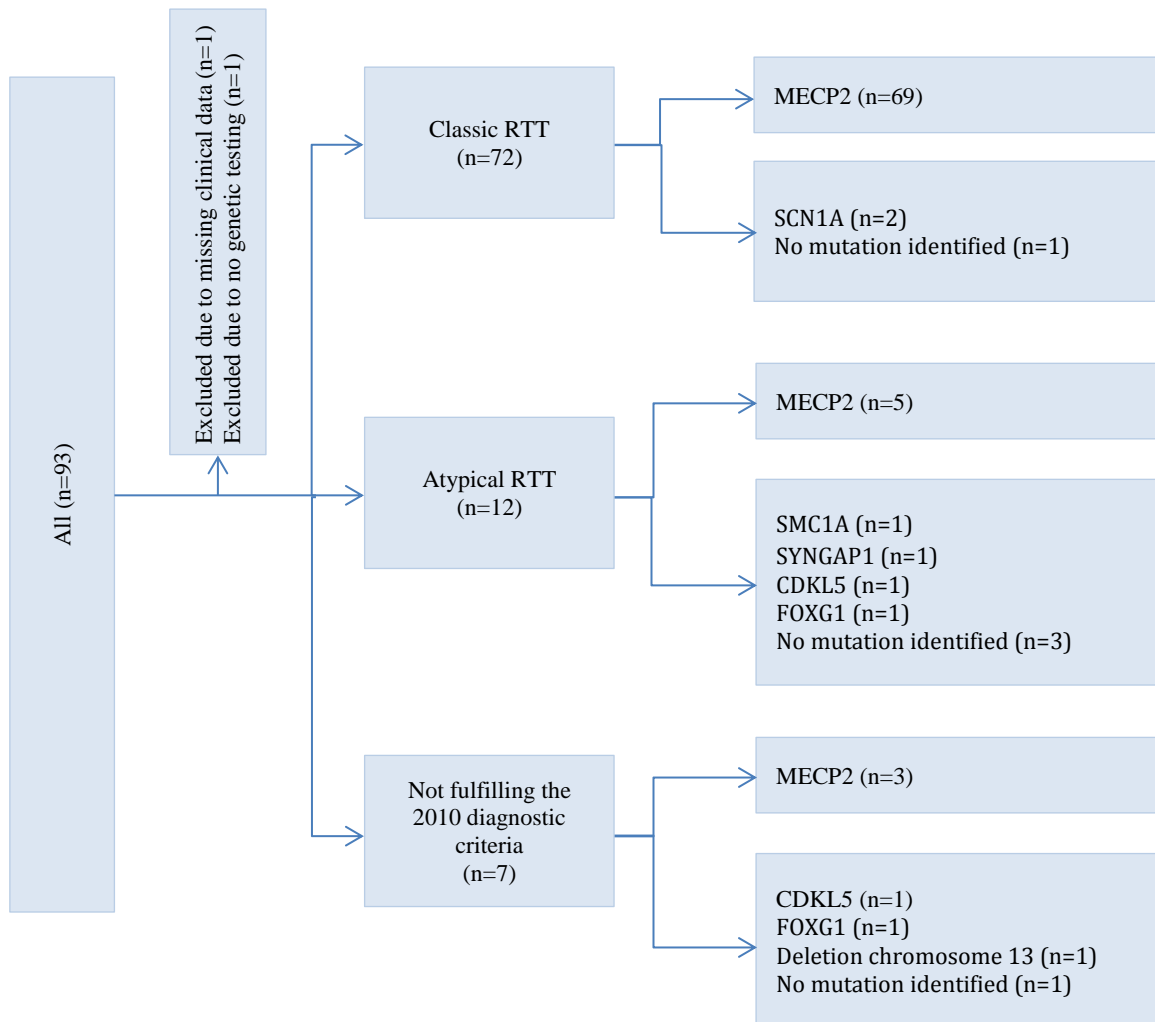
Figure 1. Genotypes and phenotypes in the present sample
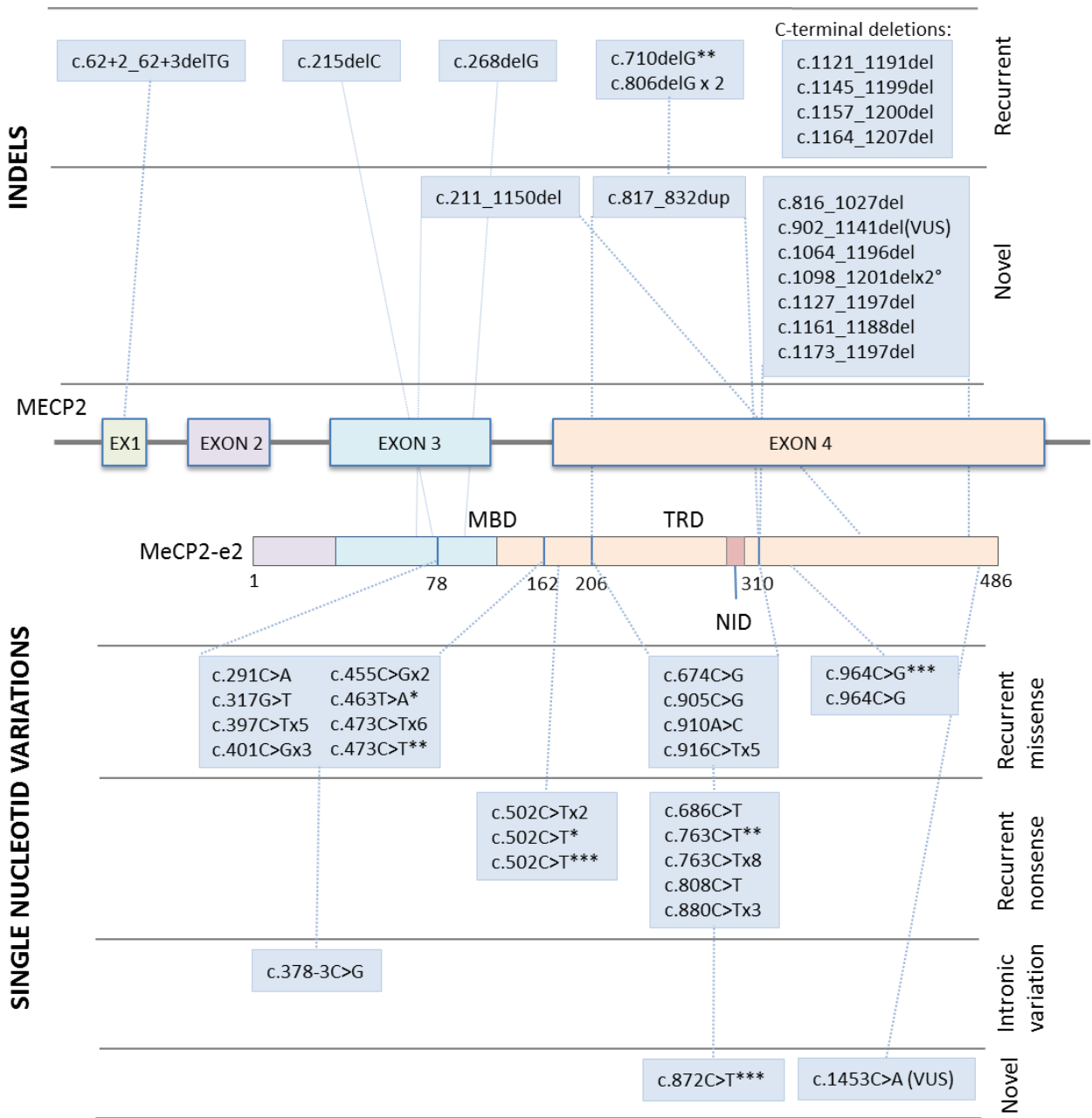
b)

Large deletions:
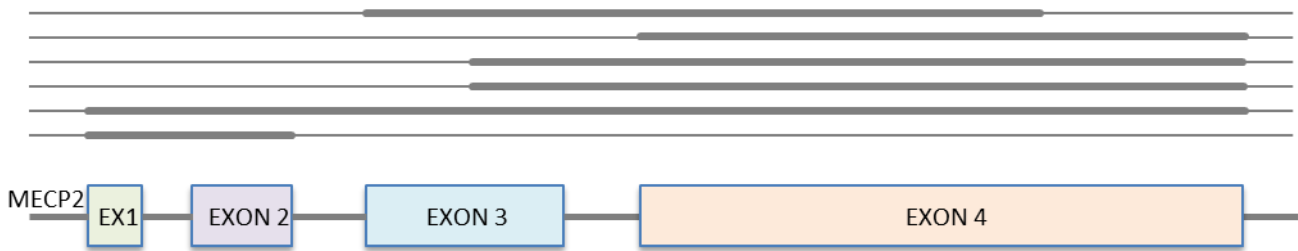
Figure 2. The distribution of mutations in *MECP2* in the present sample illustrated in accordance to the *MECP2* gene and the MeCP2-e2 protein. (The other transcript MeCP2-e1 is for simplicity not included in the figure). In the MeCP2-e2 protein the important functional areas of Methyl-CpG-binding domain (MBD), Transcriptional repression domain (TRD) and NCOR-SMRT interaction domain (NID) are marked, as are the first and last amino acid in MBD and TRD. a) Indels and point mutations of 71 individuals. Their phenotype is marked (*Atypical RTT, mild; **Atypical RTT severe; ***Not fulfilling RTT diagnostic criteria; °Monozygotic twins; All others: classic RTT.*) b) Six individuals had large deletions (illustrated by one line each, the bold lines illustrate the deletion in accordance to the schematic gene). All five had classic RTT.

Figure 3. Rett syndrome Severity Scores in individuals divided into groups based on genotype.

Supplementary table 1. Individuals with two mutations in *MECP2*

	Mutation	Novel	Pathogenicity
1	c.910A>C	-	Pathogenic
	c.1123_1191del69	-	Unknown
2*	c.1098_1201del	X	Pathogenic
	c.1276_1277dupAG	X	Likely pathogenic
3*	c.1098_1201del	X	Pathogenic
	c.1276_1277dupAG	X	Likely pathogenic
4	c.964C>G	-	Pathogenic
	c.1145_1199del	X	Likely pathogenic

monozygotic twins

II

Epilepsy in classic Rett syndrome: Course and characteristics in adult age

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ARTICLE INFO

ABSTRACT

Purpose: Rett syndrome (RTT) is a neurodevelopmental disorder that almost exclusively affects females. Epilepsy is a major clinical feature, but its long-term course in RTT has not been sufficiently explored. This study addresses the development of the epilepsy in adults with RTT.

Methods: Available females diagnosed with RTT in Norway were asked to participate. Parents/caregivers were interviewed, the girls/women were examined and their medical records reviewed. Participants were categorized according to age, epilepsy, seizure patterns and mutation severity groups. RTT severity was assessed (epilepsy score excluded).

Results: 70 females with classic RTT were included. A presumed pathogenic mutation in *MECP2* was found in 96%. The presence of active epilepsy (seizures last five years) was similar in all age groups above the age of ten: 11 (65%) in adolescents (11–20 years), 9 (60%) in young adults (21–30 years) and 14 (67%) in participants above 30 years of age. Tonic-clonic seizures within the last year were present in 55, 67 and 64%, and ≥ weekly seizures occurred in 27, 45 and 50% in the respective age groups. Among participants with active epilepsy, 69% had unremitting seizures, whereas 31% had experienced remissions for more than six months during the last five years. In the oldest group (> 30 years), only 19% had obtained seizure control for > 5 years, and 14% had never experienced seizures. Seizure activity correlated with RTT severity score, whereas the relationship to mutation type remained ambiguous.

Conclusion: Epilepsy continues to be a major concern in adults with RTT. Two thirds of women above 30 years of age remained with active epilepsy and 50% of them had seizures at least weekly.

1. Introduction

Rett syndrome (RTT, OMIM 312,750) is a neurodevelopmental disorder with a prevalence around 1 in 10 000 live female births (Fehr et al., 2011; Laurvick et al., 2006). In the majority of girls and women with RTT mutations in the *MECP2* gene have been identified (Amir et al., 1999). In its classical form, RTT is characterized by an apparently normal early development during the first 6–18 months of life. Then a regression of communication and motor skills follows, leaving these girls with severe cognitive and physical impairments (Neul et al., 2010).

Epilepsy is one of the main clinical features of RTT, and affects approximately 70–90% of the females during their lifetime (Nissenkorn et al., 2015; Pintaudi et al., 2010; Tarquinio et al., 2017). The seizure disorder is a major concern in many families and affects quality of life of both the girl/woman with RTT and her family members (Bahi-Buisson et al., 2008). Several studies have revealed a wide variability of epileptic features in RTT (Nissenkorn et al., 2015; Pintaudi et al., 2010), but little scientific attention has been given to the course of epilepsy into adult age.

Life expectancy in RTT has increased considerably during the last 50 years (Freilinger et al., 2010). The latest survival analysis reports greater than 70% survival at 45 years (Tarquinio et al., 2015). Thus, we

are facing a growing population of aging females diagnosed with RTT. A few studies from the last decade address RTT and aging on a general basis. These studies are contradictory concerning the seizure disorder. One study reports an improvement of epilepsy in adult age (Halbach et al., 2013), while two claim that epilepsy frequently still is a major concern in adulthood (Anderson et al., 2014; Vignoli et al., 2012). Studies concerning epilepsy in relation to age usually limit their focus to adolescence and early adulthood and lump the relatively few subjects older than 20 years into one group (Bao et al., 2013; Jian et al., 2007; Pintaudi et al., 2010). The course of epilepsy in later adulthood age is thus essentially unexplored.

The aim of the present paper is to describe the diversity of epilepsy in a population of females with RTT, and to address the development of the seizure disorder in adulthood.

2. Methods

2.1. Recruitment

In this population-based cross-sectional project, recruitment took place from 2014 to 2017. Invitation to participate was distributed to families or guardians of females with RTT or a RTT-like disorder through the Norwegian Rett Syndrome Association (n = 126) and Frambu, the Norwegian Resource Centre for Rare Disorders, (n = 116). The rate of overlapping was high, as only 165 subjects with RTT had been reported to the Norwegian Patient Registry from the Specialist Health Services in 2013. Lists of names from these sources were not revealed to the study group. In addition, some females were referred directly from habilitation clinics and neurologists.

Consent to participate was given on behalf of 93 subjects. Ascertainment of the diagnosis of the identified subjects was based on key clinical features independent of molecular findings, according to the latest consensus criteria (Neul et al., 2010). *CDKL5*- and *FOXG1*-disorders as well as conditions with RTT-like features and *MECP2* mutations not fulfilling the RTT criteria were defined as RTT-like disorders. Of the 93 subjects, 74 had classic RTT, ten had atypical RTT, seven had RTT-like disorders and two did neither have RTT nor a RTT-like disorder. Exclusion of two individuals with classic RTT due to mutations in *SCN1A,* which might influence the epilepsy, and missing clinical data for two subjects, left 70 individuals available for analysis.

2.2. Clinical data

Parents/caregivers were asked to complete a questionnaire covering information on the demographic background and the development of motor skills. We then met the families at their local hospital or in their homes. A clinical examination, including growth parameters, level of contact, presence of stereotypies and respiration abnormalities as well as assessment of muscle tone, deep tendon reflexes, coordination and scoliosis, was performed mainly by the first author. In addition a semi-structured interview with parents/caregivers took place. Pregnancy and birth, development, communication skills, other clinical symptoms and results of previous genetic testing were addressed. Epilepsy-specific information covered the ascertainment of epileptic seizures, age of seizure onset, the history of seizure types, seizure frequency, anti-epileptic drug (AED) treatment and any remissions. The potential pitfall of inaccurate reporting received particular attention. A review of medical records was thus carried out to complete the data sets. If information from interviews and records did not completely correspond, details recorded in writing at the time of the event were considered more reliable.

2.3. Genetic analyses

In participants without known mutations prior to inclusion (due to either negative or no testing), genetic sequencing ad modum Sanger and multiplex ligation-dependent probe amplification (MLPA) of *MECP2* were performed. If the results of these tests were negative, exome-based high throughput sequencing analysis with bioinformatic filtering of a panel of genes known to cause intellectual disability and/or epileptic encephalopathies was performed, using an Illumina hiseq 2500 platform. During the research study, the number of genes in the panel analyses available from the laboratory increased from 45 to 1400. Single patient analysis of 45 genes was performed for three participants and a trio (patient, mother, father) analysis of 1400 genes was performed for one participant. Samples with negative findings in the 45 gene panel were not reanalyzed with a larger panel.

2.4. Data categorization

MECP2 mutations were classified into two groups, according to expected phenotypic severity based on previous reports (Cuddapah et al., 2014); severe (T158 M, R168X, R255X, R270X, large deletions) and mild (R133C, R294X, R306C, other point mutations, c-terminal truncations). Age was partly used in the analyses as a continuous variable, and partly categorized into four subgroups: 1–10 years, 11–20 years, 21–30 years, and above 30 years. Head circumference was categorized using normative z-scores (Rollins et al., 2010). Disease severity was quantified according to the Rett syndrome Severity Scale with scoring of seven parameters from 0 (absent/normal) to 3 (severe) (Kaufmann et al., 2012). When analyzing RTT severity versus epilepsy, the seizure sub-score was subtracted.

Seizure categorization was based on semiological features. According to the recently revised ILAE seizure classification (Fisher et al., 2017), seizure types were identified as either focal onset motor seizures or unknown onset tonic-clonic or other motor seizures, comprising myoclonic, tonic or atonic elements. EEG findings could not be systematically assessed in this study. Dubious epileptic symptoms with low symptom burden and little or no impact on quality of life, including discrete episodes with behavior arrest only, had to be disregarded. Care was taken not to interpret non-epileptic events as epileptic seizures (i.e. unspecific twitching, jerking, head turning, trembling, staring, laughing and respiratory abnormalities)(Glaze et al., 1998).

Active epilepsy was defined as seizures within the last five years (ILAE Commission Report, 1997). Seizure frequency within the last year was categorized as ≥ daily; < daily ≥ weekly; < weekly ≥ monthly; < monthly > yearly; or seizure free.

Seizure patterns were divided into four categories. Group 1: never seizures; group 2: diagnosed with epilepsy, but seizure free for more than five years; group 3: active epilepsy with remissions more than six months within last five years; group 4: persistent seizures without remissions.

2.5. Statistical analysis

The descriptive analyses include mean and standard deviations or median and inter quartile range for continuous data, and absolute and relative frequencies for categorical data. Independent samples *t*-test or multiple linear regression were used to compare groups with continuous variables. Chi Square or Fisher's Exact Test were used for categorical variables. To assess the frequency of seizures, both cross-sectional and retrospective longitudinal data were analyzed. Significance level is ≤ 0.05. Statistical analyses were performed using SPSS for windows version 23.

Ethics approval was obtained from the Regional Committee for Medical Research Ethics. Parental/guardian consent was obtained prior to inclusion.

Table 1
The distribution of seizure patterns in 70 patients with classic Rett syndrome.

Seizure patterns	Classic RTT N (%)
Group 1: Never seizures	21 (30)
- *1a: No AEDs*	*16 (23)*
- *1b: With AEDs*	*5 (7)*
Group 2: Seizure free last five years	10 (14)
- *2a: AEDs discontinued*	*2 (3)*
- *2b: With AEDs*	*8 (11)*
Group 3: Active epilepsy with seizure remissions and relapses last five years	12 (17)
Group 4: Active epilepsy without seizure remissions and relapses	27 (39)
- *4a: Remissions, but not last five years*	*7 (10)*
- *4b: Never remissions*	*20 (29)*

AED: Anti-epileptic drug.

3. Results

3.1. Epilepsy in classic RTT

At inclusion median age was 21 years, ranging from 1 to 66 years (IQR 14–34 years). Epilepsy had been diagnosed at some point in 70% of the participants.

Median age of first seizure was 4 years (range 7 months – 40 years, IQR 3–7 years). Seizure onset occurred in four participants between 11 and 20 years of age, and in one participant above 20 years. Table 1 shows the distribution of the seizure patterns among the 70 participants. All individuals with active epilepsy received antiepileptic drugs (AEDs); five individuals with epileptiform EEG activity never diagnosed with epilepsy also used AEDs.

Fig. 1 illustrates the relationship between age and seizure pattern. Active epilepsy (group 3 and 4), occurred in 65% of adolescents (11–20 years), 60% in young adults (21–30 years) and 67% in older adults (> 30 years). Among the children (1–10 years), only five participants (29%) had developed epilepsy. None of the children had epilepsy for as long as five years; three had experienced remissions for more than six months. The distribution of seizure patterns did not differ much with age in participants above ten years of age (Fig. 1). Ten participants with previously diagnosed epilepsy had been seizure-free for more than five years. Two of them had discontinued AED treatment (Table 1), and had been seizure free for at least ten years and off medication for 23 and six years, respectively. The seizure disorders of the five participants with seizure onset after ten years of age varied considerably and did not seem to be essentially different from those with earlier onset.
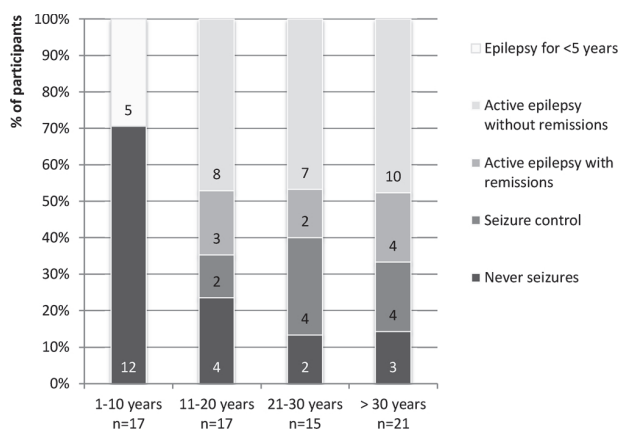
Fig. 1. The relationship between age and seizure patterns within the last five years in females with classic RTT.

3.2. Seizure frequency

Seizure frequency within the last year prior to inclusion did not differ notably between the age groups, but ≥ weekly seizures tended to occur more often in children below 10 years (60%) compared to adolescents (27%). However, the frequency of seizures showed a tendency to increase again in adults (45–50%) (Table 2). Fig. 2 neatly illustrates the mean seizure frequency at different ages according to the retrospective longitudinal data. Seizures were more frequent in the early age groups, but remained relatively stable from early adolescence through adulthood, although with a slight increase in the oldest participants, in line with the findings in Table 2.

3.3. Seizure types

The presence of tonic-clonic seizures tended to increase slightly with age. In the oldest group, 64% of participants with active epilepsy had tonic-clonic seizures during the last year prior to inclusion, whereas less than 50% of individuals below 20 years had this type of seizures (Table 2). Other seizure types were more equally distributed among the age groups. There was no correlation between seizure type and seizure patterns. The proportion of participants having more than one seizure type was close to 40% in the three oldest age groups; in the youngest group only 10% had multiple seizures types (Table 2).

3.4. Mutations

Mutation analyses were completed for 68 of the 70 participants, and of these, 67 (99%) had a *MECP2* mutation. One had negative test for *MECP2* as well as for the applied gene panel. Three of the mutations in *MECP2* could not be classified into either of the two groups of expected phenotypic severity (Cuddapah et al., 2014). Age at inclusion differed between mutation groups (Table 3). In participants below 20 years of age, mean severity score was significantly lower in those with "mild" mutations compared to those with "severe" mutations (9.5 vs 13.3). In participants above 20 years there was no such trend (15.6 vs 14.9). The same pattern was found for epilepsy characteristics; participants under 20 years with mild mutations had a tendency to a lower prevalence of active epilepsy and a lower seizure frequency compared to the severe mutation group, whereas in participants above 20 years, the results were inverse (Table 3).

3.5. Seizure patterns and clinical severity

Mean score on the Rett Syndrome Severity Scale was 9.9 in seizure pattern group 1 (never seizures), 12.6 in group 2 (seizure-free last five years), 12.2 in group 3 (active seizures with remissions) and 13.8 in group 4 (active seizures without remissions). To control for age and mutation type confounders, multiple regression analysis was performed; the adjusted mean global severity increased by 2.9 from seizure pattern group 1 to 4 (p = 0.001, Table 4).

4. Discussion

4.1. Age, epilepsy and seizure patterns

The present study includes a considerable proportion of females with RTT in Norway. More than half the participants were older than 20 years, and almost one third were above 30 years. No other study with a main focus on epilepsy has included such a large proportion of adults and aging females with RTT. Thus, this cross-sectional sample provides a unique opportunity to study the impact of epilepsy in adulthood.

The prevalence of active epilepsy was similar across the age groups after the age of ten. Approximately two thirds of these participants had experienced seizures within the last five years. The percentage of seizure-free participants during the last year did not increase after the age

Table 2
Seizure frequency and seizure types within last year by age in females with active epilepsy and classic RTT.

Age	N	Seizure frequency N (%)			Seizure types N (%)			
		≥ Weekly	< Weekly ≥ monthly	< Monthly	Tonic-clonic	Focal motor	Other motor	> 1 seizure type
1-10 years	5	3 (60)	0 (0)	2 (40)	1 (20)	3 (60)	2 (40)	1 (10)
11-20 years	11	3 (27)	2 (18)	6 (55)	6 (55)	5 (45)	2 (18)	5 (45)
21-30 years	9	4 (45)	2 (22)	3 (33)	6 (67)	3 (33)	1 (11)	3 (33)
> 30 years	14	7 (50)	5 (36)	2 (14)	9 (64)	8 (57)	4 (29)	7 (50)
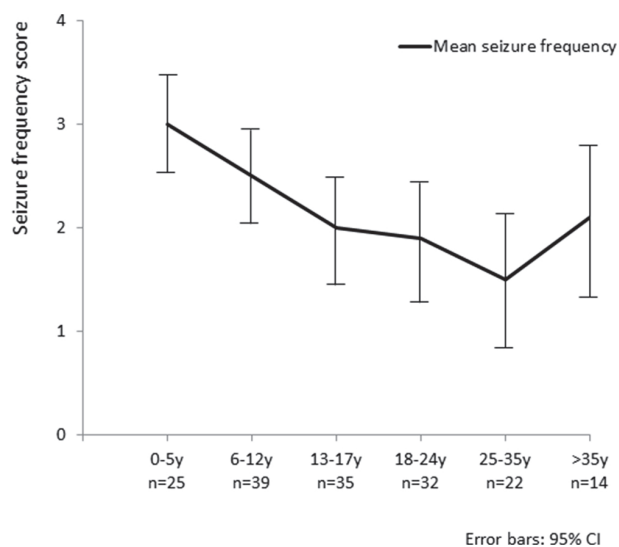
Fig. 2. Longitudinal relationships between age and mean seizure frequency in females with classic RTT ever diagnosed with epilepsy. Seizure frequency scores: 0 = no seizures last year; 1 = ≥yearly, < monthly; 2 = ≥monthly, < weekly; 3 = ≥weekly, < daily; 4 = ≥ daily.

Table 3
Mutation groups in relation to RTT severity, age groups and epilepsy characteristics.

	Mild mutations		Severe mutations		
		N		N	p-value
Age[a]	27.1 ± 17.0	38	17.5 ± 11.5	26	0.009[1]
RTT severity[a]					
1-20 years	9.5 ± 2.7	15	13.3 ± 3.6	17	0.002[1]
> 20 years	15.6 ± 2.7	20	14.9 ± 2.2	9	ns[1]
Active epilepsy[b]					
1-20 years	6 (38)	16	10 (59)	17	ns[2]
> 20 years	16 (73)	22	5 (56)	9	ns[2]
Age of seizure onset[a]					
1-20 years	4.1 ± 1.7	7	3.1 ± 1.1	11	ns[1]
> 20 years	5.1 ± 2.9	19	7.3 ± 6.5	6	ns[1]
≥Weekly seizures[c]					
1-20 years	0	6	6 (60)	10	0.034[3]
> 20 years	10 (63)	16	0	5	0.035[3]

[a] Mean ± SD; [b] n(%); [c] n(% of those with active epilepsy).
[1] Independent sample t-test; [2] Chi square; [3] Fisher exact.

of 30 years. This is in contrast to the common notion of an improvement and sometimes a remission of epilepsy in adult age that has prevailed ever since the first reports on the natural history of RTT (Naidu et al., 1986; Steffenburg et al., 2001). However, some recent studies have demonstrated results adhering to this notion (Glaze et al., 2010; Halbach et al., 2013), others have found, like the present paper, that epilepsy is a major concern in adulthood (Anderson et al., 2014; Vignoli

et al., 2012).

In a large multicenter prospective study on the longitudinal course of epilepsy based on data from the Rett Syndrome Natural History Consortium, three distinct seizure patterns emerged: a) no seizures, b) frequent remissions and relapses, and c) unremitting and persistent seizures (Tarquinio et al., 2017). In that study, information on seizure activity the last six months was collected at annual or semi-annual visits to the clinic. The remitting-relapsing pattern was identified in 41%, whereas only 16% had never experienced remission. In the present cross-sectional retrospective study, we applied the same seizure pattern categories, but extended the observation periods to the last five years. For only 17%, remissions for more than six months were reported, while 39% had not had remissions. Unsurprisingly, more children had never had seizures compared to adults. The discrepancies in the two studies are probably for the most part due to different methodologies: retrospective recall and medical records in the present study and prospective follow-up in the American study. The term remission was used for absence of seizures exceeding six months at completion in the American study, whereas in the present study terminal remission was conventionally defined as absence of active epilepsy (5 years seizure-free) (Sillanpaa et al., 2017). Hence, the two studies cannot be compared in these respects.

Seizure frequency tended to differ with age; it was highest in young children with recent seizure onset, although the number of young children with epilepsy was low. Seizure frequency decreased in adolescence and early adulthood, but there was a trend towards a slight increase later in adulthood, in contrast to previous ideas. This tendency was also apparent in the retrospective longitudinal data (Fig. 2). Half of the women above 30 years had seizures at least weekly. More adults had tonic-clonic seizures compared to children and adolescents and more women above 30 years had multiple seizure types.

Seizure types and episodic behavioral abnormalities are multiple in RTT and are often difficult to differentiate on a clinical basis. Very few participants in this sample had undergone ictal video-EEG recordings due to spells of uncertain significance, but only seizure types clearly identified from the current operational ILAE seizure classification (Fisher et al., 2017) were acknowledged in the present study. Seizure semiologies and EEG characteristics in RTT are consistent with both focal and generalized seizures (Dolce et al., 2013; Steffenburg et al., 2001), and often fall within the category of unknown onset (Fisher et al., 2017). Importantly, the epilepsy of RTT is an example of "combined generalized and focal epilepsies", along with some other genetic epilepsies, such as Dravet Syndrome. This particular type of epilepsy has only recently been acknowledged as a separate entity by the International League Against Epilepsy (Scheffer et al., 2017).

4.2. Mutation groups

There is a general consensus about the association between genotype and general phenotype in RTT (Cuddapah et al., 2014). In contrast, the association between genotype and epilepsy remains unclear and results have been somewhat conflicting (Bao et al., 2013; Cardoza et al., 2011; Nissenkorn et al., 2015). One recent study suggested that seizure

Table 4
The relationship between RTT severity and seizure patterns adjusted for age and mutation groups by multiple regression analysis.

Variable	Unadjusted effect	95% CI	p-value	Adjusted effect	95% CI	p-value
Seizure pattern group 2 vs 1	2.695	0.623-4.767	0.012	1.477	−0.775-3.729	0.194
Seizure pattern group 3 vs 1	1.762	−0.190-3.714	0.076	1.417	−0.509-3.343	0.146
Seizure pattern group 4 vs 1	3.364	1.782-4.947	< 0.001	2.851	1.226-4.476	0.001
Age	0.074	0.029-0.118	0.002	0.076	0.028-0.123	0.002
Mutation group severe vs mild	0.958	−0.609-2.526	0.226	1.626	0.301-2.951	0.017

frequency is not strongly associated with mutation type (Tarquinio et al., 2017).

In the present sample, the overall correlation was weak, and epilepsy features were almost identical in participants with so-called mild and with severe mutations. However, the age distribution in the two groups was strikingly skewed. The mean age of participants with mild mutations was significantly higher than in the severe mutation group. Children and adolescents with mild mutations had significantly lower mean global severity and less frequent seizures, compared to participants with mutations associated with more severe disease. In contrast, adults with mild mutations had a trend to higher global severity scores and more severe epilepsy. They even had earlier seizure onset than adults with mutations considered more severe.

We can only speculate on the cause of the age difference in the two mutation groups. A survival effect might be operative. Life expectancy may generally be shorter in individuals with RTT who have severe mutations and higher global severity as well as hazardous seizure disorders (Tarquinio et al., 2015). However, the trend to a milder overall phenotype (including seizure frequency) in women with RTT reaching advanced age in the group with mutations previously associated with more severe disease was striking. These trends are a surprising finding, and should be further investigated with larger samples.

4.3. Epilepsy and global clinical severity

The scores on the Rett Syndrome Severity Scale correlated significantly with the seizure pattern severity, with mean scores increasing from seizure pattern group 1 (without epilepsy) to group 4 (active epilepsy without remission). Due to the wide age range in the present sample, aging and deteriorating health were regarded as a potential bias (Cianfaglione et al., 2016; Cuddapah et al., 2014). When adjusted for age and mutation group, the association was still significant. This finding is in line with other studies (Jian et al., 2007; Tarquinio et al., 2017), although these used different scales for clinical severity. Jian et al. (2007) found an association between RTT severity and parent-reported seizure rate, while Tarquinio et al. (2017) compared participants with and without epilepsy and found that global severity scores were higher in those with epilepsy.

RTT is a condition that highlights the current discussion on the differentiation between a "developmental encephalopathy" and an "epileptic encephalopathy" where the epileptic activity itself contributes to cognitive and behavioral impairments beyond what might be caused by the underlying condition alone. According to the 2017 revised ILAE epilepsy classification (Scheffer et al., 2017), the concept of epileptic encephalopathy should be applied more widely than just for some severe epilepsies of childhood with bilateral and abundant epileptiform activity. Even in the self-limited focal epilepsies of childhood, there is evidence of a widespread impact of the epileptic disease process on cognitive functions (Wickens et al., 2017). The present findings cannot determine whether the more severe overall RTT phenotype simply is associated with more severe epilepsy, or if the clinical epileptic activity itself influences the severity of the developmental

disorder. Further research should endeavor to clarify whether RTT is a "developmental encephalopathy with epilepsy" or a combined "developmental and epileptic encephalopathy" where both factors play a part (Scheffer et al., 2017). If the latter is true, early intense anti-seizure treatment might have the potential to ameliorate the overall clinical consequences of RTT.

4.4. Limitations and strengths of the study

It is challenging to distinguish between epileptic and non-epileptic seizures in RTT. In Norway, all patients with epilepsy are routinely examined with interictal EEG recordings, but in this disorder EEG is universally abnormal, and the diagnosis of epilepsy should not rely on interictal abnormalities (Tarquinio et al., 2017). The study design with parental reports might have influenced the results by over-reporting of epileptic seizures (Glaze et al., 2010). Tarquinio et al. (2017) report that physicians diagnosed seizures in attacks that parents believed were non-epileptic in 3% of the cases, whereas parents suggested seizures in 4% of episodes that physicians considered to represent other types of spells. The problem of inappropriate seizure recording is probably as common in adults, as caregivers in group homes are often multiple, unexperienced and may be responsible for the individuals for only shorter periods. Nevertheless, care was taken not to interpret typical episodic RTT behavior, such as midline stereotypies, hyperventilation and autistic features as epileptic seizures. On the other hand, subtle non-motor seizures with behavior arrest or impaired awareness only may not have been clinically recognized.

Of course, a recall bias of historical data may be present in this kind of study. To minimize this source of error we reviewed medical records for most participants. Only large scale prospective studies will ultimately determine to what extent the validity of this study is influenced by these factors, as well as by the relatively low number of participants in some subgroups.

Nevertheless, a unique strength of the present study is its population-based character, reducing the selection bias of specialized clinics and yielding a wide age span. In spite of the high proportion of adults in this study compared to previous ones, a somewhat skewed distribution towards lower age might well be present. Families having a daughter with RTT in the younger age groups may be more active in the parent association, and parents with newly diagnosed children may make use of more services from the Resource Center for Rare Disorders. Thus, a larger proportion of families with younger girls with RTT may have received the invitation to participate. Although this was a nationwide study, the number of participants was below 60% of those registered with RTT in the Norwegian Patient Registry (n = 165).

Moreover, the general awareness of the RTT phenotype is probably higher among child neurologists than among adult neurologists due to the characteristic history of RTT features in early childhood. In adult age, difficult-to-treat epilepsy is usually the symptom that brings individuals with RTT to the attention of the specialist health care, whereas individuals without seizures or with resolved and well controlled epilepsy often are treated on a less specialized health care level.

Hence, RTT might more often remain unrecognized in individuals without seizures. Even if recognized, the broader and more common diagnostic categories of severe intellectual disability and autism spectrum disorder may be applied for this rare disorder for the sake of ease in a busy clinical practice.

It has been suggested that the RTT phenotype may have a broader genetic background than previously recognized which may cause an overlap with other genetic disorders (Ehrhart et al., 2018). Hence, we chose not to include two individuals with Rett features harboring *SCN1A* mutations and early seizure onset due to a possible link to Dravet syndrome. Nevertheless, we decided to keep three individuals with classic RTT without identified mutations according to the diagnostic criteria for RTT.

Another strong point is the fact that almost all participants in this study were personally examined by one clinical investigator (the first author), with extensive knowledge about RTT. The same person interviewed the parents or caregivers of almost all participants and organized and collected all data in a uniform manner.

5. Conclusions

In the present sample, two thirds of females with RTT still have active epilepsy in adult age. The most common seizure pattern in individuals above the age of 30 was relentlessly unremitting seizures, whereas some experienced remissions and relapses. For a minority of individuals with previously diagnosed epilepsy long-lasting seizure control was achieved, while a few never developed seizures.

Several publications convey the view that the seizure disorder in RTT usually improves or remits in adult age. This notion needs to be modified. The present results confirm that epilepsy frequently remains as a major concern in advancing age of females with RTT. Continued specialist epilepsy service is needed in these individuals.

Declarations of interest

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Medical issues in adults with Rett syndrome – a national survey

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Medical issues in adults with Rett syndrome – a national survey

Objectives: To examine main health issues in a population of females with Rett syndrome, with a focus on individuals aged 36 or older. *Methods:* A national survey including 85 females, divided into a younger (1–20 years), a middle (21–35 years) and an older group (36–66 years). Data include clinical examination, medical records and parental interviews. Prevalences of six main medical issues (scoliosis, ambulation, growth, respiration, gastrointestinal dysmobility and epilepsy) and severity scores in the three groups were compared. *Results:* Mean severity scores were 11.8, 15.1 and 13.7 (from younger to older), and the difference between the younger and the middle group was significant. No other significant prevalence differences were observed. *Conclusions:* Most main medical issues in Rett syndrome continued to be a major concern in adulthood, but health did not seem to decline with increasing age. The results emphasize the need for clinical follow-up throughout adulthood.

Keywords: Rett syndrome; ageing; adulthood; morbidity; clinical management

1. Introduction

Improved living conditions and better health care have contributed to increased life expectancy all over the world.[1] Whilst the increase has been considerable in the general population, it has been dramatic in the population with intellectual disabilities. In the 1930s, the average life span of males with intellectual disabilities was 15 years; today the expected longevity is 64 years.[2, 3] Hence, health professionals increasingly have to manage the needs of adults and elderly people with intellectual disabilities.

Rett syndrome (RTT, OMIM 312750) is a severe neurodevelopmental disorder affecting approximately 1:9-10.000.[4, 5] RTT is characterized by an apparently normal early development followed by neurological regression affecting motor, cognitive and communication skills, and is mainly found in females. More than 95 percent of females with classic RTT have a mutation in the *MECP2* gene.[6] The severity of the syndrome is associated with the type of mutation and where it is located on the gene.[7]

In the original cohort presented by Andreas Rett in 1966 the survival rate at 32 years was 10 percent.[8,9] Today, more than 70 percent of women with RTT live past their 50th birthday.[10] Hence, there is a growing population of adults and elderly people with RTT. However, like for many other developmental disorders, research on the health of older adults with RTT is scarce. When scientific insights into the physical and psychological challenges of adults with intellectual disabilities are lacking, there is a risk that important medical aspects may be overlooked and treatable conditions left untreated. The consequence may be less optimal health and lower quality of life. The available literature on health in older adults with RTT includes one longitudinal study which reports on health in general and a few studies addressing individual clinical characteristics.[11-15] Also other studies of health in adults with RTT include older participants but do not differentiate between adults of different ages.[16-18] The present study compares health issues in a sample of individuals with RTT split into three age groups, with a special focus on individuals aged 36 or older.

2. Methods

This cross-sectional study is a sub-study of a multidisciplinary national survey of females diagnosed with RTT in Norway.

2.1 Participants

An information letter was distributed to families or guardians of females with RTT and RTT-like disorders through the Norwegian Rett Syndrome Association (n=126) and Frambu Resource Centre for Rare Disorders (n=116). The rate of overlapping was high, as only 168 subjects with RTT had been reported to the Norwegian Patient Registry in 2013. Names from these sources were not available to the project. In addition, some participants were referred directly from habilitation clinics and neurologists.

 Ninety-three families gave consent to participate. The diagnoses were reviewed in accordance with the 2010 consensus criteria.[6] Seven individuals did not fulfil the diagnostic

criteria of RTT and were excluded. Six individuals with clinical RTT were excluded due to mutations in other genes (SCN1A, SMC1A, CDKL5, FOXG1), which might influence the phenotype, and one due to missing clinical data. The final sample included 71 females with classic and 8 with atypical RTT, with a mean age of 23 years (SD 15, range 1–66). All parts of Norway were represented. The sample was divided into a younger group (1–20 years, n=40), a middle group (21–35 years, n=22) and an older group (36–66 years, n=17). In the younger group, 90 percent lived with their parents, while 85 percent of the adults in the middle and older groups lived in residential facilities.

All participants without a known mutation were offered genetic analyses (one participant was not tested). Participants with negative results on earlier tests were retested with an exome-based high throughput sequencing (HTS) analysis with bioinformatic filtering of a panel of genes known to cause intellectual disability and/or epileptic encephalopathies. Participants with no prior testing were first tested for mutations in *MECP2* (Sanger sequencing and MLPA), and if the results of these tests were negative the exome-based HTS analysis was performed. During the diagnostic workup, the number of genes in the diagnostic gene panel for intellectual disability available from the laboratory increased from 45 to above 1400. When the number of genes increased the approach changed from a single patient analysis to a trio analysis with analyses of proband, father and mother. Seventy-four had a presumed pathological *MECP2* mutation, and in four participants no pathological mutations were found.

2.2 Measures

Measures included information about the six main clinical characteristics of RTT: ambulation, scoliosis, growth, gastrointestinal dysmobility, epilepsy and respiratory irregularities. Ambulation was categorized in an ordinal fashion ('walking independently', 'walking with support' or 'not walking'), both as present skills and as the best skills so far in life. Declines

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in walking skills were categorized as change 'from being ambulant to non-ambulant' or 'from walking independently to walking with support'. Scoliosis was categorized as 'present' or 'not present', and as 'with surgery' or 'not surgery'. Growth was categorized in accordance with weight, height and head circumference. Body mass index was calculated and categorized according to the Norwegian reference standard.[19] Gastrointestinal dysmobility includes presence of reflux or constipation, and associated medical treatment and/or surgery. 'Active epilepsy' was defined as seizures within the last five years.[20] There were two types of respiratory irregularities: hyperventilation and breath holding. For correlations between genotypes and phenotypes, *MECP2* mutations were classified into two groups, according to expected phenotypic severity based on a previous report;[7] severe (T158 M, R168X, R255X, R270X, large deletions) and mild (R133C, R294X, R306C, other point mutations, c-terminal truncations). Finally, on the participants were assessed with the Rett Syndrome Severity Scale.[21] A questionnaire included information about demographic background and development of motor skills, while a semi-structured interview addressed pregnancy and birth, development, communication skills, and medical history. A clinical examination included growth parameters, level of contact, presence of stereotypies and respiratory abnormalities, and assessment of muscle tone, deep tendon reflexes, coordination and scoliosis.

2.3 Procedures

Assessments and interviews were made between 2013 and 2017. Parents or other caregivers completed the questionnaire about demographic background and development of motor skills prior to the clinical assessment. The researchers met the families at their local hospital or in their home, where the clinical examination was carried out. The interviews with parents or caregivers were conducted during the same visit. Medical records were reviewed to supplement and complete the data.

2.4 Statistical analysis

The descriptive analyses include mean and standard deviations or median and inter quartile range for continuous variables, and absolute and relative frequencies of categorical measures. Chi square or Fisher's exact test were used to compare groups on categorical measures, and one-way ANOVA with post hoc tests on continuous measures. Missing data were handled by restricting analyses to individuals with complete data on the variables included in the particular analysis. Significance level was ≤0.05. All statistical analyses were performed with SPSS for Windows, Version 23.

2.5 Ethics

Ethical approval was obtained from the Regional Committee for Medical Research Ethics, South East Norway (No. 2012/1572). Consent from parents or guardians was obtained prior to inclusion.

3. Results

Thirty-five of the 40 participants in the younger group (87%), 20 of the 22 participants in the middle group (91%), and 16 of the 17 participants in the older group (94%) had classic RTT. The proportion of individuals with a *MECP2* mutation presumed to give a milder phenotype was significantly highest in the oldest group (Table 1).

3.1 Motor function and scoliosis

In the total sample, 57 individuals (72%) had been walking with or without support at some point in life. Among the 53 individuals for whom information about early walking was available, the median age of onset of walking was 1;11 years;months (IQR=1;5-2;0, range =0;9–6;0). The majority (n=31, 59%) had learned to walk between age 19 months and three years. Twenty (38%) walked before 18 months, and two individuals after three years (at five and six, respectively). There was a non-significant trend for early walkers (before 18 months) to show less decline in walking skills than later walkers (25 vs 42%, p=0.200).

The ambulation status at the time of inclusion was quite evenly distributed between independent walking (n=27), walking with support (n=22) and non-ambulation (n=30). Around 40 percent of the participants in all three age groups were non-ambulant (Table 1). However, motor development differed significantly between the age groups. In the older group, all participants had been walking at some point in life, while in the younger and middle groups, one third had never been ambulant (Table 1). Six individuals in the older group and two in the younger group later became non-ambulant (Table 1). In addition, 12 individuals showed decline from independent walking to walking with support: two in the older group, eight in the middle group and two in the younger group (Table 1). The median age for decline in walking skills was 14 years (range 8–45 years). Only three individuals lost walking skills after age 20; one of these had medullary disease and one started the decline in adolescence (from independent walking to walking with support) and became non-ambulant at the age of 37. Five individuals did not retain walking skills after a surgery (three for scoliosis, one for hip dislocation and one for medullary meningioma). Figure 1 illustrates the decline in walking skills in adolescence and the stabilization in adulthood.

Scoliosis was the medical condition affecting most individuals (86%). The highest prevalence was in the middle group (100%), in the older group the prevalence was slightly lower (88%) (Figure 2). Of the 37 adults (>20 years) with scoliosis, eighteen (49%) had undergone scoliosis surgery (Figure 2).

3.2 Growth and gastrointestinal dysmobility

Body mass index was available for 73 subjects. Twenty-seven (37%) were underweight and 16 (22%) overweight. Underweight had the lowest prevalence in the older group (4 of 16, Table 1). Twenty-eight participants had a gastrostomy feeding tube, with the lowest prevalence in the older group (Table 1). Fourteen individuals used the tube for most nutrition while the rest had mainly oral intake of food and used the tube for extra liquid and/or

medication.

Some kind of gastrointestinal distress was reported in 74 individuals (94%) at the time of inclusion. Constipation was the most frequent symptom, with a prevalence of 82% in the younger, 95% in the middle and 77% in the older age group (Figure 2c). Information about treatment of constipation was available for 72 individuals. Fifty-six (78%) used medication and one had been through surgery. Reflux was present in 35 individuals (45%) and 27 (38%, data missing for eight individuals) used antacids, H2 blockers or proton pump inhibitors. Five individuals had undergone reflux surgery. The prevalence of reflux showed a non-significant trend of decrease with increasing age (Figure 2d).

3.3 Epilepsy

The prevalence of active epilepsy at the time of inclusion was 57%. In addition, 11 individuals (14%) had previously been diagnosed with epilepsy, but had been seizure free for at least the last five years. Median age of first seizure was 3;6 years;months (range 0;2–40;0 , IQR 3;0– 7;0). Figure 2e shows the prevalence of active epilepsy in each age group and the prevalence of participants with seizures at least monthly. There are no significant differences between the age groups, but a trend toward a higher prevalence of active epilepsy and more frequent seizures with increasing age. All individuals but one (98%) with active epilepsy used anti-epileptic medications, and 26 (59%) used anti-epileptic polytherapy.

3.4 Respiratory irregularities

Breath holding was the most frequent respiratory dysfunction with a prevalence of 60% in the younger group, 73% in the middle and 69% in the older age group (Figure 2e). Thirty-five per cent in the younger, 68% in the middle and 59% in the older age groups showed episodes of hyperventilation (Figure 2e).

3.5 Global severity

There is a significant difference in mean severity score between the younger and the middle

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age group (Table 1). The middle group had a higher average Rett Syndrome Severity Scale score than the younger group. The older group had a lower mean than the middle group but the difference was not significant.

4. Discussion

This population-based cross-sectional study examined six main health issues in individuals with RTT, with a focus on adults aged 36 or older. There were no significant differences in the prevalence of these health issues in the three age groups (1–20, 21–35, and 36–66 years). Overall, the results demonstrate stability of health conditions and a need for life-span follow-up.

The older group had a lower average score on the severity scale and lower prevalences, although non-significant, of several other measures (e.g. scoliosis, underweight, reflux and constipation) compared to the middle group. It is noticeable that the older group had a higher prevalence of mutations associated with a milder phenotype than the two other age groups, and that all the participants in this group had been ambulant at some point in their life. This might indicate a "healthy survivor" bias in RTT, also suggested in other papers.[18, 22] In the future there may, as a consequence of new therapeutic approaches increasing survival, see more severe RTT phenotypes among the oldest individuals.

The presence of preserved walking skills in the older females is in line with reports from Italy and Australia.[17, 18] However, in a Danish study only three of 27 females aged above 30 years were non-ambulant and 12 (44%) walked without support.[23] The fact that all participants in the older group had been able to walk supports the results from the North-American Natural History study, suggesting that walking may be a positive sign of longevity.[10] One third of the participants did however experience a decline in walking skills. An important developmental finding is that the decline in walking skills happened mainly before the age of 20, which is in line with earlier reports of stability of gross motor skills in

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adulthood.[24] Although there are some differences in methodology, together the studies indicate that gross motor skills may be maintained into older adulthood. This is important, because compared to walking independently, walking with support has been found to be strongly associated with a sedentary life style, with its negative impact on health.[25] Being non-ambulant is a known risk factor for morbidity and mortality.[10] In addition, Andrews and associates found that non-ambulant girls and women with RTT were less involved in activities outside the home.[26]

Non-ambulation is also associated with increased risk for development of scoliosis, possibly due to more severe neurological impairment affecting muscle tone in non-ambulant girls.[27] In the present study, more participants in the older group than in the middle group had been ambulant; and this might explain the slightly lower prevalence of scoliosis in older adults than in younger adults. Still, scoliosis affected almost all of the adults (aged 21-66) and nearly half had been through surgery. The guidelines for management of scoliosis in RTT strongly recommend physiotherapy in individuals with scoliosis.[28] Studies indicate that intensive training may improve walking skills in adults with RTT or even bring back walking skills that have been lost for decades.[29,30] Together with knowledge about the positive health effects of physical activity and of remaining ambulant,[15, 27, 31] the results emphasize the need for physiotherapy and physical activity in adults with RTT.

The results of the present study support earlier reports of around 40% with underweight in the population with RTT.[11, 14] However, the prevalence of overweight (21%) was much higher than the 1–9% reported in other studies,[11, 14, 17] even though the BMI threshold for overweight in children and adolescents is slightly higher in the Norwegian classification system than the norms of WHO.[19, 32] There was less underweight in the older group, although the difference did not reach statistical significance, a trend contrary to what is reported in other studies.[16, 33] The difference cannot be explained by more use of gastrostomy

tubes because this was significantly less frequent in the older group, but might be explained by the healthy survivor effect. However, the results seem to indicate a trend of less nutritional problems in general that surpasses the effect of a possible healthy survivor bias. Different classification methods prohibit direct comparisons of former and present reports, but in the 1990s around 60% of females with RTT were reported to be underweight,[33, 34] which is clearly higher than in the more recent reports. Both an increased focus on nutrition and more use of gastrostomy tubes may have contributed to less underweight in this group. However, the prevalence of underweight in individuals with RTT is still high, possibly related to feeding difficulties, oromotor dysfunction, autonomic dysfunction and apraxia.[35] There is increased risk of morbidity and premature mortality in underweight individuals.[10] It is therefore important that health professionals attend to nutritional needs and take appropriate action when required.

The trend of reflux showing a decreasing prevalence with increasing age did not reach significance in the present study, but similar developmental trends have been reported in other studies.[14, 18] Constipation may seriously affect the well-being of the individual.[36] A prevalence of constipation of around 80% in all three age groups is in line with other studies,[14, 18] and demonstrates the importance of addressing this issue.

Unlike most other medical issues described here, and contrary to the results of other studies,[11, 37, 38] epilepsy was most frequent in the older group. In spite of a possible healthy survivor bias, epilepsy was a major concern in this group. Epilepsy in the present cohort is discussed in more detail in a previous publication.[13]

The prevalence of breathing disturbances in the present study is slightly lower than in a recent US study,[15] and higher than reports from Italy and the UK.[16, 18] This discrepancy might be explained by study designs involving parental reports and by the waxing and waning pattern of respirational dysfunction, which both are found to reduce the reliability of reported

prevalence of respiratory dysfunction.[15] In the present study, breathing disturbances were maintained across age groups, in line with two longitudinal studies.[11, 15]

An limitation of the present study is the relatively low number of participants. The population-based design however do limit the selection bias of studies based on samples from specialized clinics. Both the age range and the geographical distribution indicate that the present sample is representative of the Norwegian population diagnosed with RTT. It is however likely that the proportion of individuals with undiagnosed RTT differs with age. The oldest participants were born before diagnosis of RTT was established, and it took even longer before the RTT variants were described.[39] It is therefore likely that the clinical variation is smaller among adults with a diagnosis of RTT than in children and adolescents. This might influence the results, but since RTT variants include both milder and more severe phenotypes than classic RTT the direction of this bias is difficult to estimate. A healthy survivor bias will probably skew the results toward a better health in the oldest group. Another limitation of this study is the parental reporting, which may be influenced by the fact that almost all children and adolescents lived at home, while just a few of the adults did. Parents might be less informed of their child's health when they are not living together, which may have led to an underreporting of symptoms in adults. We have tried to limit this bias by including personnel from residential homes in interviews when possible, and by collecting complementary information in the participants' medical records.

The health issues investigated here do not include all conditions affecting the health of adults with RTT, and apart from medical conditions, wellbeing, participation in social activities and communication are issues of high importance that should be subject to further research. Still the results of the present study point to issues that will be important for clinicians treating adults with RTT. The presence of good walking skills and nutritional status in many participants aged 36 or older supports former findings that these are factors that may

contribute to increased longevity and emphasizes the need for interventions that focus on nutrition and physical activity in individuals with RTT of all ages.

In summary, the results show continuity in health throughout adulthood. Thus, the medical conditions investigated here, which mainly have been described in children and adolescents with RTT, continue to be important in later adult life. Epilepsy, scoliosis, breath holding and constipation affected more than 60% of the participants aged 36 or older. Both epilepsy and constipation are conditions where good medical care and proper treatment could improve quality of life, which emphasizes the need for regular medical follow-up of adults with RTT.

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Table 1. Diagnosis, mutations, ambulation skills, growth and severity scores based on age groups

		1-20 years n (%)	21-35 years n (%)	>35 years n (%)	Total n (%)	p
Diagnosis						
Rett syndrome	Classic	35 (87)	20 (91)	16 (94)	71 (90)	0.501[1]
	Atypical	5 (13)	2 (9)	1 (6)	8 (10)	
Mutations						
MECP2 mutation[a]	Yes	37 (92)	21 (95)	16 (94)	74 (94)	1.000[1]
	No	3 (8)	1 (5)	1 (6)	5 (6)	
Presumed phenotypic severity based on *MECP2*-mutation[b]	Mild	17 (47)	10 (50)	13 (87)	40 (56)	0.028[2]*
	Severe	19 (53)	10 (50)	2 (13)	31 (44)	
Ambulation						
Ambulation at the time of inclusion	Ambulant without support	18 (45)	3 (14)	6 (35)	27 (34)	0.085[2]
	Ambulant with support	7 (18)	10 (45)	5 (30)	22 (28)	
	Non-ambulation	15 (37)	9 (41)	6 (35)	30 (38)	
Best walking skills ever in life	Ambulant without support	22 (55)	11 (50)	13 (76)	46 (58)	0.021[1]*
	Ambulant with support	5 (13)	2 (9)	4 (24)	11 (14)	
	Non-ambulation	13 (32)	9 (41)	0 (0)	22 (28)	
Deterioration of walking skills	Yes	4 (15)	8 (61)	8 (47)	20 (35)	0.007[2]*
	No	23 (85)	5 (39)	9 (53)	37 (65)	
Growth and nutrition						
BMI classification	Underweight	15 (42)	8 (38)	4 (25)	27 (37)	0.827
	Normal weight	13 (36)	9 (43)	8 (50)	30 (41)	
	Overweight	8 (22)	4 (19)	4 (25)	16 (22)	
Gastrostomy feeding tube[c]	Yes	15 (39)	11 (50)	2 (12)	28 (36)	0.043[2]*
	No	24 (61)	11 (50)	15 (88)	50 (64)	
Rett syndrome severity score						
RSSS	Mean (95% CI)	11.79 (10.64-12.94)	15.10 (13.76-16.43)	13.69 (11.79-15.58)	13.12 (12.29-13.95)	0.002[3]*

1: Fisher exact test; 2: Chi square test; 3:Oneway ANOVA; *: Significant; a:One individual was not tested; b: Three individuals with MECP2-mutations were not categorized because their mutations was not described in Cuddapah et al [7]; c: Missing data in one individual
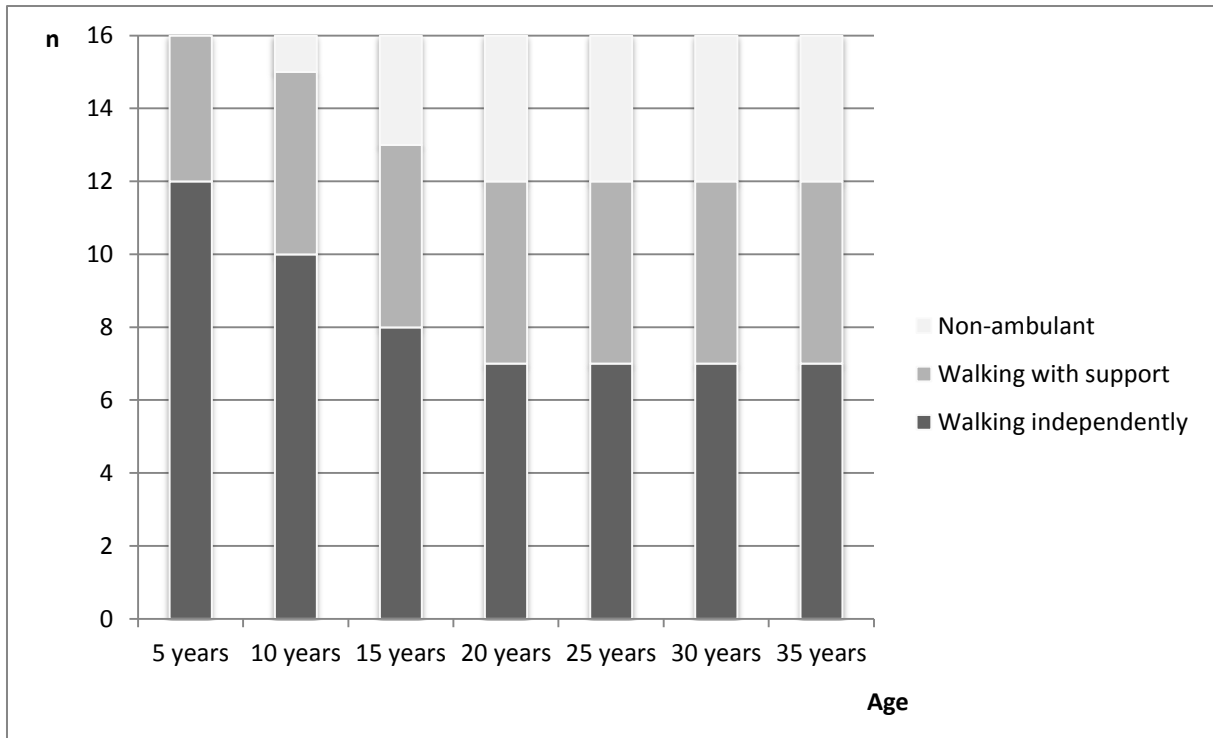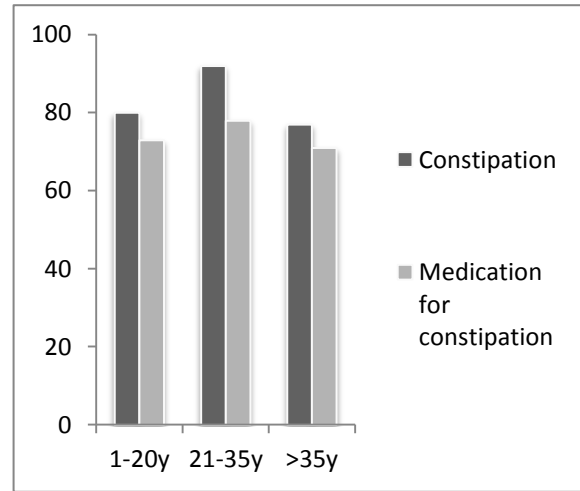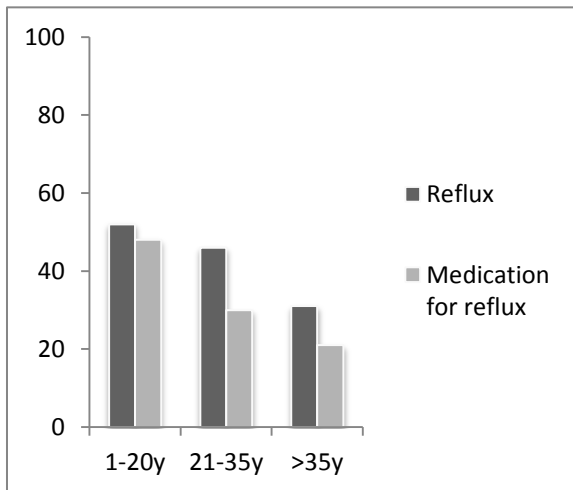
Figure 1. Ambulation status at different ages in the participants aged 36 or older (data missing in one individual)
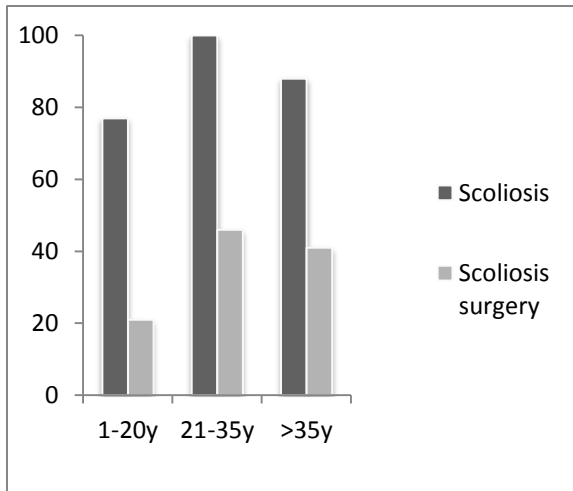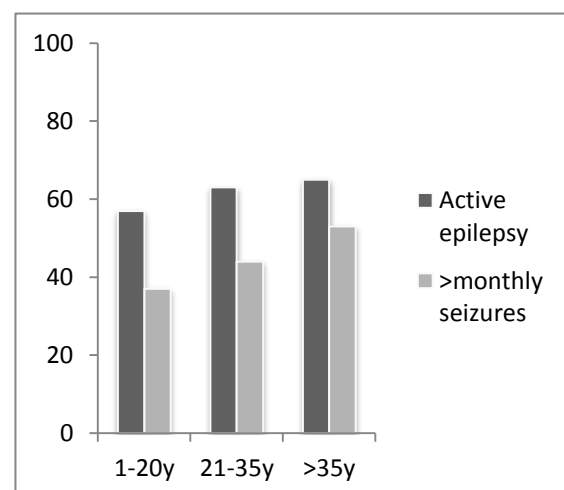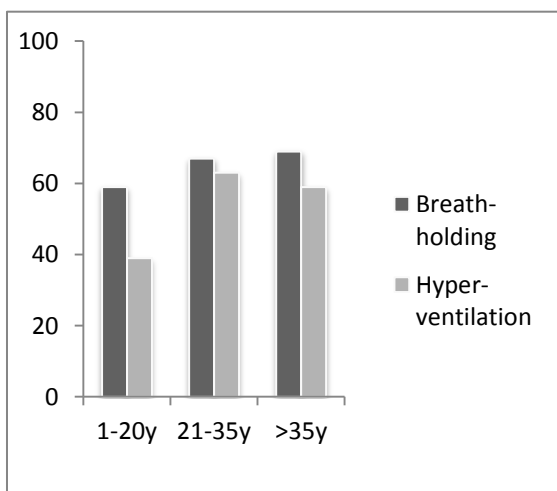
Figure 2. Cross-sectional point prevalence of a) scoliosis and scoliosis surgery, b) constipation and treatment, c) reflux and treatment, d) active epilepsy and frequency of seizures, and e) Breath holding and hyperventilation.

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BMC Medical Genetics

CASE REPORT

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De novo mutations in *SCN1A* are associated with classic Rett syndrome: a case report

Mari Wold Henriksen[1,2*†], Kirstine Ravn[3†], Benedicte Paus[2,4], Stephen von Tetzchner[5] and Ola H Skjeldal[6]

Abstract

Background: Rett syndrome (RTT) is a neurodevelopmental disorder. In more than 95% of females with classic RTT a pathogenic mutation in *MECP2* has been identified. This leaves a small fraction of classic cases with other genetic causes. So far, there has not been reported any other gene that may account for the majority of these cases.

Case presentation: We describe two females who fulfill the diagnostic criteria for classic RTT, with pathogenic de novo mutations in *SCN1A*, which usually leads to Dravet syndrome. The developmental history and clinical features of these two females fits well with RTT, but they do have an unusual epileptic profile with early onset of seizures. Investigation of mRNA from one of the females showed a significantly reduced level of *MECP2* mRNA.

Conclusions: To our knowledge, this is the first report suggesting that *SCN1A* mutations could account for a proportion of the females with classic RTT without *MECP2* mutations. As a consequence of these findings *SCN1A* should be considered in the molecular routine screening in *MECP2*-negative individuals with RTT and early onset epilepsy.

Keywords: Rett syndrome, Epilepsy, Genetics, *SCN1A*, Dravet syndrome

Background

Rett syndrome (RTT, OMIM 312750) is a severe neurodevelopmental disorder, characterized by an apparently normal development the first 6–18 months, followed by regressive loss of acquired skills [1]. The current diagnostic criteria for classic RTT require a period of regression, loss of acquired purposeful hand skills and acquired spoken language (if any), gait abnormalities and stereotypic hand movements. Exclusion criteria include grossly abnormal psychomotor development in the first 6 months of life or known brain injury [1]. In more than 95% of females with classic and 50% with atypical RTT, a pathogenic mutation in *MECP2* has been identified [1]. Mutations in 69 other genes have in recent years been associated with RTT and RTT-like disorders [2, 3], including a girl with a RTT-like condition and a mutation in *SCN1A* [4]. The present study reports two females fulfilling the diagnostic criteria for classic RTT [1] with de novo

mutations in *SCN1A*. Pathogenic mutations in *SCN1A* are known to cause Dravet syndrome [5] and have not to our knowledge been associated with classic Rett syndrome.

Case presentations
Case 1

Case 1 is a 19 years old woman (for timeline see Fig. 1). She was born at 37 weeks gestation with a birth weight of 2890 g, length 47 cm, and a head circumference of 32 cm. Pre- and neonatal periods were normal. She had her first seizure, a prolonged febrile seizure, at 5 months of age. She developed afebrile focal seizures and intractable generalized seizures, both myotonic, tonic and tonic-clonic. She has had several episodes with convulsive status epilepticus. Her early development, however, was unremarkable. She developed normal hand function, including a pincer grip, and started to use a few words, 15 at the most. She began walking independently at 17 months. However, from around 15 months of age her development slowed down and she gradually lost acquired skills. She stopped using her hands, her words disappeared and her gait became broad-based and ataxic. She developed midline rubbing hand stereotypies, although not very intense, and bruxism. She often had breath-holding spells and infrequently she hyperventilated.

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Fig. 1 Timeline case 1
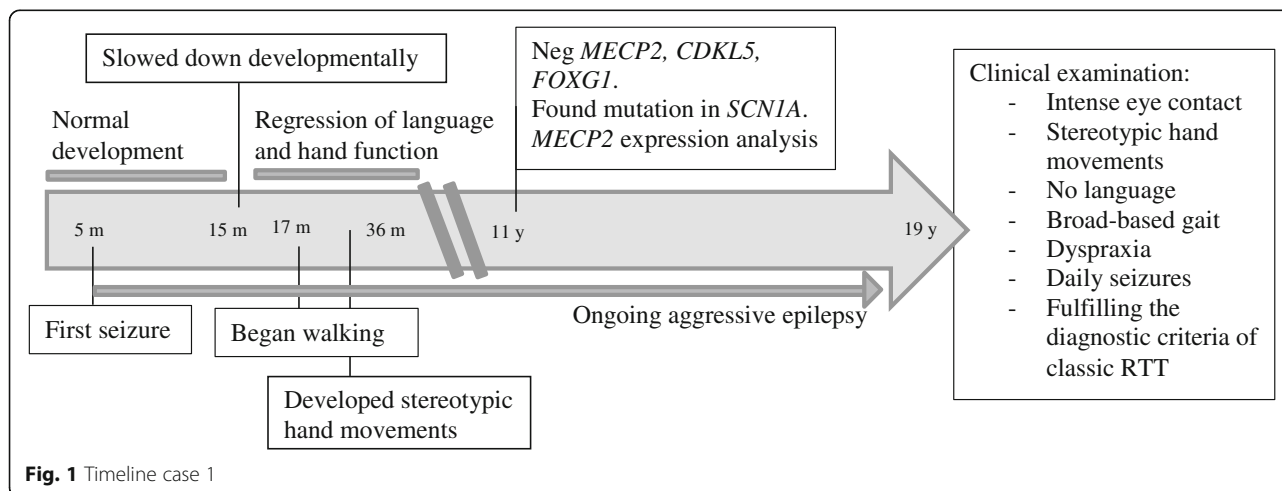
Her sleep pattern was impaired with night time screaming spells and occasionally laughing spells. Between one and 2 years of age, she developed autistic traits. She had a deceleration of head growth from 50th to 10th percentile.

The clinical examination at 19 years revealed a woman with intense eye contact and ongoing stereotypic hand movements with hand dyspraxia. She had a broad-based gait with notable ataxia. Breath holding and teeth grinding were observed. She was only 141 cm tall, but had normal weight for height. Her musculature was generally hypotonic and she had a slight scoliosis. Her epilepsy was still aggressive with daily seizures (focal, tonic and tonic-clonic), despite intense anti-epileptic treatment. Her clinical signs and symptoms were consistent with classic RTT, fulfilling the criteria of this disorder.

CT and MRI scans of the brain were unremarkable. At the age of eleven *MECP2, CDKL5*, and *FOXG1* were analyzed with Sanger sequencing of all exons with flanking intron regions and MLPA kits P015C, P395 and P189 from MRC-Holland, all with normal results. Due to the aggressive epilepsy *SCN1A* was Sanger sequenced and this disclosed the novel splice variant NG_011906.1:g.76169G > C, (NM_001165963.2): c.4284 + 1G > C. Using Alamut Visual software (Interactive Biosoftware, France) and the guidelines of American College of Medical Genetics and Genomics and the association for Molecular Pathology (ACMG) [6], this variant was scored as pathogenic. Parental testing indicated that the mutation was de novo. Two splice mutations (c.4284 + 1G > T and c.4284 + 1G > A) affecting the same splice site, have previously been reported in Dravet syndrome [7, 8]. Because she fulfilled the criteria for RTT, but no mutation in *MECP2* was identified, a *MECP2* gene expression analysis, performed on mRNA isolated from her fibroblasts was performed. This analysis indicated that her *MECP2* expression level was more than 80% reduced compared to three female controls (Fig. 2).

Case 2

Case 2 is a 32 years old woman (for timeline see Fig. 3). She was born at 40 weeks of gestation with a birth weight of 3830 g, length 52 cm, and a head circumference of 36 cm. Pre- and neonatal periods were normal. At 7 months, she had her first seizure, a febrile bilateral tonic-clonic seizure. Between one and 2 years of age her epilepsy became more severe, with daily generalized seizures. The frequency of seizures declined somewhat when she reached school age, but her epilepsy remained drug resistant, with several bilateral tonic-clonic seizures every week. Besides the epilepsy, her development was apparently normal the first 12–15 months. She sat independently at 7 months. At 1 year, she used a few words and had an appropriate use of hands. She learned to walk when she was 24 months old. When she was between 12 to 15 months of age she started to lose acquired skills. Her hand function deteriorated gradually, her words disappeared and she no longer seemed to show interest in her surroundings. She developed bruxism and hand-washing stereotypies. She could walk independently until school age, but then she gradually needed support when walking. Through childhood her sleep pattern was significantly disturbed with both screaming and laughing spells. Her respiration has however never been affected.

The clinical examination revealed a 32 years old woman who could walk a few meters with support, had ataxic and apraxic hand movements, but not hand stereotypies. She had no language but gave intense eye contact. Her muscle tone was normal. She had a slight scoliosis. Her epilepsy was still a major concern, with daily to weekly bilateral tonic-clonic seizures. She fulfilled the criteria of classic RTT.

Genetic analyses of *MECP2* at the age of 18 gave negative results (Sanger sequencing and MLPA kit P015 from

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Fig. 2 The relative expression level of *MECP2* (the alternative transcripts coding for isoforms MeCP2_E1) in cultured fibroblasts of three female controls and Case 1. The *MECP2* gene expression analysis was performed on mRNA isolated from her fibroblasts, using Applied Biosystems 7500 Fast Real-Time PCR System (ABI, Foster City, CA), with pre-designed assay (TaqMan MGB probe HS01598237). *GAPDH* served as an endogenous control. The RQmin and RQmax with the confidence interval set at 95%:Female1; RQ Min 0.77, RQ Max 1.29, Female2; RQ Min 0.85, RQ Max 1.14, Female3; RQ Min 0.787, RQ Max 0,99, Patient; RQ Min 0.07, RQ Max 0,13

MRC-Holland). As a participant in a national survey of females with RTT she was recently retested by applying whole exome sequencing (WES) using Agilent SureSelect Target Enrichment Kit (Agilent Technologies, Santa Clara, CA) on Illumina HiSeq 2500 with pair-end runs. Alignment, mapping, and variant calling were performed using Genome Analysis Tool Kit (GATK). Reads were mapped to the reference sequence (GRCh37/hg19). Following bioinformatic filtration, analysis of coding regions and intron/exon boundaries of 1479 predefined genes (including *FOXG1*, *CDKL5* and *SCN1A* with a 100% coverage at a depth > 10×) was performed. WES disclosed

the variant, NG_011906.1:g.76130G > T, NM_001165963.1: c.4246 G > T, p.(Asp1416Tyr) in *SCN1A*. Using Alamut Visual software (Interactive Biosoftware, France) and ACMG criteria [6] this novel variant was scored as pathogenic. Parental testing indicated that the mutation was de novo. This is a **novel** variant, but mutations affecting the same amino acid have been reported in Dravet syndrome [9].

Discussion and conclusions

We present two females with clinical pictures consistent with classic RTT and who fulfill the diagnostic criteria for this disorder [1], but without mutations in the coding regions of

Fig. 3 Timeline case 2

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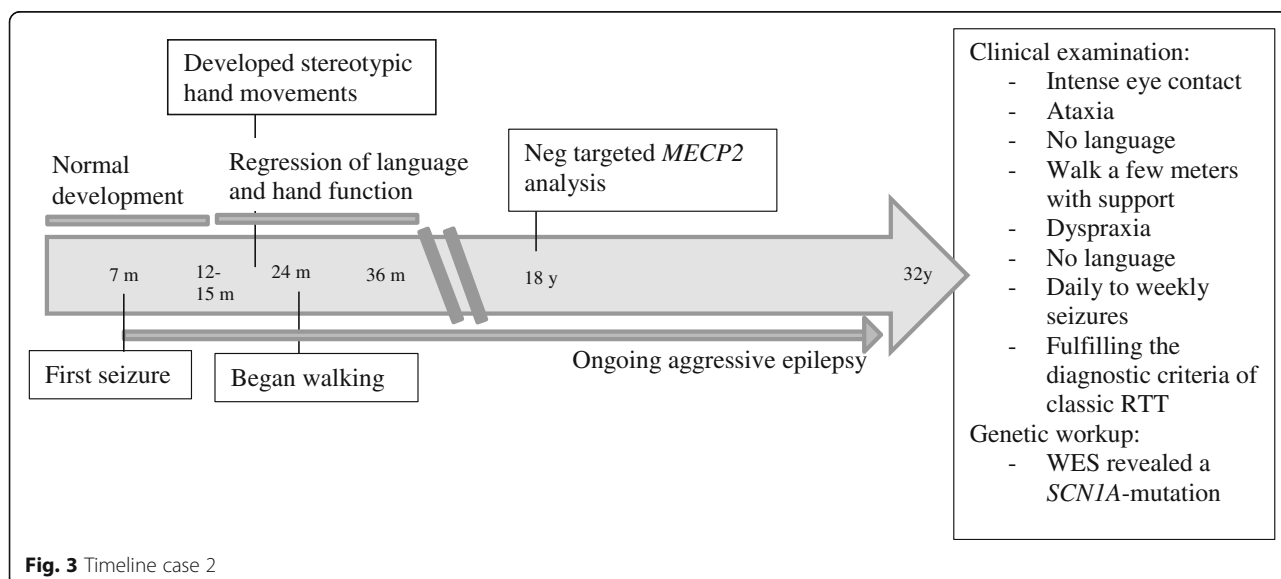
MECP2, CDKL5 and FOXG1. However, deep intronic mutations and duplications/deletions of exons not covered by the MLPA analysis, have not been excluded.

Further genetic analyses revealed presumed pathogenic de novo mutations in SCN1A in both. More than 80% of individuals with pathogenic mutations in SCN1A have Dravet syndrome [10]. Both females do have clinical features associated with this syndrome, like early seizure onset, prolonged febrile seizures, status epilepticus, and drug resistant epilepsy [5]. Dravet syndrome has no clearly defined diagnostic criteria and the phenotypic spectrum is wide. These case reports show that there may be a clinical overlap between features of RTT and other neurodevelopmental disorders, such as Dravet syndrome. This is a challenge for disease classification and diagnosis. Strict and robust criteria are necessary for making consistent diagnoses and sorting out differential diagnosis. Recognizing potential confusion, the revised RTT criteria suggest specifying both phenotype and mutation [1].

Finding the molecular basis is important in clinical practice, for prognosis and genetic counseling, and it may have implications for treatment. It may also be essential for better understanding of the pathophysiology. For instance, in Case 1, harboring the SCN1A splice site mutation, quantitative gene expression analyses showed a reduced level of MECP2 mRNA in fibroblasts, although no MECP2 mutation was detected. In order to evaluate the significance of this finding further research is demanded. Both females presented here participated in a national survey of the Norwegian population of females with RTT. This survey includes 93 participants with RTT and RTT-like disorders, 74 with classic RTT. A total of 12 participants did not have mutations in MECP2, three in the group with classic RTT, including the two females presented here (2.7% of the participants with classic RTT in this cohort). The presence of these two cases in the Norwegian RTT cohort indicates that SCN1A mutations could account for a significant part of the population of females with classic RTT without MECP2 mutations. Although fulfilling the diagnostic criteria for classic RTT their epileptic profile is atypical with early seizure onset and prolonged febrile seizures. The possibility that the two females' phenotype might be a result of two mutations, one SCN1A and one rare intronic variation in MECP2 or CDKL5, seems unlikely with our present knowledge.

In the cohort of 74 individuals with classic RTT these two individuals and two others were the only ones with seizure onset before regression. The findings in this paper could lead to justifying the inclusion of SCN1A in the molecular routine screening for MECP2-negative individuals with RTT and early onset epilepsy.

Abbreviations
ACMG guidelines: Guidelines of American College of Medical Genetics and Genomics and the association for Molecular Pathology; GATK: Genome Analysis Tool Kit; MLPA: Multiplex Ligation-dependent Probe Amplification; RTT: Rett syndrome; WES: Whole Exom Sequencing

Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
MWH collected clinical information and was a major contributor to the writing of the manuscript. KR conducted the MECP2 gene expression analysis and was a major contributor to the writing of the manuscript. OHS led and supervised the project, contributed in writing and interpretation. SvT and BP contributed with interpretation of data and critical reviews of the article. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Informed parental consent was obtained for both females. Ethics approval was obtained from the Regional Committee for Medical Research Ethics, South East Norway (ethical agreement no. 2012/1572).

Consent for publication
Written informed consent for publication of their clinical details was obtained from the parents of the patients. A copy of the consent form is available for review by the Editor of this journal.

Competing interests
The authors declare that they have no competing interests.

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