Cytogenetic and Molecular Cytogenetic Analyses of Brain Tumours

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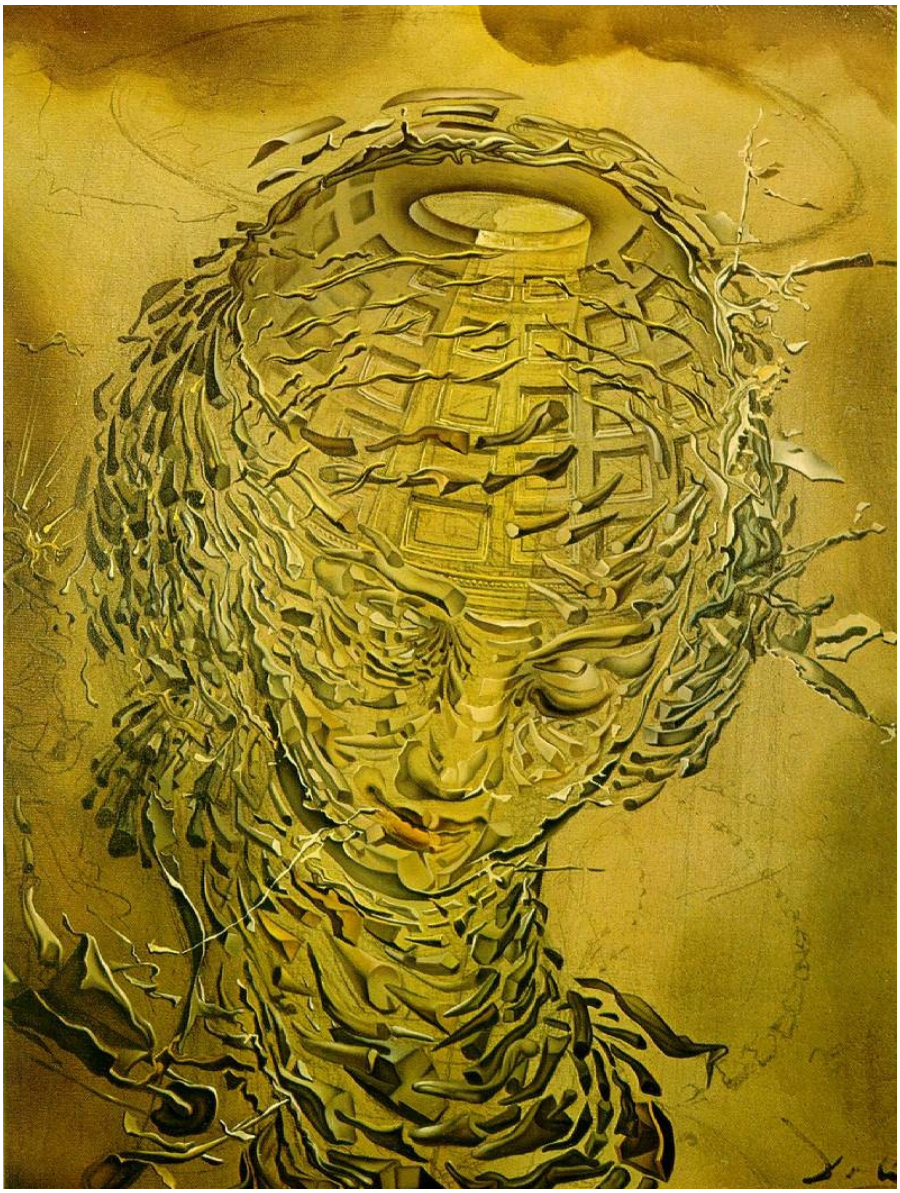

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Cover Illustration

"Tête Raphaëlesque éclatée"/"Raphaelesque Head Exploding" was painted in 1951 by Salvador Dali (1904-1989). Dali was fascinated by renaissance painters, particularly Rafael (1483-1520) and Piero della Francesca (1415-1492), and often borrowed motifs and themes from their paintings in his own work. The skull section in this painting is based on the dome of the Pantheon in Rome where many saints and patrons of the arts are buried, including Raphael. The female face in Dali's painting is recognisable as the face of Rafael's Madonna. Following the Hiroshima atomic bomb in 1945, Dali painted several fragmented figures and heads. The halo and dark clouds appearing above Madonna's head resemble photographs of atomic explosions (Weyers, 2005).

I have always been fascinated by this painting, and to me, the fragmenting head may well represent the collection of acquired genomic aberrations that give rise to tumours of the brain. Nevertheless, Dali's initial intentions with the painting are still relevant. The painting reminds us of the daunting risks of radiation to the public, a topic that has once again become the subject of discussion with the explosions and fires at the Fukushima Dai-ichi nuclear power plant in March 2011.

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Acknowledgements

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To Jacob and Ola for giving me endless joy every day.

Hanne-Sofie Spenning Dahlback.

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Summary

Cancer is the phenotypic result of susceptible somatic target cells acquiring one or more oncogenic chromosomal and/or gene-level mutations. Screening the whole tumour genome is therefore a natural starting point when trying to understand the pathogenetic mechanisms behind tumour development.

Gliomas and paediatric embryonal tumours are challenging neoplasms both diagnostically and therapeutically. Overlapping histopathological features results in high interobserver variability when they are diagnosed phenotypically and there is a call for an alternative grouping of tumours that could hopefully provide information about prognosis as well as key insights into molecular disease mechanisms that may eventually be translated into more effective and individualised therapies.

The overall aim of this thesis was to characterise brain tumours by genome-wide as well as more specific molecular cytogenetic techniques. As each cytogenetic technique has its strengths and limitations, there is a clear need for a multi-modal approach when analysing tumour genomes. Our multi-modal approach involved analysis of chromosomes from brain tumour cells by various combinations of G-banding, comparative genomic hybridisation (CGH) (chromosomal and array-based), multiplex FISH (M-FISH), and, in some instances, locus-specific FISH and DNA ploidy analysis. This combined approach identified a non-random pattern of genomic aberrations in all the examined disease entities. This may prove to be of diagnostic value as well as help identify genomic subsets of patients with high-risk disease that could benefit from early or more intensive anti-neoplastic therapy.

List of papers

This thesis is based on studies reported in the following articles, referred to in the text by their Roman numerals:

I. Dahlback H.S.S., Brandal P., Meling T.R., Gorunova L., Scheie D., Heim S. (2009) "Genomic Aberrations in 80 Cases of Primary Glioblastoma Multiforme: Pathogenetic Heterogeneity and Putative Cytogenetic Pathways". Genes, Chromosomes & Cancer 48:908-924.

II. Dahlback H.S.S., Gorunova L., Brandal P., Scheie D., Helseth E., Meling T.R., Heim S. (2011) "Genomic Aberrations in Diffuse Low-grade Gliomas". Genes, Chromosomes & Cancer 50: 409-420.

III. Dahlback H.S.S., Gorunova L., Micci F., Scheie D., Brandal P., Meling T.R., Heim S. (2011) "Molecular Cytogenetic Analysis of a Gliosarcoma with Osseous Metaplasia". Cytogenetic and Genome Research 134: 88-95.

IV. Dahlback H.S.S., Brandal P., Krossnes B.K., Fric R., Meling T.R., Meza-Zepeda L.A., Danielsen H.E., Heim S. (2011) "Multiple Monosomies Are Characteristic of Giant Cell Ependymoma". Human Pathology, in press.

V. Dahlback H.S.S., Brandal P., Gorunova L., Widing E., Meling T.R., Krossnes B.K., Heim S. (2011) "Genomic Aberrations in Paediatric Gliomas and Embryonal Tumours". Genes, Chromosomes & Cancer, in press.

"The art of simplicity is a puzzle of complexity"

— Douglas Horton (1891-1968)

General Introduction

Historical background

Human beings have always been intrigued by the brain and by the diseases that affect it. Archaeologists have found trephined skulls dating back to the Mesolithic era, perhaps as far back as 10,000 BC (Missios, 2007). The skulls show evidence of new bone formation over the burr holes, indicating that these early patients most likely survived the crude procedures (Verano and Williams, 1992; Greenblatt et al., 1997).

The earliest descriptions of cancer and of diseases affecting the head and skull are found in the ancient Egyptian Edwin Smith Papyrus from 1600 BC (Breasted, 1991). This script outlines rational treatments in a culture where medicine, magic, and superstition were inseparable. Interestingly, for ulcers or tumours of the breast, the author(s) noted: "there is no cure".

The word *cancer* dates back to Hippocrates (460-370 BC), by many considered the father of medicine because he made an attempt to establish a naturalistic theory of how diseases arise. He was the first to use the word *karkinoma* to describe a tumour because it reminded him of a crab (*gr. karkinos*) with its central body and leg-like projections. This was later translated to the Latin word *cancer* by Celsus (25 BC- 50 AD). Hippocrates provided, amongst multiple great achievements, detailed descriptions of several tumours visible on the surface of the human body but not of internal ones; opening the human body was against Greek tradition (von Staden, 1992; Missios, 2007). His humor theory suggested that disease is caused by an imbalance of the four bodily fluids (black and yellow bile, blood, and phlegm).

In his view, treatment should always aim to restore the missing balance and should therefore consist of diets, blood-letting, and/or laxatives (Karpozilos and Pavlidis, 2004).

In the 16th and 17th centuries, it became more acceptable for doctors to dissect bodies to get an overview of human anatomy, but also to understand disease processes (Kaye and Laws, 1995). However, the current genetics-based theories of cancer did not originate until the late 19th and early 20th century (see below).

The birth of cytogenetics

With the publication of the "Origin of Species" in 1859 by Charles Darwin and Georg Mendel's "Experiments on Plant Hybrids" (1865), the realm of genetics could have gotten off to a flying start. Unfortunately, neither Darwin nor Mendel lived to see Mendel's work on the breeding of peas rediscovered and verified in 1900 by Correns, von Tschermak, and de Vries (Charlesworth and Charlesworth, 2009).

Human cytogenetics as a research discipline began with the work of Arnold (1879) and Flemming (1879; Speicher et al., 2010), who were the first to examine human mitotic chromosomes. They described structures related to the cell nucleus that had a high affinity for aniline dyes, and in 1888 these were named chromosomes (*gr. chromos* = colour and *soma* = body) (Waldeyer, 1888). However, it was only following the re-discovery of Mendel's laws which clarified inheritance that chromosomes were associated with the material of heredity, the Mendelian "factors", later called genes in the Sutton-Boveri theory of 1902 (Crow and Crow, 2002; Satzinger, 2008).

The cytogenetics of the first half of the 20th century mainly focussed on determining the number of chromosomes in man. The 1950s offered two major breakthroughs in that respect, which paved the way for this relatively new medical discipline. The introduction of hypotonic treatment of cells before fixation (Hsu, 1952), causing cells to swell, resulted in improved chromosome spreading in metaphase plates. Secondly, the use of mitotic spindle poisons such as colchicine, an alkaloid derived from the autumn crocus, *Colchicum autumnale*, was shown to inhibit spindle formation during mitosis (Ford and Hamerton, 1956) and arrest the cells in metaphase, thus increasing the number of cells available for cytogenetic analysis. Individual chromosomes could now be reliably counted and analysed, and finally it was established that the correct chromosome number of man was 46 (Tijo and Levan, 1956). Cytogenetics was recognised as a useful diagnostic tool when chromosomal imbalances were linked to constitutional syndromes, i.e. when trisomy 21 was shown to be the cause of Down's syndrome (Lejeune et al., 1959).

The next milestone for cytogenetics was the development of banding techniques. Caspersson et al. (1968) were the first to successfully stain plant chromosomes with quinacrine dihydrochloride, also called quinacrine mustard (Q-banding), and they applied this technique to human chromosomes shortly after (1970). The banding pattern is thought to be the result of variations in the content of base pairs AT (adenine-thymine) and GC (guanine-cytosine) across human chromosomes; AT-rich areas fluoresce intensely with Q-banding (Weisblum and De Haseth, 1972). Sumner et al. (1971) introduced G-banding that produces an almost identical banding pattern by treating chromosome preparations with a salt solution at 60°C, or with a proteolytic enzyme such as trypsin, before staining with Giemsa. G-banding leads to stable and

effective banding without the need for fluorescence and is currently the most widely-used banding technique.

Cancer cytogenetics

In 1890, von Hansemann (1858-1920), a pathologist who had trained with the famous cellular pathologist Rudolph Virchow in Berlin, performed the first systematic study of cell division in malignant tumour cells and discovered mitotic nuclear irregularities. These studies and the re-discovery of Mendelian genetics 10 years later, inspired Theodor Boveri to ask the fundamental question of what causes normal cells to become neoplastic. In his thesis "Zur Frage der Entstehung maligner Tumoren" ("On the Origin of Malignant Tumours")(1914), Boveri stated that "a malignant tumour cell is a cell with a specific defect; it has lost properties that a normal tissue cell retains". In this work he introduced the somatic mutation theory of cancer, which suggested that cancer is the net result of cells acquiring an abnormal chromosomal constitution, and that tumour growth is based on passing this "incorrect chromosome combination... on to daughter cells". In addition to these insightful interpretations that have laid the foundation for genetic cancer research ever since, Boveri also predicted several now well-established concepts such as "teilungshemmende" and "teilungsfördernde" factors (now known as tumour-suppressor genes and oncogenes), cell-cycle check-points, and cancer predisposition (Balmain, 2001).

Methodological difficulties in cytogenetics left the mutation theory of Boveri uncorroborated for over 40 years until improvements in harvesting techniques first allowed the characterisation of normal human chromosomes, and subsequently made visualisation of chromosomal rearrangements in tumour samples possible. In 1960, Nowell and Hungerford, working in Philadelphia, described a small marker, the

Philadelphia chromosome, in bone marrow cells of patients with chronic myeloid leukaemia (CML). This represented the first consistent chromosomal abnormality linked to a specific malignancy, and it seemed to verify Boveri's theory that cancer was indeed a result of the acquisition of such aberrations. In the article (Nowell and Hungerford, 1960a), the authors suggested such a causal relationship, but this bold statement was met with initial scepticism as the general belief at that point was that a chromosomal abnormality could be an associated, but not the causative, phenomenon. The invention of banding techniques (Caspersson et al., 1968, 1970b), however, gave rise to a surge of cytogenetic research that resulted in the discovery of several neoplasia-associated chromosomal aberrations, the first of which was monosomy 22 in meningiomas (Mark et al., 1972; Zankl and Zang, 1972). The Philadelphia chromosome itself, first thought to represent a deleted chromosome 22 (Caspersson et al., 1970a), was in 1973 shown to be the product of a reciprocal translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34;q11) (Rowley, 1973). More than a decade later, the t(9;22) was shown to result in a recombination of the genes *BCR* (from 22q11) and *ABL1* (from 9q34) to form the *BCR/ABL1* fusion gene (Daley et al., 1990; Heisterkamp et al., 1990) that produces an oncoprotein with abnormal tyrosine kinase activity (Lugo et al., 1990).

The Philadelphia chromosome story was to be the first of many similar cytogenetic successes where acquired chromosomal abnormalities specific to various neoplastic diseases were identified (Heim and Mitelman, 2009; Mitelman et al., 2011). This has vastly increased our pathogenetic understanding of various cancer entities, but has also provided opportunities to utilise the new knowledge to develop effective and selective therapies, like imatinib (Gleevec) for CML (Druker, 2008).

Human cytogenetic nomenclature

The methodological advances and the discoveries in CML during the second half of the 20th century created a massive increase in the interest in cytogenetics as a medical science. As several laboratories started examining human chromosomes, a variety of nomenclature systems were proposed. This called for an easy-to-use, exact, reproducible, and standardised classification system on which all could agree and that would aid exchange of information between the researchers in the field. The first system was suggested following a conference in Denver, Colorado, in 1960: "A Proposed Standard System of Nomenclature of Human Mitotic Chromosomes". The current system for human cytogenetic nomenclature is based on the consensus reached at several subsequent international conferences, after which recommendations were published in the form of "An International System for Human Cytogenetic Nomenclature". In 1991, Guidelines for Cancer Cytogenetics (ISCN, 1991) were published as the standing committees grew increasingly aware that the acquired chromosome aberrations of neoplastic diseases could not be adequately described by the nomenclature used to characterise constitutional aberrations. These publications have since been fused into one guideline. The most recent is "An International System for Human Cytogenetic Nomenclature (2009)", abbreviated ISCN (2009).

The chromosomal complement of an individual, cell line, or tissue sample is called a karyotype (gr. *karyon*=cell*, typos*=pattern), which is defined by both the number and appearance of the chromosomes in the cells. By contrast, the karyogram (*gr. karyon*=cell*, gramma*=image) (Fig. 1) is the systemised image of chromosomes prepared by drawing, digitised imaging, or photograph of a single metaphase. Chromosomes are classified according to size, location of the centromere, and banding pattern along each

arm. Autosomes are numbered 1 to 22 in order of decreasing length (with the exception of chromosome 21 which is shorter than 22) and the sex chromosomes are referred to as X and Y.

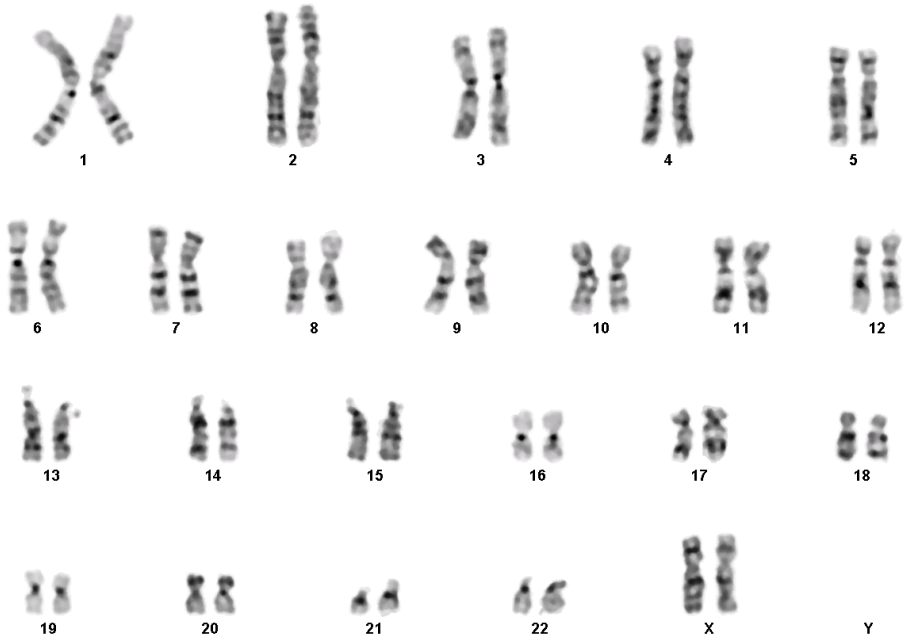

Figure 1: *Karyogram of a G-banded metaphase showing a normal female karyotype 46,XX.*

Each chromosome consists of two arms (Fig. 2): the short arm is called the p-arm (*fr. petit*=small) and the long arm q – simply because this was the next letter in the alphabet. A chromosomal region is defined as areas lying between two specific landmarks with distinct morphological features such as centromeres, ends of chromosome arms (telomeres), and also certain characteristic bands. A band is a chromosomal area that is distinguishable from its vicinity due to lighter or darker

staining intensities. Regions and bands are numbered consecutively from the centromere and out along each arm.

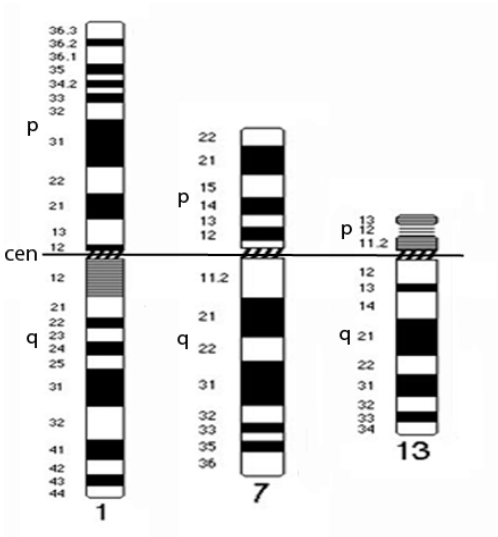

Figure 2. *Human chromosomes are classified into three main groups based on the position of the centromere: metacentric (left), sub-metacentric (centre), and acrocentric (right) chromosomes: The centromere (cen) divides the chromosomes in two; the short p-arm and the long q-arm. Regions, bands, and possible sub-bands are given on the left side of each chromosome.*

The written karyotype starts with the total number of chromosomes, followed by comma, and the sex chromosome constitution. Hence, the normal karyotype is "46,XY" for males and "46, XX" for females. This is followed by a description of the autosomal aberrations, presented in a numerical order, and separated by a comma. The aberrations are described using a combination of symbols and single- or three-letter abbreviations (Table 1). Numerical changes are listed before structural aberrations for each chromosome. Mathematical '+' and '–' symbols are used to indicate gained and missing chromosomes. In rearrangements involving one chromosome only, the chromosome number is indicated within a first parenthesis followed by the two brakpoints in a second parenthesis. If more than one chromosome is involved, a semicolon (;) is used to

Table 1. Common abbreviations in cytogenetic nomenclature

Abbreviation	Aberration	Description
add	*additio*	additional material of unknown origin
cp	composite karyotype	aberrations that are shared by cells with roughly the same abnormal chromosome complements
del	deletion	an interstitial or terminal loss of or from a chromosomal segment
der	derivative chromosome	a structurally rearranged chromosome involving two or more chromosomes or multiple aberrations within a single chromosome
dic	dicentric chromosome	chromosome with two centromeres
dmin	double minute	small acentric circular DNA fragments
i	isochromosome	mirror image of one chromosome arm
idem		from Latin meaning "the same", indicates the stemline karyotype in a subclone
inc	incomplete	unable to recognise all chromosomal elements in the metaphase
ins	insertion	a chromosomal segment moved to an interstitial position on the same or another chromosome
inv	inversion	rotated (180°) chromosomal segment
mar	marker chromosome	an unrecognisable rearranged chromosome
r	ring chromosome	a circular chromosome resulting from the break and fusion of two or more chromosome arms
t	translocation	a chromosomal segment moves from the original position to another chromosome; can be balanced or unbalanced

separate them both in the first and second parenthesis. If one of the involved chromosomes is a sex chromosome, this is listed first. If not, the chromosomes are listed in numerical order (except in insertions where the receptor chromosome is listed first). Breakpoints are given subsequently, also within parenthesis, and if more than one chromosome is involved, breakpoints are given in the same order as the chromosomes, with a semicolon separating them. For example in paper III, the t(3;21)(q13~21;q21~22) designates a translocation between chromosomes 3 and 21 with the breakpoints situated in or between chromosome bands 3q13 and 3q21 and

between 21q21 and 21q22, and del(13)(q13q22) is an interstitial deletion of the long arm of chromosome 13 of the material between bands 13q13 and 13q22.

A clone is a population of cells that stem from a single progenitor somatic cell. In cancer cytogenetics, however, a clone may not necessarily consist of cells that are completely homogenous karyotypically, as subclones may have evolved during tumour development. It is internationally accepted that two mitoses sharing the same structural or gained chromosome or three mitoses missing the same chromosome, signify the presence of a clone. Multiple clones may be cytogenetically related, i.e. share one or more aberrations, or they may be unrelated, in which case they have no aberrations in common.

The most common chromosome number in a tumour cell population is called the modal number. As humans are normally diploid with 46 chromosomes, the modal number of a clone may be described as near-diploid if it is more or less 46. Similarly, the modal number may be hypodiploid or hyperdiploid if it is below or above this level. The same prefixes are used to describe other ploidy levels (e.g. triploid, near-triploid, hypotriploid, etc). If the number of chromosomes in a tumour cell population showing structural aberrations is normal, this tumour is described as pseudodiploid. Aneuploidy is an abnormal balance of chromosomes in a cell (Duesberg and Li, 2003).

Molecular cytogenetics

Chromosome banding techniques remain useful in routine cytogenetic diagnostic service and research. They provide genome-wide screening at a relatively low cost and offer a unique visualisation of the genome. Although the success rate and sensitivity of G-banding analysis has dramatically improved, the frequency of genomic aberrations in some tumours may be underestimated because of the inability to obtain analysable

metaphases. Furthermore, while karyotyping is ideal for revealing genetic heterogeneity in cancer (Pandis et al., 1994), the identification of all clonal genetic aberrations is sometimes difficult because of the overwhelming complexity of changes, poor metaphase quality, and/or low mitotic yield. In addition, chromosome preparations are dependent on live cells for culturing and that the tumour cells divide in vitro, and are limited by the resolution they offer (>5Mb) (Heim and Mitelman, 2009). The analysis is time-consuming and requires substantial cytogenetic experience. There is also a theoretic possibility of in vitro generated aberrations. When using short-term cultures, however, such aberrations have not been shown to be a problem (Pandis et al., 1994).

The discovery of deoxyribonucleic acid (DNA) as the hereditary material of the cell (Avery et al., 1944) and the subsequent deduction of the double-helix structure of DNA (Watson and Crick, 1953) paved the way for molecular cytogenetics. By using appropriately labelled nucleic acid sequences as probes to DNA targets within the cell, one manages to bridge the gap between banding cytogenetics and molecular genetics.

In 1969, Pardue and Gall demonstrated that satellite DNA, in the form of repetitive DNA, could be hybridised to denatured chromosomes in situ on microscope slides using radioactively labelled DNA probes that were detected by autoradiography. These isotopic DNA/RNA probes could not be used for mapping single copy genes, as it was impossible to make them sufficiently radioactive to allow detection. Advances in recombinant DNA technology in the late 1970s, however, allowed construction of DNA libraries and cloning of DNA fragments in bacteria in sufficient quantity to give good hybridisation signals and in the early 1980s, fluorescence in situ hybridisation (FISH) was introduced – a technique that revolutionised gene mapping (Langer et al., 1981; Langer-Safer et al., 1982). This technique is based on the inherent organisation of DNA into two antiparallel complementary strands. After denaturing “target DNA” (tumour

DNA in our case) on metaphase spreads or interphase nuclei on a slide, single-stranded DNA probes are allowed to form hybrid double-stranded complexes with matching genomic sequences. The probes are then labelled with a fluorophore prior to hybridisation, to allow detection by fluorescence microscopy. The advantages of FISH include being able to detect several genomic sequence targets simultaneously because fluorophores of different wavelengths can be combined in the same experiment. In addition, the techniques are rapid to perform, highly sensitive, and there is a flexibility with regards to probes making both whole genome screening and unique sequences down to the gene level possible to target.

In the following, the molecular cytogenetic techniques used in the experiments that form the backbone of this thesis will be described in more detail (Fig. 4).

Genome-wide screening techniques

Comparative genomic hybridisation

Comparative genomic hybridisation (CGH) (Kallioniemi et al., 1992) is a genome-wide screening technique where tumour and normal DNA compete to hybridise to normal human metaphases in order to measure copy number alterations in the tumour genome. Equal amounts of tumour and normal genomic reference DNA are labelled by nick translation using different fluorochromes (a green fluorophore for tumour DNA and a red fluorophore for normal DNA). In the presence of unlabelled Cot-1 blocking DNA, used to prevent cross-hybridisation of highly repetitive sequences, the two differently labelled DNAs are co-hybridised to normal human metaphase spreads. Under normal circumstances, both DNAs would have equal opportunities to hybridise to a specific locus. However, copy number imbalances in the tumour genome will alter the green-to-red fluorescence ratio observed on the target loci in the metaphases; gains and losses will make the target appear more green or red, respectively. The metaphases are also counterstained using 4'-6-diamidino-2-phenylindole (DAPI) allowing chromosome identification based on their inverted DAPI appearance prior to fluorescence ratio analysis. Digital images of 10-15 metaphases are captured and the fluorescence ratio profiles are measured along the length of each chromosome by means of a Cytovision System (Applied Imaging, Santa Clara, CA, USA).

One advantage that CGH has over karyotyping and M-FISH (see below) is the ability to use DNA extracted directly from the tumour. This eliminates problems such as low mitotic index, poor quality metaphases, and the possibility of in vitro generated aberrations. Furthermore, it is possible to use archival material such as frozen samples and formalin-fixed paraffin-embedded (FFPE) material for this analysis, although the success rate of CGH of FFPE tissue has been reported to be highly variable mainly due to

the degradation of DNA and the fixation process (Isola et al., 1994; Brandal et al., 2003, 2004).

CGH is, however, unable to detect balanced aberrations such as translocations, inversions, and insertions; these do not cause changes in copy number and thus generate no relative imbalance in DNA content between tumour and normal DNA. Another limitation of CGH is that it requires samples of high tumour content. A tumour sample often consists of a mixture of tumour parenchyma, stroma, and normal tissue. To be able to detect copy number aberrations, the sample must contain at least 50% neoplastic parenchyma DNA compared to normal or stromal DNA. In theory, this problem may be overcome by the pathologist micro-dissecting the sample so that the material is known to represent more than 50% tumour, but when tumour samples are small, like in brain tumour surgery where sufficient resection margins must always be weighed against the attempt to preserve normal brain function, this may not be possible. As CGH is also only able to visualise the average number and type of copy number imbalances, tumour heterogeneity will remain hidden, unless CGH is used in combination with other genome-wide screening techniques such as karyotyping.

Conventional/chromosomal/metaphase CGH (cCGH) has detected deletions of tumour DNA down to the 10-12 Mb level (Kallioniemi et al., 1994; Bentz et al., 1998) and amplifications as small as 2 Mb have been reported (Joos et al., 1993; Piper et al., 1995). In an attempt to account for some of the expected variation detected in chromosomes of healthy individuals, Kirchhoff et al. (1998; 1999) introduced dynamic standard reference intervals (D-SRI) instead of fixed diagnostic thresholds. This has been shown to reduce the number of false positive and false negative results, thereby increasing sensitivity and specificity, and regions that previously had to be excluded from the analysis such as the distal half of the p-arm of chromosomes 1, 19, and 20 as well as

telomeric areas, could now be analysed. The resolution level of CGH with these modifications is approximately 3Mb.

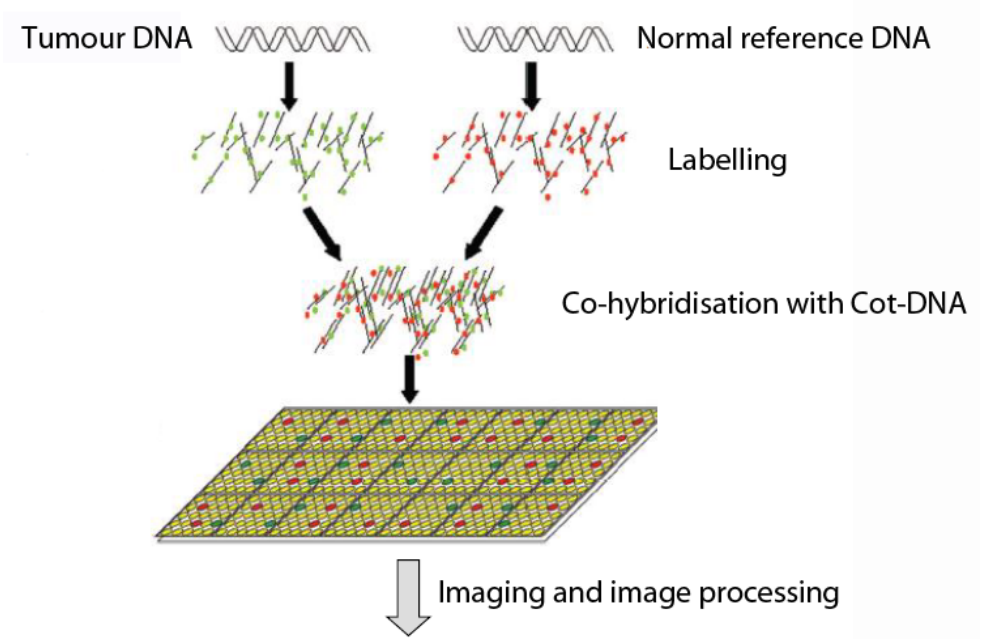

Figure 3: *Schematic presentation of CGH (in this case aCGH) where differently labelled tumour and normal DNA competes to hybridise to a chip onto which DNA fragments spanning the entire human genome have been applied. This is imaged and computer software calculates fluorescence intensities.*

Array-CGH (aCGH) was introduced to further enhance the resolution level of CGH. This variant of CGH utilises known DNA fragments fixed in a matrix system as target sequences (Fig. 3) rather than metaphases used in cCGH. Current array platforms are based on relatively large human sequences cloned into bacterial artificial chromosome libraries (BAC arrays) or on shorter single-stranded oligonucleotides (oligonucleotide arrays) which may or may not include single-nucleotide polymorphisms (SNP arrays). The latter may assess genomic imbalances that are not reflected as copy number aberrations as well, e.g. loss of heterozygosity (LOH) by uniparental disomy (Bignell et al., 2004).

Multicolor fluorescence in situ hybridisation

Though FISH with specific probes requires some knowledge of underlying aberrations, in 1996 a genome-wide variant of FISH was introduced that could paint not only small segments but entire human chromosomes, all 23 pairs, simultaneously in a single experiment. Variants of this technique include well-established cytogenetic techniques such as multiplex-FISH (M-FISH), spectral karyotyping (SKY), and cross-species colour banding.

Multiplex-FISH (M-FISH) (Speicher et al., 1996) is a genome-wide screening technique based on all 24 human chromosomes being labelled with a different combination of five fluorochromes to create a unique colour signature for each chromosome. The technique makes use of a combinatorial probe-labelling approach, generating colour combinations that exceed the number of fluorophores used. As the number of useful combinations of *N* fluors is 2^N-1, only five fluorophores are needed to obtain the 31 different colours used to distinguish between chromosomes. Different sets of fluorophores such as fluorescein isothiocyanate (FITC), Texas-Red, and cyanine dyes (e.g. Cy3 and Cy5) can be used for the M-FISH technique whereas DAPI is used as counterstain. Each fluorochrome is detected separately with a narrow bandpass excitation/emission filter across the spectral interval, and the degree of fluorescent intensity of each of the fluorochromes in the different images is then analysed by the M-FISH capturing system. The images may be viewed separately to allow the researcher to manually identify each chromosome. However, the images may also be superimposed to obtain a final composite image of the metaphase where the computer software identifies each chromosome, and the composite image may in turn be used to create a "painted karyogram" where each chromosome pair is uniquely and uniformly stained instead of presenting the band-like pattern obtained by G-banding.

M-FISH offers an easy, rapid way of karyotyping and has proved useful in both tumour cytogenetics and pre- and post-natal diagnostic settings (Uhrig et al., 1999; Speicher et al., 2000). It is particularly useful in the analysis of complex tumour metaphase spreads where conventional banding analysis fails to identify the components of highly rearranged chromosomes such as marker chromosomes, but also to detect subtle interchromosomal rearrangements that are otherwise below the resolution level of conventional banding methods. Although fresh metaphases are preferred for optimal chromosome staining, previously G-banded preparations may be used, and are in fact very useful when attempting to identify very complex rearrangements or when the aberration in question is only present in a small subclone (Micci et al., 2001). Like CGH, M-FISH paints the whole chromosome with one colour for its entire length and is therefore unable to detect intra-chromosomal rearrangements such as small deletions, duplications, and inversions. It is also unable to provide details of breakpoints involved in an aberration. In addition, the cytogeneticist must be aware that fluorescent signals suggesting insertion of material from nonhomologous chromosomes may not always constitute true insertions but rather false positive "flares" due to superimposed fluorescence from two adjacent chromosome segments in the breakpoint of a translocation. Such false positives are particularly important to exclude when the "inserted" chromosome material has a characteristic fluorochrome combination that is a mixture of the fluorochrome profile of the two adjacent chromosome segments. Another common pit-fall in M-FISH interpretation may occur when the computer program uses DAPI as a counterstain to determine chromosome contours. This stain is sometimes less intense at the telomeric ends of chromosomes, and if care is not taken, fluorescent signals beyond these boundaries may be lost (Lee et al., 2001).

Due to the inherent limitations of M-FISH, quite similar to those of SKY (Schröck et al., 1996), such techniques cannot replace conventional banding analysis but should be used for what they were designed, as a complement to banding techniques to further elucidate complex chromosomal rearrangements.

Specific FISH techniques

A **whole chromosome painting probe (WCP)** (Pinkel et al., 1988) is made of DNA sequences labelled with a fluorochrome and hybridising along the entire length of a given chromosome. Such probes are generated either by chromosome flow-sorting or microdissection of normal metaphase chromosomes, subsequent DNA amplification, and fluorochrome labelling (Meltzer et al., 1992; Telenius et al., 1992). The latter can also be used to create colour banding along regions or the entire length of one or more chromosomes (Chudoba et al., 2004).

The use of WCPs provides easy and rapid visualisation of a specific chromosome in a metaphase or interphase cell and may help identify both numerical and structural aberrations. WCPs are of particular value when trying to determine the nature of a marker chromosome in situations where the potential candidate chromosomes are few. The principle limitations of the approach are the same as for M-FISH.

A **locus-specific probe (LSP)** can be manufactured by amplifying genomic DNA after inserting it into various vectors such as cosmids, fosmids, bacterial artificial chromosomes (BACs), and yeast artificial chromosomes (YACs). The different vectors can hold different sizes of DNA, i.e. BACs can hold 100-300kb inserts (Shizuya et al., 1992) whereas YACs can take up to 1500kb inserts of DNA (Burke et al., 1987). LSPs can

target unique sequences down to gene level. They are particularly useful to map chromosome breakpoints and may be applied to both metaphase and interphase cells. Examination of the latter may be resorted to in cases with low mitotic activity in cell culture.

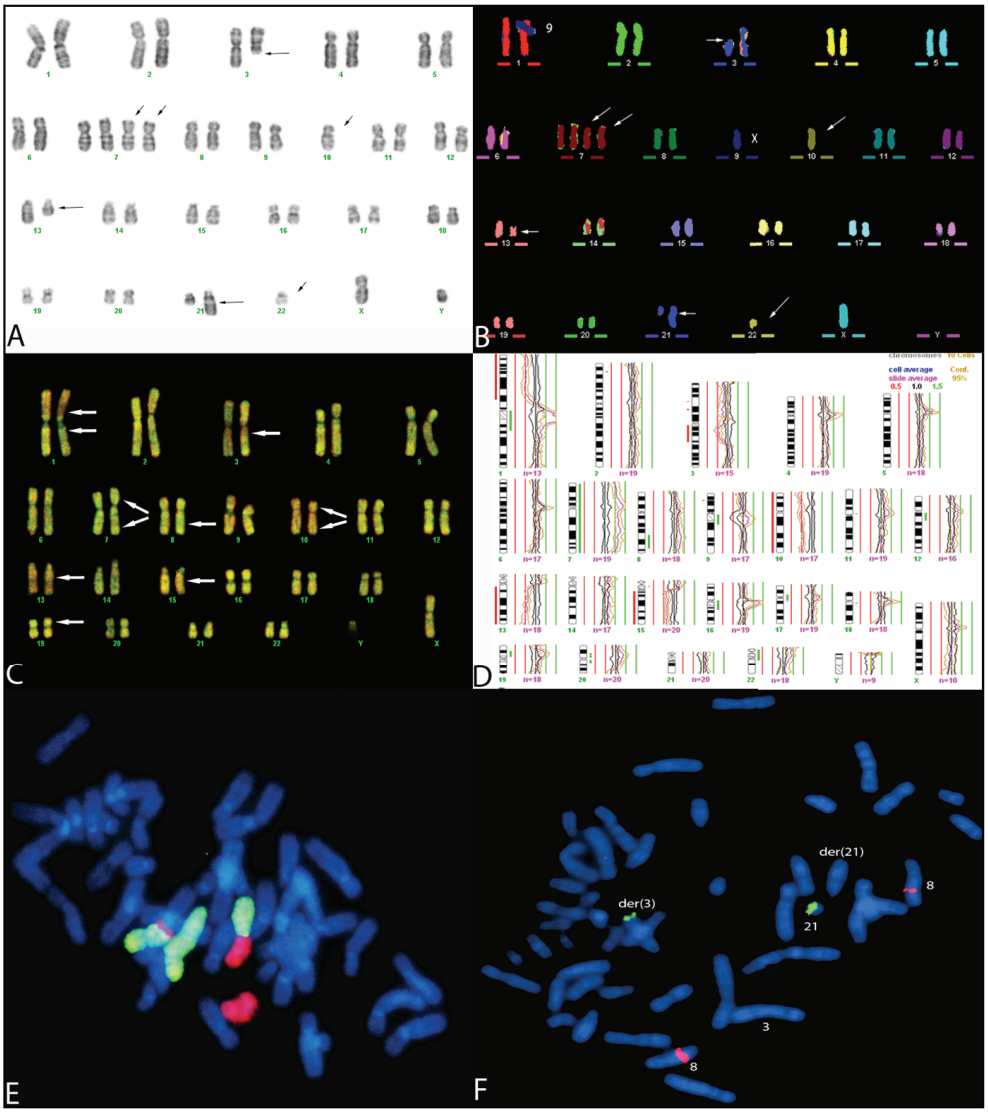

Figure 4. *Examples of genome-wide screening techniques* ***(A-D)*** *and specific FISH techniques used in this thesis* ***(E-F)****.* ***(A)*** *G-banded karyogram of a gliosarcoma with bone differentiation in Paper III showed a complex karyotype:46,XY,t(3;21)(q13~21;q21~22),+7,+7,–10,del(13)(q13q21~22),–22. Arrows indicate gained, lost and rearranged chromosomes.* ***(B)*** *M-FISH karyogram showing the same aberrations.* ***(C)*** *and* ***(D)*** *show the CGH karyotype and profile of a glioblastoma in Paper I. The arrows point to the significant gains and losses revealed by CGH analysis: rev ish enh(1q11-23,7,8q22-24,19p13) dim(1p21-pter,3q13-21,10, 13q12qter,15q11qter).* ***(E)*** *Image of a metaphase hybridised with whole chromosome painting probes for chromosomes 4 (green) and 17 (red).* ***(F)*** *FISH image of a metaphase using the AML1/ETO probes. ETO is labelled in red and maps to chromosomal band 8q22, whereas AML1 is labelled in green and maps to chromosomal band 21q22.*

"From the brain and the brain only arise our pleasures, joys, laughter and jests, as well as our sorrows, pains, griefs, and tears.... These things we suffer all come from the brain, when it is not healthy, but becomes abnormally hot, cold, moist or dry."

— Hippocrates (460 BC - 370 BC)

Brain tumours

Malignant tumours of the central nervous system (CNS) account for merely 1-2% of all cancers in adults and are therefore considered rare. Still they remain among the top 10 causes of cancer-related deaths (CBTRUS, 2011). The incidence increases with age and there is a slight female preponderance. The overall age-adjusted incidence rate of brain tumours is 18.7 per 100,000 person-years, and 7.2 per 100 000 person-years for malignant brain tumours (CBTRUS, 2011). In Norway, the age-adjusted incidence of CNS tumours is approximately 12-15 per 100,000 person-years (Fig. 5) and the total number of new cases of CNS tumours was 882 in 2008 (Cancer Registry of Norway, 2009).

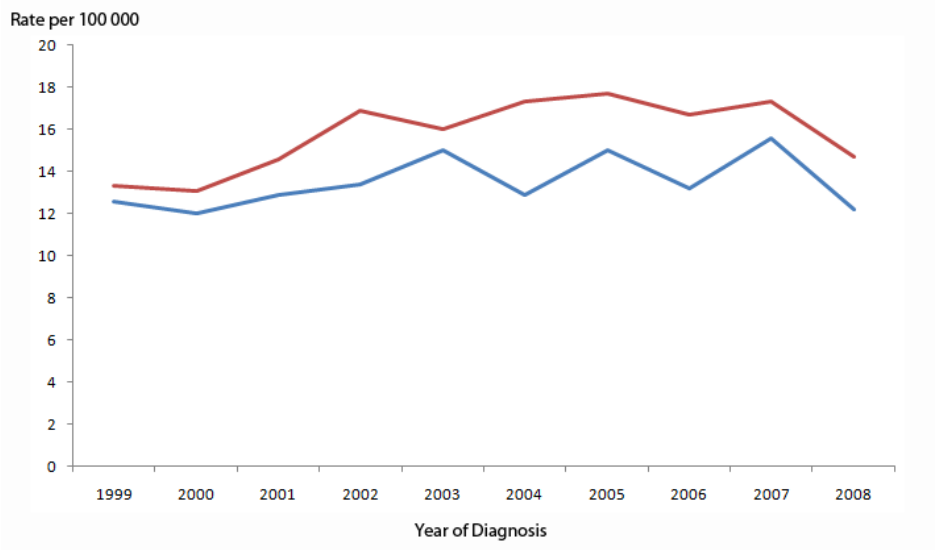

Figure 5: *Incidence rates for brain tumours in men (blue) and women (red) in Norway over the past 10 years. Note the slight female preponderance. Raw data were obtained from the Cancer Registry of Norway (2009).*

CNS tumours represent the largest group of solid cancers (39-51%) in childhood (Parkin et al., 1999; Stiller et al., 2006), and in the Nordic countries these tumours have

surpassed leukaemias and now constitute the most common malignancy in children (Cancer Registry of Norway, 2009; Schmidt et al., 2011). Despite steady improvements in prognosis over the past decades, CNS tumours remain the leading cause of cancer mortality in children (U.S. Cancer Statistics Working Group, 2010).

Some hereditary syndromes such as tuberous sclerosis and neurofibromatosis types 1 and 2 go with a genetic predisposition to the development of brain tumours. Nevertheless, these syndromes have been estimated to account for only 1-2% of such tumours (Narod et al., 1991).

Two recent genome-wide association studies (GWAS) of gliomas in adult patients and controls of European ancestry suggested glioma risk loci to exist on 5p15.33(*TERT*), 9p21 (*CDKN2A-CDKN2B*), 11q23.3 (*PHLDB1*), and 20q13 (*RTEL1*) (Shete et al., 2009; Wrensch et al., 2009). Further studies are needed to determine the true role of genetic predisposition in these patients, including to establish beyond doubt where the loci are found and which genes reside in them.

The only established environmental risk factor for brain tumours is exposure to ionising radiation. The survivors of the Hiroshima atomic bomb showed a high incidence of meningiomas and the incidence was inversely related to the distance from the hypocentre (Shintani et al., 1999). Relatively small therapeutic doses of radiation for diseases such as tinea capitis and acute lymphoblastic leukaemia have also been associated with an increase in CNS tumours (Neglia et al., 1991; Salvati et al., 1991; Karlsson et al., 1998). Furthermore, the development of a second primary brain tumour is more common in patients treated with radiotherapy than in those who are not (Salminen et al., 1999). The diagnostic use of x-rays has been linked to meningiomas but not to gliomas (Wrensch et al., 2000, 2002).

Data on the use of hand-held mobile phones and brain tumour risk remain inconclusive (Wrensch et al., 2002), whereas allergies seem to be inversely associated to glioma risk (Bondy et al., 2008).

Brain tumour classification

Harvey W. Cushing and Percival Bailey created the first main classification of brain tumours in 1926 based on microscopic appearance, natural history, and prognosis and their histopathological approach still influences present-day neurosurgical practice. In 1949, Kernohan et al. introduced the concept of histological differentiation of brain tumours, and the Ringertz system (1950) suggested different brain tumours to have originated from different brain cells. In 1979, the World Health Organization (WHO) published a classification system that encompassed all CNS tumours instead of focusing merely on intracranial lesions. Revised in 1999 and 2007, this remains the currently accepted classification (Louis et al., 2007).

Gliomas

Gliomas, meningiomas, and embryonal tumours account for the majority of primary intracranial neoplasms (Central Brain Tumor Registry of the United States (CBTRUS), 2011). The term "glioma" was first used by Rudolf Virchow in 1860 (Gonzales, 1995) to describe these tumours because they were presumed to derive from the glial cells (*gr. glia*=glue) of the brain. The term "glia" was first used in the 1850s (Somjen, 1988) by early anatomists who thought these cells constituted "the glue" that held neurons together (Rothwell, 2009). In later years, glial cells have been shown to

constitute a very diverse group both anatomically and physiologically, their only common feature being that they make up the non-neuronal elements of the CNS.

The diversity of glial cells

Astrocytes are the most numerous glial cells by far. Some are in contact with neurons and are important in the transferral of nutrients, while others make up the blood-brain barrier. Furthermore, recent studies have unravelled additional important functions of astrocytes that include regulation of potassium concentration around neurons, pH homeostasis, adjustment of voltage across the neuronal membrane, glutamate uptake at synapses, and modulation and control of synaptic activity through release of neurotransmitters (Rothwell, 2009; Kimelberg and Nedergaard, 2010). Other glial cells include **oligodendrocytes** that produce the myelin that envelops axons and speed up the conduction of electrical impulses (Allen and Barres, 2009), **ependymal cells** that line the ventricles of the brain and the spinal canal and mediate flow of cerebrospinal fluid through the central nervous system (Del Bigio, 1995), and **microglia** that constitute the immune cells of the nervous system and may also play a role in synaptic remodelling (Graeber, 2010).

For each glial cell, there are neoplasms that span a broad spectrum of biological aggressiveness. The WHO classification (Louis et al., 2007) recognises four main groups of gliomas: astrocytomas, oligodendrogliomas, oligoastrocytomas, and ependymomas. Of these, the astrocytic and oligodendroglial tumours are the most common by far (Louis et al., 2007; Central Brain Tumor Registry of the United States (CBTRUS), 2011).

Gliomas: Incidence, treatment, and outcome

Astrocytic tumours are largely divided into four subgroups ranging from the low-grade pilocytic astrocytoma (WHO grade I) and diffuse astrocytoma (WHO grade II) to the high-grade anaplastic astrocytoma (WHO grade III), and glioblastoma (WHO grade IV). These tumours account for the large majority of gliomas, grades I-III and glioblastomas making up 14% and 54% of gliomas, respectively (CBTRUS, 2011). Pilocytic astrocytoma (PA) is the most common childhood glioma. This tumour is also seen in young adults, albeit less frequently, but is rare in those over 50 years of age (Ohgaki and Kleihues, 2005).

Low-grade astrocytomas (WHO grade I and II tumours) in adults are usually slow-growing tumours that are locally infiltrative (Kleihues and Cavenee, 2000) and rarely metastasise outside the CNS (Johannesen et al., 2003). These tumours, especially the diffuse astrocytomas (WHO grade II), have a propensity to evolve into more aggressive high-grade anaplastic astrocytomas or glioblastoma multiforme (Shafqat et al., 1999). In adult patients, agreement on the optimal therapy of low-grade gliomas has not been reached. Early or delayed radiotherapy after tumour resection or biopsy is the most common treatment, but although early radiotherapy has been shown to delay tumour progression, no overall survival benefit has been reported for early compared with delayed radiotherapy (Johannesen et al., 2003; van den Bent et al., 2005). In addition, there are concerns regarding the spectrum of radiation-induced toxicities that may follow (Douw et al., 2009). Chemotherapy is used in the initial treatment of patients with unresectable or large residual tumours after surgery, and in cases of post-radiation tumour progression (Soffietti et al., 2010). While survival rates for patients with pilocytic astrocytomas are excellent (96% alive at 10 years), the prognosis worsens with increasing grade; five-year overall survival rates are reported at 65% and 31-39% for

patients with diffuse and anaplastic astrocytomas, respectively (Okamoto et al., 2004; Stupp et al., 2007; Chaichana et al., 2010; Matar et al., 2010). Estimates have varied greatly, however, both between and within the different glioma entities with regards to prognosis. Some of the observed difference may be accounted for by the considerable interobserver classification variation in these tumour groups (Coons et al., 1997; Prayson et al., 2000). More objective markers, capable of identifying gliomas that will progress and the timeframe associated with progression, are sorely needed.

The term **glioblastoma** (WHO grade IV) is used synonymously with **glioblastoma multiforme** (GBM) to describe the most common malignant primary brain tumour in adults. The age-adjusted incidence rate is 3.2 per 100,000 person-years (CBTRUS, 2011). In 2005, Stupp et al. showed that treating glioblastoma patients with the now gold-standard combination of tumour resection (as radical as possible) and radiotherapy with concomitant and adjuvant temozolomide increased the median overall survival by 2.5 months to 14.6 months (Stupp et al., 2005, 2009). Prognostic factors such as age, functional status, extent of surgical resection, type of cytotoxic therapy, and methylation status of the *MGMT* gene promoter have all been suggested to have an impact on overall survival (Hegi et al., 2005; Helseth et al., 2010).

Most glioblastoma patients are elderly and present with a relatively short clinical history without evidence of a precursor lesion; these tumours are often called primary glioblastomas (WHO grade IV). There is, however, a subgroup of glioblastomas that arise in younger patients through progression from diffuse or anaplastic astrocytomas; these are called secondary glioblastomas (Louis et al., 2007). Molecular markers are yet to be discovered that can reliably distinguish between these two, but some evidence suggests that they develop through different genetic pathways (Fig. 6) (Ohgaki and Kleihues,

2007). Recently, somatic mutations of the isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) genes were identified in 50-80% of low-grade astrocytic and oligodendroglial tumours as well as in secondary glioblastomas (Kloosterhof et al., 2011). By contrast, these mutations are present in less than 5% of primary glioblastomas (Yan et al., 2009) and may therefore turn out to be relatively reliable markers of secondary glioblastoma (Ohgaki and Kleihues, 2011).

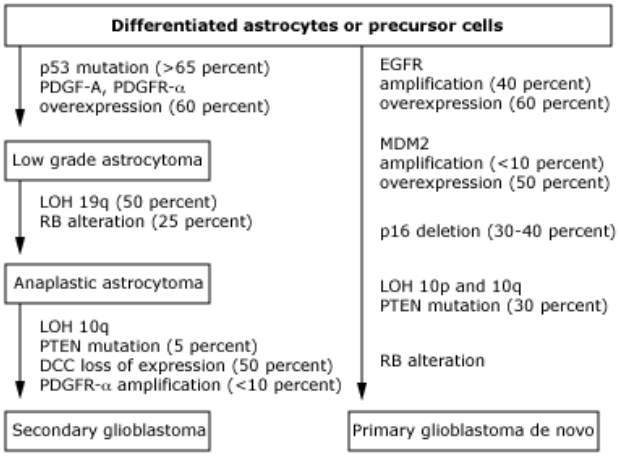

Figure 6: *Genetic pathways in the development of primary and secondary glioblastoma. LOH: loss of heterozygosity; RB: retinoblastoma gene; PDGF: platelet-derived growth factor gene; PDGFR: platelet-derived growth factor receptor gene; DCC: deleted in colon cancer gene.* From Kleihues et al. (1999).

Oligodendroglial tumours include both pure **oligodendrogliomas** and **oligoastrocytomas**; the latter is a mixture of both oligodendroglial and astrocytic components (Louis et al., 2007). "Pure" oligodendrogliomas (WHO grades II and III) account for 5-7% of gliomas (Rodriguez and Giannini, 2010; CBTRUS, 2011). The true incidence of oligoastrocytomas (WHO grades II and III) is difficult to estimate as there has been quite some controversy surrounding the diagnostic criteria for these tumours. This has resulted in high interobserver variability and a highly variable reported

incidence (Rodriguez and Giannini, 2010). Current treatment regimens consist of surgery in low-grade tumours, whereas chemotherapy with procarbazine, CCNU and vincristine (PCV), temozolomide regimens, and/or radiotherapy is reserved for patients with anaplastic tumours or recurrent tumours (Van den Bent et al., 2008).

The presence of a 1p/19q-codeletion (Fig. 7), which in most cases is mediated through an unbalanced t(1;19)(q10;p10)(Jenkins et al., 2006), has been associated with improved prognosis (Cairncross et al., 1998, 2006; Smith et al., 2000; Bromberg et al., 2009). The median survival duration has been reported at 12-15 years for 1p/19q codeleted patients with low-grade oligodendroglial tumours and 5-8 years for patients without the deletion (Jenkins et al., 2006). Similarly, in anaplastic tumours, the codeletion is associated with a median survival time of more than 6-7 years compared to a survival time of 2-3 years in the absence of this aberration (Cairncross et al, 2006; van den Bent et al., 2006). Five-year overall survival rates for patients with WHO grade II oligodendrogliomas and patients with WHO grade III anaplastic oligodendrogliomas and oligoastrocytomas range from 68 to 96% and 31 to 66 %, respectively, depending on 1p/19q-status (Cairncross et al., 2006; Jenkins et al., 2006).

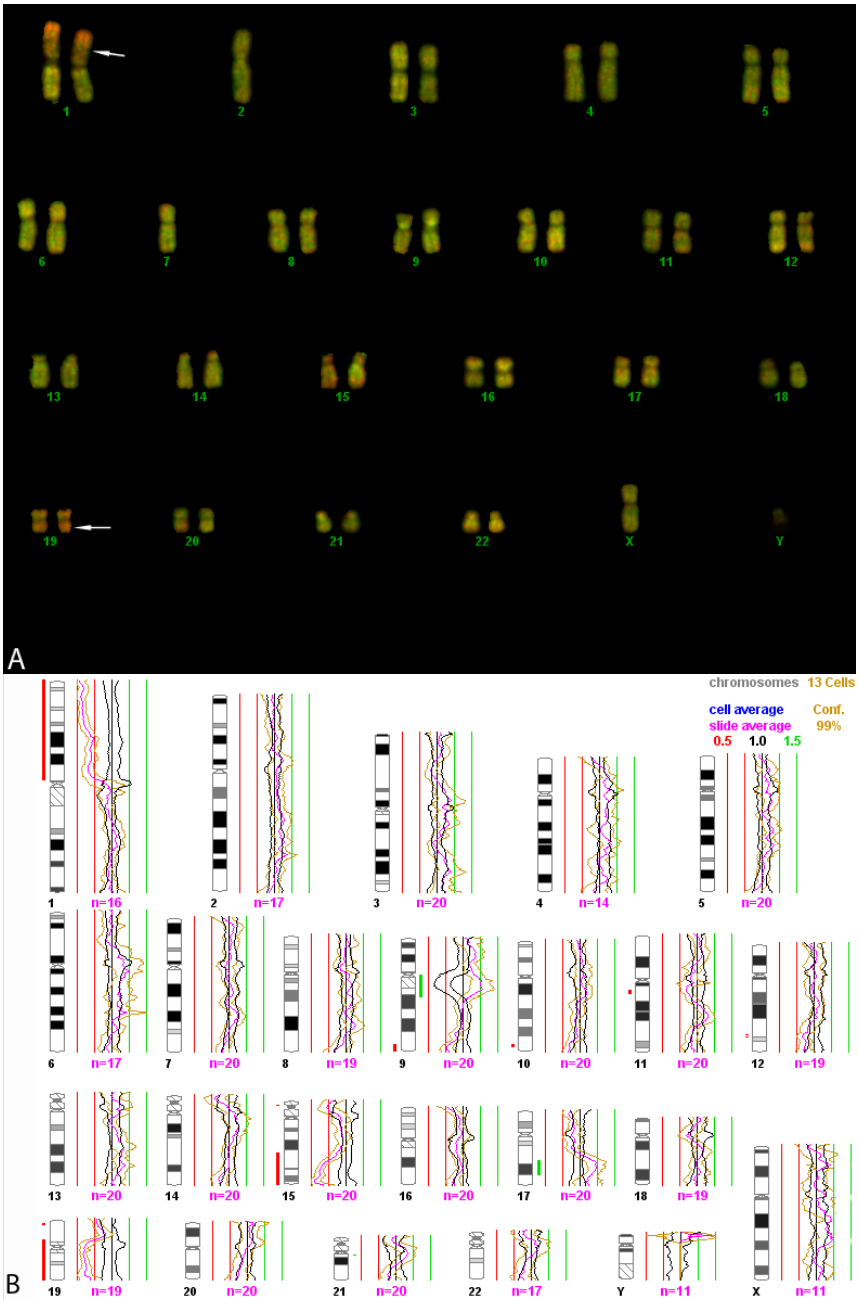

Figure 7: *CGH karyogram and profile of an oligodendroglioma showing the 1p/19q-codeletion. The deleted areas are seen in red and the codeletion is marked with arrows.*

Ependymomas are rare neuroepithelial tumours that are thought to arise from the ependymal cells of the cerebral ventricles or spinal cord. They account for approximately 2% of all primary intracranial tumours, 6% of all gliomas, and are more common in children than in adults. These tumours show remarkable histopathological heterogeneity (Louis et al., 2007; Ruda et al., 2008; CBTRUS, 2011). The World Health Organisation currently recognises four main groups of ependymal tumours: subependymoma, myxopapillary ependymoma, ependymoma (including the cellular, papillary, clear cell, and tanycytic subtypes), and anaplastic ependymoma; however, other rare variants also exist such as giant-cell ependymoma (Zec et al., 1996). There is still considerable controversy regarding the therapeutic management of ependymoma patients. Surgical resection is the mainstay of treatment whereas radiotherapy is usually reserved for patients with anaplastic (WHO grade III) ependymoma or when residual disease is visible after the initial surgical procedure (Metellus et al., 2010). The role of chemotherapy in these patients remains to be clarified, however (Gilbert et al., 2010). The reported 5- and 10-year overall survival rates for adults with intracranial ependymoma range between 62-85% and 44-81%, respectively. Unfortunately, little is known about the pathogenesis and prognostic determinants of these tumours (Metellus, 2007, 2010; Ruda et al., 2008).

Paediatric embryonal tumours

Medulloblastomas account for 20% of all childhood brain tumours (CBTRUS, 2011). The incidence has a bimodal pattern with one peak between ages 3 and 4 and another between ages 8 and 9 (Crawford et al., 2007). Standard treatment includes surgery, chemotherapy, and, depending on the age of the patient, radiotherapy. The aim of irradiation is to destroy disseminated microscopic disease that cannot be detected in cerebrospinal fluid or by MRI; however, this modality is best avoided in children under the age of 3 years due to the severe neurological morbidity associated with it (Ellison, 2010). Five-year progression-free survival in children with average risk (patients older than 3 years with less than 1.5cm^2 of residual tumour and non-disseminated disease) treated with multimodality therapy is 80-85%, whereas for high-risk disease (children younger than 3 years with more than 1.5cm^2 residual tumour, evidence of tumour dissemination or diffuse anaplasia) and for infants, the survival rate is 60-65% and 30-35%, respectively (Gajjar et al., 2006; Packer et al., 2006; Carrie et al., 2009; Grundy et al., 2010).

Medulloblastomas are among the brain tumours that have been most eagerly studied at the molecular level. In the last decade, it has been increasingly understood that they constitute a heterogenous group of tumours with regards to histopathology, genetic aberration patterns, and likely cell of origin (Gibson et al., 2010; Eberhart, 2011). The use of a molecular classification in conjunction with clinicopathological parameters has been suggested to aid in the identification of patients with low- and high-risk disease, and tumours with distorted signalling pathways such as sonic hedgehog (Shh) and *Wnt* may be targeted by specific therapies in the future (Thompson et al., 2006; Kool et al., 2008; Ellison et al., 2011; Northcott et al., 2011).

CNS-primitive neuroectodermal tumours (CNS-PNETs) are embryonal tumours that are most commonly found in supratentorial sites. They make up a mere 2-3% of paediatric CNS tumours (Gaffney et al., 1985; Pollack, 1994; Rickert and Paulus, 2001). Children with such tumours were traditionally treated with a combination of chemotherapy and radiotherapy, quite like medulloblastoma patients, but showed 3-year overall survival rates below 50% (Timmermann et al., 2002; Larouche et al., 2006; Fangusaro et al., 2010). Young children (<3 years) are particularly vulnerable (3-year survival of less than 20%) and difficult to treat, again because of the detrimental side-effects of radiotherapy (Timmermann et al., 2006). High-dose marrow ablative chemotherapy followed by autologous haematopoietic cell rescue has been shown to improve prognosis (5-year overall survival of 49%), but life prospects for these children remain dismal (Fangusaro et al., 2008).

Atypical teratoid/rhabdoid tumours (AT/RT) are highly malignant tumours that most commonly affect children in the first two years of life. Like for CNS-PNETs, prognosis is poor and reported 5-year overall survival rates range between 18.2% and 50% (Garre and Tekautz, 2010). AT/RTs account for 1-6% of all paediatric brain tumours (Woehrer et al., 2010) and two-thirds have components that resemble medulloblastomas or CNS-PNETs (Rorke et al., 1996). A distinguishing feature compared with the tumours above is the presence of a mutation in the tumour-suppressor gene *hSNF5/INI1* (Biegel et al., 2002).

AT/RTs progress and disseminate rapidly. They are notoriously unresponsive to cytotoxic therapy and are mostly fatal (Garre and Tekautz, 2010) although an AT/RT variant with a relatively favourable prognosis was recently described: cribriform

neuroectodermal tumour (CRINET) (Hasselblatt et al., 2009). These are non-rhabdoid ventricular tumours that show loss of INI1 protein.

How to best manage children with AT/RT remains unclear, including which agents are the most efficient or whether high-dose or multiagent chemotherapy improves prognosis (Garre and Tekautz, 2010).

Aims of the study

Cancer is the phenotypic result of the acquisition of one or more chromosomal and/or gene-level mutations by susceptible somatic target cells. Many of these aberrations involve sufficient quantities of DNA to be microscopically visible when the chromosomes condense during mitosis, and can therefore be registered as numerical or structural chromosomal alterations. Screening of the whole tumour genome is therefore a natural starting point when trying to understand the pathogenetic mechanisms behind tumour development. The information one obtains by such investigations may also increase our understanding of neoplastic clonal evolution (whether a given neoplasm evolves from one or several transformed cells and how genomic complexity changes over time) as well as provide diagnostic and prognostic insights of great clinical importance.

Gliomas and paediatric embryonal tumours are tumour entities that are challenging both diagnostically and therapeutically. This accentuates the call for an alternative grouping of tumours that could also hopefully provide information about prognosis as well as key insights into molecular disease mechanisms that may eventually be translated into more effective and individualised therapies (Brandal et al., 2010). Our knowledge of chromosome aberrations in haematological malignancies is much more complete than in solid tumours, largely because the latter have been more difficult to characterise cytogenetically. Tumours of the central nervous system are no exceptions to this (Mitelman et al., 2011). Furthermore, the majority of CNS tumours examined were investigated using merely one cytogenetic technique, often with heterogenous groups of tumours being analysed together. Although in the busy life of cancer diagnostics and research, preference is mostly given to the most modern

techniques, more time-consuming and seemingly out-dated methodologies (like banding cytogenetics) may have unique qualities that are quickly and conveniently "forgotten" by the fashion-minded, and so important information may be lost (or remain undiscovered) in uni-technological studies. The prinicipally best approach must be to combine several relevant techniques in order to get an un-biased and broad overview of the tumour genome before concentrating on more specific targets. In that respect, the 9;22-translocation in CML is a good example; it took 40 years to get from the detection of the cytogenetic aberration (detectable only by a suitable screening technique) until an effective, specific therapy was invented (Druker, 2008), illustrating how the various steps in genetic cancer research are intertwined and dependent upon one another before final success is reached.

The overall aim of this study was thus two-fold. We wished to analyse brain tumours using the range of cytogenetic techniques available to us. This would help establish the advantages and disadvantages of each technique in the detection of genomic aberrations in these tumours. Secondly, and perhaps more importantly, we wished to use the information obtained by the application of these methods to increase our pathogenetic understanding of brain tumours, to look for aberration patterns that could suggest alternative pathogenetic classifications as well as possible histopathological-cytogenetic and clinico-cytogenetic correlations that might eventually become clinically useful.

Specific aims of each study and article

Paper I: ***Genomic Aberrations in 80 Cases of Primary Glioblastoma Multiforme: Pathogenetic Heterogeneity and Putative Cytogenetic Pathways.*** We wished to use a combination of G-banding and HR-CGH to increase the existing knowledge of the genomic aberrations that characterise primary glioblastomas, including the more recently recognised subgroups. We also looked for non-random cytogenetic patterns that would suggest different pathogenetic pathways taken by various tumour subsets.

Paper II: ***Genomic Aberrations in Diffuse Low-grade Gliomas*****.** We aimed to gain information about the acquired genomic aberrations in low-grade diffuse gliomas. In particular, we wished to establish to what extent diffuse astrocytomas, oligodendrogliomas, and the notoriously difficult to classify oligoastrocytomas showed systematic differences and/or similarities with regards to karyotypic features and DNA copy number changes. As the current tumour classification fails to identify patients who will later have a tumour recurrence, we were particularly interested in looking for cytogenetic patterns that correlate with longer/shorter progression-free survival.

Paper III: ***Molecular Cytogenetic Analysis of a Gliosarcoma with Osseous Metaplasia*****.** Gliosarcomas consist of both a classical glial as well as a sarcomatous component. The latter may very occasionally show differentiation of bone. This is the first of these very rare gliosarcoma variants to be cytogenetically characterised and we determined its cytogenetic aberration pattern using a combination of G-banding, FISH, M-FISH, and aCGH.

Paper IV: ***Multiple Monosomies are Characteristic of Giant Cell Ependymoma*****.** A rare case of giant-cell ependymoma showed multiple monosomies by G-banding both in the initial primary tumour and in a recurrent anaplastic ependymoma. We looked to verify these findings by DNA ploidy analysis and aCGH, and compared the results with those of the literature.

Paper V: ***Genomic Aberrations in Paediatric Gliomas and Embryonal Tumours*****.** The distribution of CNS tumours in the paediatric population is very different from that of adults. Cytogenetic information, both in terms of karyotypic description and genomic profiles as determined by CGH, is extremely limited for the majority of brain tumours of children. The purpose of this study was to increase the knowledge of the acquired genetic changes that characterise these tumours. To this purpose, a combination of G-banding and aCGH was used.

Results in brief

Paper I: ***Genomic Aberrations in 80 Cases of Primary Glioblastoma Multiforme: Pathogenetic Heterogeneity and Putative Cytogenetic Pathways.*** We analysed cytogenetically 80 such tumours by a combination of G-banding and HR-CGH. Abnormal karyotypes were found in 83% of tumours. The most common numerical chromosome aberrations were +7, -10, -13, -14, -15, +20, and -22. Structural abnormalities most frequently involved chromosomes 1, 3, and the short arm of chromosome 9. HR-CGH verified these findings and revealed additional frequent losses at 1p34-36, 6q22-27, and 19q12-13 and gains of 3q26 and 12q13-15. Although most karyotypes and gain/loss patterns were complex, there was also a distinct subset of tumours displaying simple karyotypic changes only. There was a statistically significant association between trisomy 7 and monosomy 10, and also between +7/-10 as putative primary aberrations and secondary losses of 1p, 9p, 13q, and 22q. The low number of tumours in the rarer histological tumour subgroups precludes definite conclusions, but there did not seem to be any clear-cut cytogenetic-pathological correlations, perhaps with the exception of ring chromosomes occurring in giant cell glioblastomas. Our findings demonstrate that although GBM is a pathogenetically very heterogeneous group of diseases, distinct genomic aberration patterns exist.

Paper II: ***Genomic Aberrations in Diffuse Low-grade Gliomas.*** We investigated 38 WHO grade II astrocytomas, oligodendrogliomas, and oligoastrocytomas using a combination of G-band chromosome analysis and high-resolution comparative genomic hybridisation (HR-CGH). Abnormal karyotypes were found in 41% of tumours.

Karyotypes of astrocytomas and oligodendrogliomas were near-diploid, while oligoastrocytomas also displayed near-tetraploid clones. The most common aberrations were losses of chromosomes X, Y, 3, 4, 6, and 11 and gains of chromosomes 8 and 12. The only recurrent structural rearrangement was del(6)(q21). HR-CGH analysis verified karyotyping findings but also revealed frequent losses at 1p, 17q, and 19q and gains of 7q, 10p, 11q, and 20p. Among the tumours were two gemistocytic astrocytomas, a subgroup of diffuse astrocytomas with a particular predisposition to progression but not studied cytogenetically before; one showed a near-diploid, complex karyotype with structural aberrations of chromosomes 1, 3, and 11, whilst both displayed simple aberrations including loss of 11p by HR-CGH. Tumours with complex chromosomal aberrations had a generally higher tendency to show aggressive tumour behaviour (shorter progression-free survival) than did tumours displaying simple aberrations only (P=0.07).

Paper III: ***Molecular Cytogenetic Analysis of a Gliosarcoma with Osseous Metaplasia*****.** We present the first genomic characterisation (karyotyping followed by FISH and array comparative genomic hybridisation analysis) of a gliosarcoma with osseous metaplasia. In addition to chromosomal changes often found in gliomas (+7,-10,-13, and -22), the tumour cells also harboured a hitherto unknown (3;21)(q13~21;q21~22)-translocation. aCGH was able to confirm the whole chromosome gains and losses detected by G-banding, added new information regarding the deleted chromosome 13 narrowing down the lost segment to 13q13.3-q22.3, and revealed new deletions at 1p, 3q, 4q, 8p, and 14q, as well as gain of material at 2q, 10q, 12p, and 22q, changes not identifiable by any of the other methods.

Paper IV: ***Multiple Monosomies are Characteristic of Giant Cell Ependymoma*****.** Giant cell ependymoma (GCE), a rare ependymoma subtype, was recently recognised as a separate diagnostic entity with variations both in malignant potential and course of disease. We analysed the first supratentorial GCE using G-band karyotyping, DNA ploidy analysis, and array comparative genomic hybridisation. The tumour was hypodiploid because of multiple monosomies. This novel cytogenetic pattern seems specific for GCE as the only previous cytogenetic analysis of a GCE found similar monosomies. We were also able to analyse cytogenetically the subsequent recurrent tumour, phenotypically an anaplastic ependymoma, allowing a first insight into the genetic events involved in disease progression.

Paper V: ***Genomic Aberrations in Paediatric Gliomas and Embryonal Tumours*****.** We performed genome-wide screening of 17 paediatric gliomas and embryonal tumours using a combination of G-band karyotyping and aCGH. G-banding revealed abnormal karyotypes in 56% of tumour samples (9 of 16; one failed in culture), whereas aCGH analysis found copy number aberrations in all 13 tumours that could be examined. Pilocytic astrocytomas (n=3) showed normal karyotypes or simple non-recurrent translocations by karyotyping, but revealed the now well-established recurrent gain of 7q34 and 19q13.3 by aCGH. Our series included one anaplastic oligoastrocytoma, a tumour type that has not previously been characterised genomically in children, and an anaplastic neuroepithelial tumour (probably an oligoastrocytoma); both tumours showed loss of chromosome 14 by G-banding as well as structural aberrations of the long arm of chromosome 6, and loss of 14q, 17p, and 22q by aCGH. Three of five supratentorial primitive neuroectodermal tumours showed aberrant karyotypes; two were near-diploid with mainly structural changes while one was near-triploid with

several trisomies including gains of one copy of chromosomes 1, 2, and 7. aCGH confirmed these findings and revealed additional recurrent gains of 1q21-44, and losses of 3p21, 3q29, and 8p23. Finally, we described cytogenetically for the first time a cribriform neuroepithelial tumour (CRINET) that showed loss of 1p33, 4q13.2, 10p12.31, 10q11.22, and 22q by aCGH. This study indicates that distinct cytogenetic patterns in paediatric gliomas and embryonal tumours do exist. Because so few cases in each pathological subgroup have so far been examined, further studies using a multi-modal approach are required to learn more about the genomic patterns of these rare tumours.

Discussion

Consent and ethical considerations

The European Convention on Human Rights (2010) states that "everyone has the right to respect for his private and family life, his home and his correspondence". In medicine, the importance of confidentiality was first emphasised in the Hippocratic Oath dating back to 400 BC (Markel, 2004). The respect for privacy, trust, and confidence are all important if patients are to reveal the personal information required to make a diagnosis and offer appropriate treatment. The same applies to medical research, where the patient's autonomy and right to informed consent are required to ensure public trust.

The work that forms the basis for this thesis is the beginning of a larger study aimed to characterise brain tumours using a spectrum of techniques ranging from classical cytogenetics to the more molecular genetic tools. The study has been approved by the regional ethics committee and follows the guidelines of the World Medical Association's Declaration of Helsinki (2008); a statement of ethical principles for medical research involving human subjects, including research on human material and data. The overall aim was to collect tumour samples from brain tumours removed at the neurosurgical department and analyse them cytogenetically. In neurosurgical emergencies, time to explain research designs and consent forms is limited, hence consent forms were posted retrospectively to the patients' home addresses. Patients were asked to read the patient information, and if they wished to participate in the study, they submitted the consent form by post in a pre-stamped envelope. From January 2005 to July 2009, we received 596 samples. Of these, 66% of patients consented to taking part in the study, 17% died before they were able to give informed

consent, and another 17% never returned the form. Although not a prerequisite, one patient contacted us specifically to decline. An exemption was given by the Regional Ethics Committee and the Norwegian Directory of Health to include patients that were unable to fill out the consent form prior to death, and we were therefore able to include 83% of patients. Although this is a very good return, it is interesting that 17% of patients did not return the form. This raises questions regarding timing of consent requests, the need for, and implications of, consent in this type of medical research.

One can argue that although sending the patient information post hoc does give patients more time to consider giving informed consent, it may in fact lead to a type of inclusion bias. For instance, questions regarding the study, which could normally be raised during the hospital stay, now require the patient to actively contact the department. This may result in only the most eager and healthy individuals being recruited. In this respect, obtaining consent in the hospital, once the acute illness has settled, would be ideal. Unfortunately, this has so far proved impractical.

Obtaining informed consent from the critically ill may be both difficult and impractical, and may cause more distress than gain. For some brain tumour patients, many hurdles must be overcome in order to post a consent form. Tumours may alter mental state and cognitive function. In addition, patients may be in pain, some sedated, and there may exist uncertainty with regards to disease, prospects of treatment, and survival. Although able to make informed consent, the ability to write may be impaired, and even though the patient is able to make informed consent and sign the form, he or she may not be able to post the letter. Limited life expectancy in this patient group may be an issue, particularly if a relative returns home to find a consent form addressed to a newly deceased child. This is a particularly vulnerable group with regards to exploitation, and must therefore be protected, but possibly not at all cost. If informed

consent leads to inclusion bias, and patients with the most severe disease are unable to consent and take part in a study, it is near impossible to generalise findings. Thus, making an absolute demand of informed consent in these groups may in fact be a hinder to the possibility of future research and the prospect of evidence-based medicine for these patients (Loge et al., 2010).

During therapeutic surgical procedures, large samples are often removed even though only a small part is needed for diagnostic confirmation. This leads to a vast volume of "left-over" samples, most of which are usually left in the laboratories in case more tissue is needed in the future. However, when time has passed and laboratories are filled up, the dilemma arises with regards to what to do: to discard or to keep? Discarding saves both costs and space, but using the material for research serves future patients. In the Alder Hey inquiry (Burton and Wells, 2001), a British hospital was criticised for having removed and retained children's organs after autopsy without the knowledge of the parents. In these cases, the problem was not the storage and reuse of the material for research, it was rather that it had not been used for the intended diagnostic purposes.

Three main arguments have been posed for patients refusing to consent to the use of "left-over material"; some fear that this would result in a lack of material should the patients themselves require more diagnostic procedures, others fear the intrusion of privacy, and some argue that the body material belongs to the individual, who thus has the right to decide what becomes of it (van Diest, 2002). Self-determination over one's tissues is in any case limited: we all shed cells from our skin, cut our hair and nails, and excrete urine and faeces, without a desire to claim it back. Material can be encoded to ensure the anonymity of the individual, sufficient material may be stored to ensure the future needs of the individual and their families, and still leave some for research. To

some extent, the individual's rights must be balanced between a call for solidarity and the moral obligation to give back to those who have already shared in order to shape current medical practice.

In general, consent should be sought. However, if consent is impossible or impractical, or if it would generate more grief than gain, there might be call for conducting the research in the absence of consent, if a regulatory body has approved the research protocol. This approach has already proved effective in some countries (Australian National Health and Medical Research Council, 2007).

Methodological considerations

G-banding is a cytogenetic technique that has stood the test of time. It is simple, robust, and pure, without the need for complex, computerised algorithms. Either the aberration is there in a large enough number of cells to signify a clone, or it is not. G-banding still forms the backbone of screening techniques for karyotypic aberrations both in the diagnostic setting and in research. Sometimes, however, the tumour's mitotic index is too low, neoplastic cells fail to divide in vitro, or chromosomal rearrangements may be too complex or too small making it impossible to characterise them fully using G-banding alone.

In the studies that form the basis for this thesis, we made use of what we consider to be an ideal first approach to solid tumour cytogenetics, i.e. combining the initial screening using conventional G-banding with one or more molecular cytogenetic techniques depending on the aim of the study and the aberrations visualised by the karyotypic data. This has already proved a successful approach when analysing other solid neoplasms such as gynaecological (Micci et al., 2003, 2004), musculoskeletal

(Brandal et al., 2003; 2004), and renal tumours (Brandal et al., 2005) in our laboratory. How these techniques are best combined in CNS neoplasms was not clear, however, as experience with a multimodal approach in these tumours is surprisingly limited.

Where possible, an area of tumour sample adjacent to that used to establish cell culture was fresh-frozen on arrival to the laboratory. This allowed for DNA extraction and subsequent CGH analysis. An attempt was made to analyse all cases by both G-banding and CGH. In papers I and II, tumour samples were analysed by G-banding and high-resolution metaphase CGH (HR-CGH), whereas in papers III-V, array-based CGH (aCGH) was used in the same manner.

The main advantage of CGH is that it is independent of tissue culture, as was evident in papers I, II, and V but particularly so in paper II. Here, 28 of the 29 tumours analysed by HR-CGH showed copy number imbalances, including 17 tumour samples with apparently normal karyotypes, most likely because the mitotic index of the cultured neoplastic cells was low compared with that of the normal cells. Furthermore, CGH proved quite useful when genomically characterising gliomas, because of the high number of rearranged chromosomes that could not be completely identified. HR-CGH revealed additional information on genomic aberrations beyond that obtained by G-banding alone in 87% of the glioblastoma multiforme tumours (paper I) and 97% of the low-grade gliomas (paper II) analysed. This again is most likely due to the inherent differences of G-banding and CGH, the latter being independent of tissue culture and metaphase quality.

CGH also proved highly valuable when trying to determine exact breakpoint positions in chromosomal rearrangements. The gliosarcoma with bone differentiation presented in paper III showed a deleted chromosome 13 by both G-banding and M-FISH

analysis, however these methods were unable to clearly identify the breakpoints of the aberration as the metaphase chromosomes were relatively condensed. The breakpoint was initially thought to lie within the dark bands q21-q22. The high-resolution (~13kb) offered by aCGH proved invaluable in this case and could reveal that the deletion on chromosome 13 was in fact interstitial and spanned the region 13q13.3-q22.3.

Loss-of-heterozygosity-polymerase chain reaction (LOH-PCR) analysis is part of the routine diagnostic service in this hospital. Hence, in paper II, the HR-CGH findings of the 1p/19q-codeletion, a well-established prognostic marker in oligodendroglial tumours (Jenkins et al., 2006; Kesari et al., 2009), could be directly compared with the LOH-PCR results for 18 patients. There was good overall agreement (P=0.01). Any discrepancies observed, like the one tumour showing 1p/19q-codeletion by LOH-PCR but partial 1p/complete 19q loss by HR-CGH, is most likely due to the inherent differences between these methods. LOH-PCR is dependent on a few microsatellite markers present only on the very distal ends of 1p and 19q, whereas HR-CGH allows visualisation of whole chromosomes. Recognising the limiting factors of LOH-PCR, the routine investigative method has now been changed to multiplex ligation-dependent probe amplification (MPLA), which allows detection of DNA copy number changes in multiple loci in one reaction and has been shown to reliably identify both complete and partial loss of 1p and 19q (Jeuken et al., 2006).

Although material was not sufficient to allow direct comparison between HR-CGH and aCGH in papers III-V, the latter proved to be as useful as metaphase-based CGH in identifying copy number alterations present in the genome of brain tumour samples. Array-based methods are now gradually replacing classical cytogenetic techniques in many laboratories and have been shown to be powerful tools in disease gene discovery,

cancer research, and pre-natal diagnostics (Shinawi and Cheung, 2008). Although this technique offers whole genome screening at a high resolution level and is less time consuming than, for instance, G-banding, its sole use is problematic and should, in my opinion, best be avoided. The inability to detect balanced aberrations and copy neutral loss of heterozygosity is often acknowledged and the latter can to some extent be solved using SNP-CGH arrays. More importantly, however, aCGH is unable to detect certain types of aneuploidy (Kallioniemi et al., 1994). This was particularly evident in paper IV, where karyotypic analysis of a rare tumour, giant-cell ependymoma, and its subsequent recurrence revealed multiple monosomies that could not be detected by aCGH. Instead aCGH reported large areas of apparently gained chromosomal material. This emphasises the need for caution when interpreting aCGH findings when used as a stand-alone tool. It is a valuable technique, but should only be used as part of a multimodal approach, where the different techniques may act to complement each other. DNA ploidy analysis may be a relatively easily accessible supplementary technique in such situations and it proved very helpful when making sense of the data in paper IV. This technique was introduced in the 1960s (Caspersson and Lomakka, 1962), is easy to perform, and offers high sensitivity and specificity (Kristensen et al., 2003). It has the advantage over G-banding that it is independent of living cells, can be performed on formalin-fixed paraffin-embedded material, and may therefore be of particular value in retrospective studies where live cells are not available.

Another issue with aCGH that is somewhat related to point above, is the lack of standardised interpretation of the vast amount of data generated by this technique. Even though aCGH has been around for quite some time already, the calling of aberrations still seems difficult to standardise, partly because the quality of the DNA and hybridisation must be taken into account and partly because of the wide variety of platforms used.

Several standardisation methods are currently used that vary from the most simple ones using one fixed threshold for all samples to more statistically advanced algorithms. Following visual inspection of the array data generated in papers III-V, we applied the threshold recommended by the manufacturer (6.0), the ADM-2 aberration detection algorithm, and the Fuzzy Zero correction algorithm. The latter helps to avoid scoring long aberrations with low absolute log ratios that are likely to represent noise (Niini et al., 2010).

In general, it still remains important to visually inspect individual array plots and to be critical when applying filters and thresholds before scoring aberrations. The need for standardisation remains, however, as it is important to be able to distinguish between aberrations that have pathogenetic significance, background noise, and aberrations that are merely part of the normal spectrum of interindividual genome variation (Heim and Mitelman, 2009). Similarly, it is important to bear in mind that some acquired clonal aberrations (especially low-grade mosaicism for numerical aberrations) have also been found in non-neoplastic tissues (Lindström et al., 1991; Johansson et al., 1993). Hence, the data generated by these techniques may have to rely on both systematic comparison of tumour samples with control tissues and databases of normal human sequence variation.

We came close to illustrating what we see as an ideal approach to the cytogenetic analysis of a tumour in paper III, where a gliosarcoma with osseous metaplasia was first G-banded to find a t(3;21), a deleted chromosome 13, as well as several numerical aberrations. Findings were confirmed using M-FISH and whole-chromosome painting probes, and aCGH was used to determine more exactly the breakpoints of the interstitially deleted chromosome 13 and to identify small additional copy number aberrations that were probably below the resolution level of the other methods used. In

this case, the exclusive use of aCGH analysis would have failed to detect the balanced aberration. Locus-specific probes were used to confirm the reciprocal nature of the translocation. Lack of tumour material prevented us from completing the systematic BAC-followed-by-fosmid approach that has been so successful in identifying exact chromosomal breakpoints and the genes involved (Brandal et al., 2008; 2009; Micci et al., 2009). This case does, however, illustrate nicely what each method offers in terms of advantages and limitations and that if used in combination, they may reveal hidden "truths" about the cytogenetics of brain tumours as well as other malignancies.

Biological considerations

The overall aim of this study was to examine the genomic aberrations of brain tumours in adult and paediatric patients in the hope of increasing the existing pathogenetic knowledge about these tumours.

In paper I, 80 cases of primary, previously untreated glioblastomas were analysed using G-band karyotyping and HR-CGH. Clonal cytogenetic aberrations were detected by karyotyping in 96% of cases, in most cases giving rise to complex karyotypes with multiple numerical and structural aberrations. There was, however, a subset of tumours that displayed merely simple (≤4) changes. These included loss of the Y chromosome as the sole aberration and trisomy 7, both of which have been previously reported in both normal and cancerous tissues of the brain (Lindström et al., 1991). This does not, however, imply that all relatively simple numerical clones are merely reflections of changes outside the tumour parenchyma. As HR-CGH confirmed the in vivo existence of such "simple aberrations" in this series, the combined findings strongly

suggest that some glioblastomas do have less complex genomes, although how these function pathogenetically remains unknown.

The majority of glioblastomas showed multiple numerical and structural changes including loss of or from chromosomes 1, 6, 9, 10, 13, 14, 19, and 22, but the single most frequently lost autosome detected by both techniques was chromosome 10, with a particular clustering of breakpoints at the very distal end of 10q (10q25-26). This agrees with previous LOH studies that have suggested the presence of pathogenetically important tumour suppressor genes in this area, more specifically *MGMT*, *DMBT1*, and *FGFR2* (Watanabe et al., 1990; Rasheed et al., 1995). One should keep in mind, however, that the majority of primary glioblastomas showed loss of an entire chromosome 10 suggesting that several pathogenetically important areas may be found on this chromosome. Structural and numerical gains of chromosome 7 have been reported as one of the hallmarks of glioblastoma for more than two decades now; hence, it was not surprising to find that this was the most commonly gained autosome with a particular clustering of breakpoints around 7p11-21 (70%) and 7q11-31 (70%) by HR-CGH. Again, as the gained chromosomal regions were quite large, one should be careful putting too much emphasis on the role of the *EGFR* gene (mapping to 7p12), as it is highly likely that multiple pathogenetically important genes, possibly oncogenes, are located on this chromosome.

Statistical analysis was used to look for cytogenetic patterns that might reveal pathogenetically important alterations with a particular affinity for occurring together in more or less distinct pathways. A dominant finding in this series as well in those of others studies (Bigner et al., 1986; Jenkins et al., 1989; Mohapatra et al., 1998; Hassler et al., 2006) was that trisomy 7 and monosomy 10, the most common findings in

glioblastoma, often coexist. In fact, of the original 80 samples, only 11 did not show the +7/-10 combination. The +7 and -10 combination was significantly associated with secondary aberrations such as loss of 1p, 9p, 13q, and 22q, indicating that +7/-10-positive glioblastomas follow a preferential route when undergoing clonal evolution. Other similar associations were also found, and the existence of such genetic subgroups suggests that several distinct pathogenetic and evolutionary pathways exist for these tumours, pathways that are not necessarily reflected in the currently used histopathological classification. This may instead explain some of the observed variations in response to therapy and overall survival.

Low-grade diffuse gliomas include diffuse astrocytomas (WHO grade II), oligodendrogliomas (WHO grade II), and oligoastrocytomas(WHO grade II). This is a heterogenous group of tumours with regards to prognosis, and it is difficult to predict if and when they transform to their more aggressive counterparts. The cytogenetic and molecular cytogenetic analysis of 38 such tumours (paper II) showed that all three glioma variants displayed different genomic aberration patterns that may explain some of the variation in tumour behaviour. The 1p/19q-codeletion was present in 83% of oligodendrogliomas and in 31% of oligoastrocytomas. As oligodendroglial tumours showing this aberration have a tendency for better prognosis, these results support the need for a reclassification of tumours based partly on molecular and/or cytogenetic findings.

If copy number alterations can be linked to survival, cytogenetic markers of low-grade gliomas could become prognostically useful. At the present moment, only advanced patient age has proven to be of prognostic value (Lote et al., 1997). To establish whether cytogenetic aberrations could predict time to progression, patients

who had not received cytotoxic therapy following surgery and who had HR-CGH data available (N=23) were included in a time-to-progression analysis. Patients whose tumours displayed several (>4) chromosomes with copy number alterations showed a clear tendency to have a shorter time to progression than did patients with tumours displaying few aberrations. The difference was marginally not significant, most likely due to the lack of power as the 1p/19q-codeletion, a known prognostic indicator of favourable prognosis, showed the same marginally significant trend. It is important to repeat this study in a larger study population, as this could potentially help identify patients with low-grade gliomas who might benefit from early anti-neoplastic therapy.

Gliosarcomas represent rare glioblastoma variants that are characterised by biphasic tissue patterns with both glial and mesenchymal elements. Occasionally, the mesenchymal portion of these tumours show bone differentiation (Louis et al., 2007). In paper III, the first genomic characterisation of such a tumour was presented, revealing a novel balanced 3;21-translocation in addition to the chromosomal changes often observed in gliomas such as +7,-10,-13, and -22. The absence of relevant data for comparison prevents us from concluding whether this is a recurrent aberration, what mechanisms lie behind it, nor whether it could eventually act as a potential target for future therapy, as translocations leading to fusion genes do in some other malignancies (Druker, 2008). Literature data indicate that there is a higher frequency of balanced translocations in gliosarcomas compared with conventional glioblastomas, something that may be related to the existence of the sarcomatous portion in these tumours. Approximately 15-20% of non-cerebral mesenchymal tumours carry a specific translocation that results in a fusion gene (Mitelman et al., 2011).

Giant cell ependymomas (GCE) are rare ependymoma subtypes that show pleomorphic giant cells admixed with features of typical ependymomas. The karyotypic features of the GCE reported in paper IV were hypodiploidy with multiple monosomies leading to a stemline midway between 2n and n. This was strikingly similar to the only other cytogenetically analysed tumour of the same type (Zec et al., 1996). The genomic events involved in classical ependymoma pathogenesis remain elusive, the majority being near-diploid to near-triploid with loss of chromosome 22 representing the most common aberration by far. Hence, although the presence of monosomy 22 supports the histopathological notion that GCE have features that resemble classical ependymomas, the marked hypodiploid stemline now provides a genomic basis for its identification as an entity of its own, and may also help pathologists distinguish it from other glioma entities that contain giant cells.

Tumours of the central nervous system (CNS) represent the largest group of solid tumours in childhood (Parkin et al., 1999; Stiller et al., 2006), and with the exception of medulloblastomas, no clear aberration pattern has yet been demonstrated (Mitelman et al., 2011). Of the 17 paediatric CNS tumours examined in paper V, one third could not readily be grouped in accordance with the current WHO classification, emphasising the diagnostic difficulties neuropathologists and clinicians face. Pilocytic astrocytomas had normal karyotypes or simple non-recurrent translocations by karyotyping, but showed recurrent gain of 7q34 and 19p13.3 by aCGH. As these aberrations were present in both infratentorial and supratentorial PAs, the gains do not seem to be specific for tumours of any given anatomic site.

Glioblastomas are very rarely encountered in children, and thus cytogenetic data on them are limited (Mitelman et al., 2011). The two paediatric glioblastomas analysed

in paper V showed very different genomic profiles from those of their adult counterparts analysed in paper I. One tumour showed simple numerical aberrations by both G-banding and aCGH, whereas multiple copy number aberrations were present in the second tumour despite an apparently normal karyotype. The data, although scant, suggest that glioblastomas in childhood may be divided into two groups based on genomic complexity (Bax et al., 2010). More, probably multicentre, studies are required to determine to what extent and how the pattern of genomic aberrations is non-random in these tumours.

Anaplastic oligoastrocytomas are difficult to diagnose with certainty as was evident in the two tumours analysed in paper V. Both the anaplastic oligoastrocytoma and the anaplastic neuroepithelial tumour showed loss of chromosome 14 and structural aberrations of chromosome 6 by G-banding, admittedly as part of complex karyotypes, as well as losses of 14q and 17p and homozygous loss from 22q when analysed by aCGH. Certainly more of these tumours must be characterised before the diagnostic value of cytogenetics in this context can be determined and any non-random role of aberrations of chromosomes 6, 14, 17, and/or 22 is established. Interestingly, loss of 1p/19q was not seen in these tumours, suggesting that alternative pathogenetic pathways are involved in the development of paediatric oligodendroglial tumours compared to their adult counterparts.

Due to the rarity of CNS-PNETs, few such tumours have been investigated cytogenetically. Three of five CNS-PNETs examined by us showed abnormal karyotypes; two were near-diploid with mainly structural changes whereas one was near-triploid with trisomies for chromosomes 1, 2, and 7. As this pattern is by and large similar to that of the five previously reported abnormal cases, the combined findings suggest that CNS-

PNETs consist of separate subgroups based on ploidy status; however, this must be replicated in larger studies that should also take into account clinical parameters and survival. Recurrent gains of chromosome bands 1q21-44 and losses of 3p21, 3q26, and 8p23 were noted. Again, more studies are needed to determine the importance of these aberrations and what gene-level change they might correspond to.

Recently, Hasselblatt et al (2009) described CRINET to represent a variant of atypical teratoid/rhabdoid tumour but with a relatively favourable prognosis. In paper V, the first cytogenetic description of such a tumour revealed a normal karyotype, whereas the aCGH examination showed loss of the entire 22q, 1p33, 10p12.31, and 10q11.22 as well as homozygous loss of 4q13.2. The absence of data on more cases makes firm conclusions impossible. However, losses of 1p and 22q have been described in two and nine of the ten conventional AT/RTs analysed by conventional CGH, respectively (Wharton et al., 2003; Rickert and Paulus, 2004). This suggests a pathogenetic link between the two tumour entities, but it could be speculated that losses at chromosome 10 and homozygous loss of 4q might represent changes specific to the CRINET variant.

“Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less."
— Marie Curie (1867-1934)

Conclusions and future perspectives

Since the discovery of the Philadelphia chromosome 50 years ago, cancer cytogenetics as a medical science has come a long way. Technical developments have unfolded a plethora of possibilities, some of which have most certainly not been followed up by relevant research or in diagnostic algorithms.

The current histopathological classification of brain tumours is suboptimal, and a clear-cut diagnosis is often difficult to make in a substantial proportion of cases. Furthermore, inter-observer variability remains high (Mittler et al., 1996; Coons et al., 1997; Ohgaki and Kleihues, 2011). This is particularly evident for oligoastrocytomas, which are composed of a mixture of astrocytic and oligodendroglial components, but applies also to other diagnostic groups (Louis et al., 2007).

As cancer is thought to be the phenotypic result of the acquisition of one or more chromosomal and gene-level mutations by susceptible somatic target cells, screening the whole tumour genome is a natural starting point when trying to understand the pathogenetic mechanisms behind tumour development (Heim and Mitelman, 2009). Eventually, a full pathogenetic classification of tumours would be one of the aims, an alternative grouping of neoplastic processes that could hopefully provide both information about prognosis and key insights into more effective therapies in individual cases than the current morphology-based classification does (Brandal et al., 2010). The majority of research now focuses on looking for disease-specific genes that could be used to stratify patients according to risk. We argue that although it is time-consuming, it is important to take a step back and consider the whole tumour genome first, establish

areas of interest, and only then subject these to examinations with more molecular techniques. Otherwise, we seem to be searching blind-folded.

Genome-wide screening of gliomas

The work in this thesis has largely focussed on primary glioblastomas (paper I), low-grade gliomas (paper II), and paediatric gliomas and embryonal tumours (paper V). In addition, we have genomically characterised diagnostic rarities such as gliosarcoma with osseous differentiation (paper III) and giant-cell ependymoma (paper IV). We would like to extend the multimodal cytogenetic analysis to include also other glioma groups.

Secondary glioblastomas are currently being investigated to look for specific molecular markers (Ohgaki and Kleihues, 2011). However, to the best of our knowledge, these tumours have not yet been subjected to systematic cytogenetic analysis. In our laboratory, a study is therefore underway that aims to genomically characterise these tumours and to compare the results with those found in primary glioblastomas and low-grade gliomas. The latter is particularly interesting, as these are thought to represent the precursor lesions for secondary glioblastomas (Ohgaki and Kleihues, 2007).

As illustrated in the studies that form the basis for this thesis, oligoastrocytomas are particularly difficult to classify using the current histopathological classification (Louis et al., 2007). In our brain tumour project we have collected a large series of high-grade oligoastrocytomas in adults and these will be subjected to cytogenetic analysis. It will be particularly interesting to see if the characteristics of these tumours are similar to those of their low-grade equivalents.

The third group of tumours that we are hoping to learn more about in the near future are ependymomas. Although 108 ependymomas have so far been cytogenetically described (Mitelman et al., 2011), much remains to be learned about the acquired chromosomal aberrations that characterise these tumours, particularly those of high-grade.

Future plans for the genomically characterised tumours

Our brain tumour project started collecting tumour samples in 2005 and recruitment of new cases is still ongoing. By comparing and making use of data obtained by various techniques, the limitations of each technique are reduced. We have a unique opportunity to get a near-complete overview over the genomic and genetic profiles of these tumours. The tumours investigated in papers I and II have subsequently been analysed by quantitative methylation specific PCR (qMSP) and pyrosequencing (Håvik et al., 2011) to look for methylation of the O^6-methylguanine-DNA methyltransferase (*MGMT*) gene promoter that has been shown to be associated with a favourable prognosis in patients with glioblastoma treated with the cytotoxic drug temozolomide (Stupp et al., 2010; Weller et al., 2010). Furthermore, there are plans to compare the genomic data with gene expression data. It will be interesting to compare the expression profiles of +7/-10-positive and negative glioblastomas, tumours with simple and complex genomic aberrations, and 1p/19q-codeleted and non-1p/19q-codeleted tumours.

Future projects are envisaged to use similar approaches in the analysis of other brain tumours. For instance, fusion-gene microanalysis that makes use of the total set of reported fusion genes in different malignancies to screen tumour samples for such aberrations, looks to be another possible venture (Løvf et al., 2011).

Survival analysis

Work is ongoing to correlate the genomic characteristics of the glioblastomas reported in paper I with clinical parameters. It will be interesting to see if there are significant associations between genomic status and overall survival, but also to see if the patients that died shortly after surgery had genomic aberrations that differed from those of relatively long-term survivors. Similarly, in the future, we hope to be able to collect enough low-grade gliomas, to do a follow-up study of the survival analysis done in paper II. Here, genomic complexity was near-significant, and we hypothesised that this was due to the low power of the study. It is important to be able to repeat studies in larger study populations, to be able to confirm or reject the hypothesis that genomic complexity could be used to identify patients with low-grade tumours that are at particular risk of recurrence.

Reference list

Allen NJ, Barres BA (2009): Neuroscience: Glia - more than just brain glue. Nature 457:675-677.

Arnold J (1879): Beobachtungen über Kernteilungen in den Zellen der Geschwülste. Virchows Arch Pathol Anat 78: 279.

Australian National Health and Medical Research Council (2007): National statement on ethical conduct in medical research. Available at: http://www.nhmrc.gov.au/publications/synopses/e72syn.htm. Accessed in March 2011.

Avery OT, Macleod CM, McCarty M (1944): Studies on the chemical nature of the substance inducing transformation of pneumococcal types : Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. J Exp Med 79:137-158.

Bailey P, Cushing H (1926): A classification of the tumors of the glioma group on a histogenic basis with a correlated study of prognosis. J.B.Lippincott, Philadelphia.

Balmain A (2001): Cancer Genetics: From Boveri and Mendel to microarrays. Nat Rev Cancer 1:77-82.

Bax DA, Mackay A, Little SE, Carvalho D, Viana-Pereira M, et al (2010): A distinct spectrum of copy number aberrations in pediatric high-grade gliomas. Clin Cancer Res 16:3368-3377.

Bentz M, Plesch A, Stilgenbauer S, Dohner H, Lichter P (1998): Minimal sizes of deletions detected by comparative genomic hybridization. Genes Chromosomes Cancer 21:172-175.

Biegel JA, Kalpana G, Knudsen ES, Packer RJ, Roberts CW, et al (2002): The role of INI1 and the SWI/SNF complex in the development of rhabdoid tumors: Meeting summary from the workshop on childhood atypical teratoid/rhabdoid tumors. Cancer Res 62:323-328.

Bignell GR, Huang J, Greshock J, Watt S, Butler A, et al (2004): High-resolution analysis of DNA copy number using oligonucleotide microarrays. Genome Res 14:287-295.

Bigner SH, Mark J, Bullard DE, Mahaley MS, Jr., Bigner DD (1986): Chromosomal evolution in malignant human gliomas starts with specific and usually numerical deviations. Cancer Genet Cytogenet 22:121-135.

Bondy ML, Scheurer ME, Malmer B, Barnholtz-Sloan JS, Davis FG, et al (2008): Brain tumor epidemiology: Consensus from the brain tumor epidemiology consortium. Cancer 113:1953-1968.

Boveri T (1914): Zur Frage der Entstehung Maligner Tumoren. Translated and annotated by Henry Harris. Available in Journal of Cell Science (2008);121, supplement 1, 1-84.

Brandal P, Bjerkehagen B, Heim S (2004): Molecular cytogenetic characterization of tenosynovial giant cell tumors. Neoplasia 6:578-583.

Brandal P, Busund LT, Heim S (2005): Chromosome abnormalities in juxtaglomerular cell tumors. Cancer 104:504-510.

Brandal P, Micci F, Bjerkehagen B, Eknæs M, Larramendy M, et al (2003): Molecular cytogenetic characterization of desmoid tumors. Cancer Genet Cytogenet 146:1-7.

Brandal P, Panagopoulos I, Bjerkehagen B, Gorunova L, Skjeldal S, et al (2008): Detection of a t(1;22)(q23;q12) translocation leading to an EWSR1-PBX1 fusion gene in a myoepithelioma. Genes Chromosomes Cancer 47:558-564.

Brandal P, Panagopoulos I, Bjerkehagen B, Heim S (2009): t(19;22)(q13;q12) translocation leading to the novel fusion gene EWSR1-ZNF444 in soft tissue myoepithelial carcinoma. Genes Chromosomes Cancer 48:1051-1056.

Brandal P, Teixeira MR, Heim S (2010): Genotypic and phenotypic classification of cancer: How should the impact of the two diagnostic approaches best be balanced? Genes Chromosomes Cancer 49:763-774.

Breasted J (1991): The Edwin Smith Papyrus: Hieroglyphic transliteration, translation and commentary v1. University of Chicago Oriental Institute publications: University of Chicago Press, Chicago.

Bromberg JE, Van den Bent MJ (2009): Oligodendrogliomas: Molecular Biology and Treatment 14:155-163.

Burke DT, Carle GF, Olson MV (1987): Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. Science 236:806-812.

Burton JL, Wells M (2001): The Alder Hey affair: Implications for pathology practice. J Clin Pathol 54:820-823.

Cairncross G, Berkey B, Shaw E, Jenkins R, Scheithauer B, et al (2006): Phase III trial of chemotherapy plus radiotherapy compared with radiotherapy alone for pure and mixed anaplastic oligodendroglioma: Intergroup radiation therapy oncology group trial 9402. J Clin Oncol 24:2707-2714.

Cairncross JG, Ueki K, Zlatescu MC, Lisle DK, Finkelstein DM, et al (1998): Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. J Natl Cancer Inst 90:1473-1479.

Cancer Registry of Norway (2009): Cancer in Norway 2008 - Cancer Incidence, Mortality, Survival and Prevalence in Norway. Cancer Registry of Norway, Oslo.

Carrie C, Grill J, Figarella-Branger D, Bernier V, Padovani L, et al (2009): Online quality control, hyperfractionated radiotherapy alone and reduced boost volume for standard risk medulloblastoma: Long-term results of MSFOP 98. J Clin Oncol 27:1879-1883.

Caspersson T, Farber S, Foley GE, Kudynowski J, Modest EJ, et al (1968): Chemical differentiation along metaphase chromosomes. Exp Cell Res 49:219-222.

Caspersson T, Gahrton G, Lindsten J, Zech L (1970a): Identification of the Philadelphia chromosome as a number 22 by quinacrine mustard fluorescence analysis. Exp Cell Res 63:238-240.

Caspersson T, Zech L, Johansson C, Modest EJ, Farber S, et al (1970b): Identification of human chromosomes by DNA-binding fluorescent agents chemical differentiation along metaphase chromosomes. Chromosoma 30:215-227.

Caspersson TO, Lomakka GM (1962): Scanning microscopy techniques for high resolution quantitative cytochemistry. Ann N Y Acad Sci 97:449-463.

CBTRUS (2011): CBTRUS Statistical Report Statistical report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States, 2004-2007. Available at www.cbtrus.org. Accessed March, 2011.

Chaichana KL, Kosztowski T, Niranjan A, Olivi A, Weingart JD, et al (2010): Prognostic significance of contrast-enhancing anaplastic astrocytomas in adults. J Neurosurg 113:286-292.

Charlesworth B, Charlesworth D (2009): Darwin and genetics. Genetics 183:757-766.

Chudoba I, Hickmann G, Friedrich T, Jauch A, Kozlowski P, et al (2004): mBAND: a high resolution multicolor banding technique for the detection of complex intrachromosomal aberrations. Cytogenet Genome Res 104:390-393.

Coons SW, Johnson PC, Scheithauer BW, Yates AJ, Pearl DK (1997): Improving diagnostic accuracy and interobserver concordance in the classification and grading of primary gliomas. Cancer 79:1381-1393.

Crawford JR, MacDonald TJ, Packer RJ (2007): Medulloblastoma in childhood: new biological advances. Lancet Neurol 6:1073-1085.

Crow EW, Crow JF (2002): 100 years ago: Walter Sutton and the chromosome theory of heredity. Genetics 160:1-4.

Daley GQ, Van Etten RA, Baltimore D (1990): Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science 247:824-830.

Del Bigio MR (1995): The ependyma: a protective barrier between brain and cerebrospinal fluid. Glia 14:1-13.

Douw L, Klein M, Fagel SSAA, van den Heuvel J, Taphoorn MJB, et al (2009): Cognitive and radiological effects of radiotherapy in patients with low-grade glioma: Long-term follow-up. The Lancet Neurology 8:810-818.

Druker BJ (2008): Translation of the Philadelphia chromosome into therapy for CML. Blood 112:4808-4817.

Duesberg P, Li R (2003): Multistep carcinogenesis: a chain reaction of aneuploidizations. Cell Cycle 2:202-210.

Eberhart CG (2011): Molecular diagnostics in embryonal brain tumors. Brain Pathol 21:96-104.

Ellison DW (2010): Childhood medulloblastoma: novel approaches to the classification of a heterogeneous disease. Acta Neuropathol 120:305-316.

Ellison DW, Kocak M, Dalton J, Megahed H, Lusher ME, et al (2011): Definition of disease-risk stratification groups in childhood medulloblastoma using combined clinical, pathologic, and molecular variables. Journal of Clinical Oncology 29:1400-1407.

European Court of Human Rights: Convention for the protection of human rights and fundemental freedoms. Article 8. Available at http://www.echr.coe.int/nr/rdonlyres/d5cc24a7-dc13-4318-b457-5c9014916d7a/0/englishanglais.pdf. Accessed March, 2011).

Fangusaro J, Massimino M, Rutkowski S, Gururangan S (2010): Non-cerebellar primitive neuroectodermal tumors (PNET): Summary of the Milan consensus and state of the art workshop on marrow ablative chemotherapy with hematopoietic cell rescue for malignant brain tumors of childhood and adolescents. Pediatr Blood Cancer 54:638-640.

Fangusaro JR, Jubran RF, Allen J, Gardner S, Dunkel IJ, et al (2008): Brainstem primitive neuroectodermal tumors (bstPNET): Results of treatment with intensive induction chemotherapy followed by consolidative chemotherapy with autologous hematopoietic cell rescue. Pediatr Blood Cancer 50:715-717.

Flemming W (1879): Beiträge zur Kenntniss der Zelle und ihrer Lebenserscheinungen. Archiv für Mikroskopische Anatomie 16:302-436.

Ford CE, Hamerton JL (1956): A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes. Stain Technol 31:247-251.

Gaffney CC, Sloane JP, Bradley NJ, Bloom HJ (1985): Primitive neuroectodermal tumours of the cerebrum. Pathology and treatment. J Neurooncol 3:23-33.

Gajjar A, Chintagumpala M, Ashley D, Kellie S, Kun LE, et al (2006): Risk-adapted craniospinal radiotherapy followed by high-dose chemotherapy and stem-cell rescue in children with newly diagnosed medulloblastoma (St Jude medulloblastoma-96): Long-term results from a prospective, multicentre trial. Lancet Oncol 7:813-820.

Garre ML, Tekautz T (2010): Role of high-dose chemotherapy (HDCT) in treatment of atypical teratoid/rhabdoid tumors (AT/RTs). Pediatr Blood Cancer 54:647-648.

Gibson P, Tong Y, Robinson G, Thompson MC, Currle DS, et al (2010): Subtypes of medulloblastoma have distinct developmental origins. Nature 468:1095-1099.

Gilbert MR, Ruda R, Soffietti R (2010): Ependymomas in adults. Curr Neurol Neurosci Rep 10:240-247.

Gonzales M (2001): Classification and pathogenesis of brain tumors. In: Kaye AH, Laws ER: Brain tumors - an encyclopedic approach. 2nd edition. Churchill Livingstone, Edinburgh.

Graeber MB (2010): Changing face of microglia. Science 330:783-788.

Greenblatt S, Dagi T, Epstein ME (1997): A history of neurosurgery in its scientific and professional contexts. Section II-IV. American Association of Neurological Surgeons, Park Ridge, Illinois.

U.S. Cancer Statistics Working Group (2010): United States Cancer Statistics: 1999–2007 incidence and mortality web-based report. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute, Atlanta. Available at: www.cdc.gov/uscs. Accessed April, 2011.

Grundy RG, Wilne SH, Robinson KJ, Ironside JW, Cox T, et al (2010): Primary postoperative chemotherapy without radiotherapy for treatment of brain tumours other than ependymoma in children under 3 years: Results of the first UKCCSG/SIOP CNS 9204 trial. Eur J Cancer 46:120-133.

Hasselblatt M (2009): Ependymal tumors. Recent Results Cancer Res 171:51-66.

Hasselblatt M, Oyen F, Gesk S, Kordes U, Wrede B, et al (2009): Cribriform neuroepithelial tumor (CRINET): A nonrhabdoid ventricular tumor with *INI1* loss and relatively favorable prognosis. J Neuropathol Exp Neurol 68:1249-1255.

Hassler M, Seidl S, Fazeny-Doerner B, Preusser M, Hainfellner J, et al (2006): Diversity of cytogenetic and pathohistologic profiles in glioblastoma. Cancer Genet Cytogenet 166:46-55.

Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, et al (2005): *MGMT* gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352:997-1003.

Heim S, Mitelman F (2009): Cancer cytogenetics. 3rd edition. Wiley-Blackwell, New Jersey.

Heisterkamp N, Jenster G, ten Hoeve J, Zovich D, Pattengale PK, et al (1990): Acute leukaemia in bcr/abl transgenic mice. Nature 344:251-253.

Helseth R, Helseth E, Johannesen TB, Langberg CW, Lote K, et al (2010): Overall survival, prognostic factors, and repeated surgery in a consecutive series of 516 patients with glioblastoma multiforme. Acta Neurol Scand 122:159-167.

Hsu TC (1952): Mammalian chromosomes in vitro I. The karyotype of man. J Hered 43:167-172.

Håvik AB, Brandal P, Honne H, Dahlback HSS, Scheie D, et al (2011): MGMT promoter methylation in gliomas - assessment by pyrosequencing and quantitative methylation-specific PCR and review of the literature. Submitted manuscript.

ISCN (1991): Guidelines in cancer cytogenetics, supplement to an international system for human cytogenetic nomenclature. Mitelman F (ed). S. Karger, Basel.

ISCN (2009): An International System for Human Cytogenetic Nomenclature. Shaffer LG, Slovak ML, Campbell LJ (eds). S. Karger, Basel.

Isola J, DeVries S, Chu L, Ghazvini S, Waldman F (1994): Analysis of changes in DNA sequence copy number by comparative genomic hybridization in archival paraffin-embedded tumor samples. Am J Pathol 145:1301-1308.

Jenkins RB, Blair H, Ballman KV, Giannini C, Arusell RM, et al (2006): A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. Cancer Res 66:9852-9861.

Jenkins RB, Kimmel DW, Moertel CA, Schultz CG, Scheithauer BW, et al (1989): A cytogenetic study of 53 human gliomas. Cancer Genet Cytogenet 39:253-279.

Jeuken J, Cornelissen S, Boots-Sprenger S, Gijsen S, Wesseling P (2006): Multiplex ligation-dependent probe amplification: A diagnostic tool for simultaneous identification of different genetic markers in glial tumors. J Mol Diagn 8:433-443.

Johannesen TB, Langmark F, Lote K (2003): Progress in long-term survival in adult patients with supratentorial low-grade gliomas: A population-based study of 993 patients in whom tumors were diagnosed between 1970 and 1993. J Neurosurg 99:854-862.

Johansson B, Heim S, Mandahl N, Mertens F, Mitelman F (1993): Trisomy 7 in nonneoplastic cells. Genes Chromosomes Cancer 6:199-205.

Joos S, Scherthan H, Speicher MR, Schlegel J, Cremer T, et al (1993): Detection of amplified DNA sequences by reverse chromosome painting using genomic tumor DNA as probe. Hum Genet 90:584-589.

Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, et al (1992): Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 258:818-821.

Kallioniemi OP, Kallioniemi A, Piper J, Isola J, Waldman FM, et al (1994): Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Genes Chromosomes Cancer 10:231-243.

Karlsson P, Holmberg E, Lundell M, Mattsson A, Holm LE, et al (1998): Intracranial tumors after exposure to ionizing radiation during infancy: A pooled analysis of two swedish cohorts of 28,008 infants with skin hemangioma. Radiat Res 150:357-364.

Karpozilos A, Pavlidis N (2004): The treatment of cancer in Greek antiquity. Eur J Cancer 40:2033-2040.

Kaye AH, Laws ER (2001): Brain tumors: An encyclopedic approach. Chapt. 1. Churchill Livingstone, Edinburgh.

Kesari S, Schiff D, Drappatz J, LaFrankie D, Doherty L, et al (2009): Phase II study of protracted daily temozolomide for low-grade gliomas in adults. Clin Cancer Res 15:330-337.

Kimelberg HK, Nedergaard M (2010): Functions of astrocytes and their potential as therapeutic targets. Neurotherapeutics 7:338-353.

Kirchhoff M, Gerdes T, Maahr J, Rose H, Bentz M, et al (1999): Deletions below 10 megabasepairs are detected in comparative genomic hybridization by standard reference intervals. Genes Chromosomes Cancer 25:410-413.

Kirchhoff M, Gerdes T, Rose H, Maahr J, Ottesen AM, et al (1998): Detection of chromosomal gains and losses in comparative genomic hybridization analysis based on standard reference intervals. Cytometry 31:163-173.

Kleihues P, Cavenee WE (2000): Pathology and genetics of tumours of the nervous system. IARC press, Lyon.

Kleihues P, Ohgaki H (1999): Primary and secondary glioblastomas: From concept to clinical diagnosis. Neuro Oncol 1:44-51.

Kloosterhof NK, Bralten LB, Dubbink HJ, French PJ, van den Bent MJ (2011): Isocitrate dehydrogenase-1 mutations: A fundamentally new understanding of diffuse glioma? Lancet Oncol 12:83-91.

Kool M, Koster J, Bunt J, Hasselt NE, Lakeman A, et al (2008): Integrated genomics identifies five medulloblastoma subtypes with distinct genetic profiles, pathway signatures and clinicopathological features. PLoS One 3:e3088.

Kristensen GB, Kildal W, Abeler VM, Kaern J, Vergote I, et al (2003): Large-scale genomic instability predicts long-term outcome for women with invasive stage I ovarian cancer. Ann Oncol 14:1494-1500.

Langer-Safer PR, Levine M, Ward DC (1982): Immunological method for mapping genes on drosophila polytene chromosomes. Proc Natl Acad Sci USA 79:4381-4385.

Langer PR, Waldrop AA, Ward DC (1981): Enzymatic synthesis of biotin-labeled polynucleotides: Novel nucleic acid affinity probes. Proc Natl Acad Sci USA 78:6633-6637.

Larouche V, Capra M, Huang A, Bartels U, Bouffet E (2006): Supratentorial primitive neuroectodermal tumors in young children. J Clin Oncol 24:5609; author reply 5609-5610.

Lee C, Gisselsson D, Jin C, Nordgren A, Ferguson DO, et al (2001): Limitations of chromosome classification by multicolor karyotyping. Am J Hum Genet 68:1043-1047.

Lejeune J, Gautier M, Turpin R (1959): Etude des chromosomes somatiques de neuf enfants mongoliens. C R Hebd Seances Acad Sci 248:1721-1722.

Lindström E, Salford LG, Heim S, Mandahl N, Strömblad S, et al (1991): Trisomy 7 and sex chromosome loss need not be representative of tumor parenchyma cells in malignant glioma. Genes Chromosomes Cancer 3:474-479.

Loge JH, Kaasa S, Aass N (2010): Personer med begrenset levetid. Available at http://www.etikkom.no/fbib/temaer/forskning-pa-bestemte-grupper/personer-med-begrenset-levetid. Accessed April, 2011.

Lote K, Egeland T, Hager B, Stenwig B, Skullerud K, et al (1997): Survival, prognostic factors, and therapeutic efficacy in low-grade glioma: A retrospective study in 379 patients. J Clin Oncol 15:3129-3140.

Louis D, Ohgaki H, Wiestler O, Cavenee W (eds.)(2007): WHO classification of tumours of the central nervous system, 4th edition. Renouf Publishing Co. Ltd., Ogdensburg.

Lugo TG, Pendergast AM, Muller AJ, Witte ON (1990): Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. Science 247:1079-1082.

Løvf M, Thomassen GO, Bakken AC, Celestino R, Fioretos T, et al (2011): Fusion gene microarray reveals cancer type-specificity among fusion genes. Genes Chromosomes Cancer 50:348-357.

Mark J, Levan G, Mitelman F (1972): Identification by fluorescence of the G chromosome lost in human meningomas. Hereditas 71:163-168.

Markel H (2004): "I swear by Apollo"--on taking the Hippocratic oath. N Engl J Med 350:2026-2029.

Matar E, Cook RJ, Fowler AR, Biggs MT, Little NS, et al (2010): Post-contrast enhancement as a clinical indicator of prognosis in patients with anaplastic astrocytoma. J Clin Neurosci 17:993-996.

Meltzer PS, Guan XY, Burgess A, Trent JM (1992): Rapid generation of region specific probes by chromosome microdissection and their application. Nat Genet 1:24-28.

Mendel G (1865): Versuche über Pflanzen-Hybriden. Naturforsch Ver Brünn 4:3-47.

Metellus P, Barrie M, Figarella-Branger D, Chinot O, Giorgi R, et al (2007): Multicentric French study on adult intracranial ependymomas: Prognostic factors analysis and therapeutic considerations from a cohort of 152 patients. Brain 130:1338-1349.

Metellus P, Guyotat J, Chinot O, Durand A, Barrie M, et al (2010): Adult intracranial WHO grade II ependymomas: Long-term outcome and prognostic factor analysis in a series of 114 patients. Neuro Oncol 12:976-984.

Micci F, Panagopoulos I, Haugom L, Andersen HK, Tjønnfjord GE, et al (2009): t(3;21)(q22;q22) leading to truncation of the RYK gene in atypical chronic myeloid leukemia. Cancer Lett 277:205-211.

Micci F, Teixeira M, Scheistrøen M, Abeler V, Heim S (2003): Cytogenetic characterization of tumors of the vulva and vagina. Genes Chromosomes Cancer 38:137-148.

Micci F, Teixeira MR, Heim S (2001): Complete cytogenetic characterization of the human breast cancer cell line ma11 combining G-banding, comparative genomic hybridization, multicolor fluorescence in situ hybridization, rxfish, and chromosome-specific painting. Cancer Genet Cytogenet 131:25-30.

Micci F, Haugom L, Kristensen G, Abeler VM, Heim S (2004): Genomic aberrations in carcinomas of the uterine corpus. Genes Chromosomes Cancer 40:229-246.

Missios S (2007): Hippocrates, Galen, and the uses of trepanation in the ancient classical world. Neurosurg Focus 23:E11.

Mitelman F, Johansson B, Mertens F, (eds.)(2011): Mitelman database of chromosome aberrations and gene fusions in cancer. Available at http://cgap.nci.nih.gov/chromosomes/mitelman. Accessed April, 2011.

Mittler MA, Walters BC, Stopa EG (1996): Observer reliability in histological grading of astrocytoma stereotactic biopsies. J Neurosurg 85:1091-1094.

Mohapatra G, Bollen AW, Kim DH, Lamborn K, Moore DH, et al (1998): Genetic analysis of glioblastoma multiforme provides evidence for subgroups within the grade. Genes Chromosomes Cancer 21:195-206.

Narod SA, Stiller C, Lenoir GM (1991): An estimate of the heritable fraction of childhood cancer. Br J Cancer 63:993-999.

Neglia JP, Meadows AT, Robison LL, Kim TH, Newton WA, et al (1991): Second neoplasms after acute lymphoblastic leukemia in childhood. N Engl J Med 325:1330-1336.

Niini T, Lopez-Guerrero JA, Ninomiya S, Guled M, Hattinger CM, et al (2010): Frequent deletion of CDKN2A and recurrent coamplification of KIT, PDGFRA, and KDR in fibrosarcoma of bone--an array comparative genomic hybridization study. Genes Chromosomes Cancer 49:132-143.

Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, et al (2011): Medulloblastoma comprises four distinct molecular variants. Journal of Clinical Oncology 29:1408-1414.

Nowell PC, Hungerford DA (1960a): A minute chromosome in human chronic granulocytic leukaemia. Science 132:1497.

Nowell PC, Hungerford DA (1960b): Chromosome studies on normal and leukemic human leukocytes. J Natl Cancer Inst 25:85-109.

Ohgaki H, Kleihues P (2005): Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J Neuropathol Exp Neurol 64:479-489.

Ohgaki H, Kleihues P (2007): Genetic pathways to primary and secondary glioblastoma. Am J Pathol 170:1445-1453.

Ohgaki H, Kleihues P (2011): Genetic profile of astrocytic and oligodendroglial gliomas. Brain Tumor Pathol. Epub ahead of print.

Okamoto Y, Di Patre PL, Burkhard C, Horstmann S, Jourde B, et al (2004): Population-based study on incidence, survival rates, and genetic alterations of low-grade diffuse astrocytomas and oligodendrogliomas. Acta Neuropathol 108:49-56.

Packer RJ, Gajjar A, Vezina G, Rorke-Adams L, Burger PC, et al (2006): Phase III study of craniospinal radiation therapy followed by adjuvant chemotherapy for newly diagnosed average-risk medulloblastoma. J Clin Oncol 24:4202-4208 (2006).

Pandis N, Bardi G, Heim S (1994): Interrelationship between methodological choices and conceptual models in solid tumor cytogenetics. Cancer Genet Cytogenet 76:77-84.

Pardue ML, Gall JG (1969): Molecular hybridization of radioactive DNA to the DNA of cytological preparations. Proc Natl Acad Sci U S A 64:600-604.

Parkin D, Kramarova E, Draper G, Masuyer E, Michaelis J, et al (eds.) (1999): International incidence of childhood cancer. International Agency for Research on Cancer, Lyon.

Pinkel D, Landegent J, Collins C, Fuscoe J, Segraves R, et al (1988): Fluorescence in situ hybridization with human chromosome-specific libraries: Detection of trisomy 21 and translocations of chromosome 4. Proc Natl Acad Sci U S A 85:9138-9142.

Piper J, Rutovitz D, Sudar D, Kallioniemi A, Kallioniemi OP, et al (1995): Computer image analysis of comparative genomic hybridization. Cytometry 19:10-26.

Pollack IF (1994): Brain tumors in children. N Engl J Med 331:1500-1507.

Prayson RA, Agamanolis DP, Cohen ML, Estes ML, Kleinschmidt-DeMasters BK, et al (2000): Interobserver reproducibility among neuropathologists and surgical pathologists in fibrillary astrocytoma grading. J Neurol Sci 175:33-39.

Rasheed BK, McLendon RE, Friedman HS, Friedman AH, Fuchs HE, et al (1995): Chromosome 10 deletion mapping in human gliomas: A common deletion region in 10q25. Oncogene 10:2243-2246.

Rickert CH, Paulus W (2001): Epidemiology of central nervous system tumors in childhood and adolescence based on the new WHO classification. Childs Nerv Syst 17:503-511.

Rickert CH, Paulus W (2004): Chromosomal imbalances detected by comparative genomic hybridisation in atypical teratoid/rhabdoid tumours. Childs Nerv Syst 20:221-224.

Rodriguez FJ, Giannini C (2010): Oligodendroglial tumors: Diagnostic and molecular pathology. Semin Diagn Pathol 27:136-145.

Rorke LB, Packer RJ, Biegel JA (1996): Central nervous system atypical teratoid/rhabdoid tumors of infancy and childhood: Definition of an entity. J Neurosurg 85:56-65.

Rothwell J (2009): Meet the brain neurophysiology. Int Rev Neurobiol 86:51-65.

Rowley JD (1973): Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and giemsa staining. Nature 243:290-293.

Ruda R, Gilbert M, Soffietti R (2008): Ependymomas of the adult: Molecular biology and treatment. Curr Opin Neurol 21:754-761.

Salminen E, Pukkala E, Teppo L (1999): Second cancers in patients with brain tumours--impact of treatment. Eur J Cancer 35:102-105.

Salvati M, Artico M, Caruso R, Rocchi G, Orlando ER, et al (1991): A report on radiation-induced gliomas. Cancer 67:392-397.

Satzinger H (2008): Theodor and Marcella Boveri: Chromosomes and cytoplasm in heredity and development. Nat Rev Genet 9:231-238.

Schmidt LS, Schmiegelow K, Lahteenmaki P, Trager C, Stokland T, et al (2011): Incidence of childhood central nervous system tumors in the nordic countries. Pediatr Blood Cancer 56:65-69.

Schröck E, du Manoir S, Veldman T, Schoell B, Wienberg J, et al (1996): Multicolor spectral karyotyping of human chromosomes. Science 273:494-497.

Shafqat S, Hedley-Whyte ET, Henson JW (1999): Age-dependent rate of anaplastic transformation in low-grade astrocytoma. Neurology 52:867-869.

Shete S, Hosking FJ, Robertson LB, Dobbins SE, Sanson M, et al (2009): Genome-wide association study identifies five susceptibility loci for glioma. Nat Genet 41:899-904.

Shinawi M, Cheung SW (2008): The array cgh and its clinical applications. Drug Discov Today 13:760-770.

Shintani T, Hayakawa N, Hoshi M, Sumida M, Kurisu K, et al (1999): High incidence of meningioma among Hiroshima atomic bomb survivors. J Radiat Res (Tokyo) 40:49-57.

Shizuya H, Birren B, Kim UJ, Mancino V, Slepak T, et al (1992): Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in Escherichia Coli using an F-factor-based vector. Proc Natl Acad Sci U S A 89:8794-8797.

Smith JS, Perry A, Borell TJ, Lee HK, O'Fallon J, et al (2000): Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. J Clin Oncol 18:636-645.

Soffietti R, Baumert BG, Bello L, von Deimling A, Duffau H, et al (2010): Guidelines on management of low-grade gliomas: Report of an EFNS-EANO task force. Eur J Neurol 17:1124-1133.

Somjen G (1988): Nervenkitt: Notes on the history of the concept of neuroglia. Glia 1:2-9.

Speicher MR, Antonarakis SE, Motulsky AG (eds) (2010): Vogel and Motulsky's Human Genetics. Problems and approaches. 4th edition. Springer-Verlag Berlin Heidelberg, Berlin.

Speicher MR, Gwyn Ballard S, Ward DC (1996): Karyotyping human chromosomes by combinatorial multi-fluor FISH. Nat Genet 12:368-375.

Speicher MR, Petersen S, Uhrig S, Jentsch I, Fauth C, et al (2000): Analysis of chromosomal alterations in non-small cell lung cancer by multiplex-FISH, comparative genomic hybridization, and multicolor bar coding. Lab Invest 80:1031-1041.

Stiller CA, Marcos-Gragera R, Ardanaz E, Pannelli F, Almar Marques E, et al (2006): Geographical patterns of childhood cancer incidence in Europe, 1988-1997. Report from the automated childhood cancer information system project. Eur J Cancer 42:1952-1960.

Stupp R, Hegi M, Weller M (2010): Neuro-oncology, a decade of temozolomide and beyond. Expert Rev Anticancer Ther 10:1675-1677.

Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, et al (2009): Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 6:6.

Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, et al (2005): Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987-996.

Stupp R, Reni M, Gatta G, Mazza E, Vecht C (2007): Anaplastic astrocytoma in adults. Crit Rev Oncol Hematol 63:72-80.

Sumner AT, Evans HJ, Buckland RA (1971): New technique for distinguishing between human chromosomes. Nat New Biol 232:31-32.

Telenius H, Carter NP, Bebb CE, Nordenskjold M, Ponder BA, et al (1992): Degenerate oligonucleotide-primed PCR: General amplification of target DNA by a single degenerate primer. Genomics 13:718-725.

Thompson MC, Fuller C, Hogg TL, Dalton J, Finkelstein D, et al (2006): Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. J Clin Oncol 24:1924-1931.

Tijo JH, Levan A (1956): The chromosome number of man. Hereditas 42.

Timmermann B, Kortmann RD, Kuhl J, Meisner C, Dieckmann K, et al (2002): Role of radiotherapy in the treatment of supratentorial primitive neuroectodermal tumors in childhood: Results of the prospective German brain tumor trials HIT 88/89 and 91. J Clin Oncol 20:842-849.

Timmermann B, Kortmann RD, Kuhl J, Rutkowski S, Meisner C, et al (2006): Role of radiotherapy in supratentorial primitive neuroectodermal tumor in young children: Results of the German HIT-SKK87 and HIT-SKK92 trials. J Clin Oncol 24:1554-1560.

Uhrig S, Schuffenhauer S, Fauth C, Wirtz A, Daumer-Haas C, et al (1999): Multiplex-FISH for pre- and postnatal diagnostic applications. Am J Hum Genet 65:448-462.

van den Bent MJ, Afra D, de Witte O, Ben Hassel M, Schraub S, et al (2005): Long-term efficacy of early versus delayed radiotherapy for low-grade astrocytoma and oligodendroglioma in adults: The EORTC 22845 randomised trial. Lancet 366:985-990.

van den Bent MJ, Carpentier AF, Brandes AA, Sanson M, Taphoorn MJ, et al (2006): Adjuvant procarbazine, lomustine, and vincristine improves progression-free survival but not overall survival in newly diagnosed anaplastic oligodendrogliomas and oligoastrocytomas: A randomized European Organisation for Research and Treatment of Cancer phase III trial. J Clin Oncol 24: 2715-2722.

van den Bent MJ, Reni M, Gatta G, Vecht C (2008): Oligodendroglioma. Crit Rev Oncol Hematol 66:262-272.

van Diest PJ (2002): No consent should be needed for using leftover body material for scientific purposes. BMJ 325:648-651.

Verano J, Williams J (1992): Head injury and surgical intervention in pre-Columbian Peru. Am J Phys Anthropology Suppl. 14. 167-168.

von Hansemann D (1890): Über Asymmetrische Zelltheilung in Epitelkrebsen und deren Biologische Bedeutung. Virchows Arch A Pathol Anat 119:299–326.

von Staden H (1992): The discovery of the body: Human dissection and its cultural contexts in ancient Greece. Yale J Biol Med 65:223-241.

Waldeyer W (1888): Über Karyokinese und ihre Beziehungen zu den Befruchtungsvorgängen. Archiv für Mikroskopische Anatomie 32.

Watanabe K, Nagai M, Wakai S, Arai T, Kawashima K (1990): Loss of constitutional heterozygosity in chromosome 10 in human glioblastoma. Acta Neuropathol 80:251-254.

Watson JD, Crick FH (1953): Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. Nature 171:737-738.

Weisblum B, De Haseth PL (1972): Quinacrine, a chromosome stain specific for deoxyadenylate-deoxythymidylaterich regions in DNA. Proc Natl Acad Sci U S A 69:629-632.

Weller M, Stupp R, Reifenberger G, Brandes AA, van den Bent MJ, et al (2010): MGMT promoter methylation in malignant gliomas: Ready for personalized medicine? Nat Rev Neurol 6:39-51.

Weyers F (2005): Salvador Dali - liv og virke. Tandem Forlag GmbH, Berlin.

Wharton SB, Wardle C, Ironside JW, Wallace WH, Royds JA, et al (2003): Comparative genomic hybridization and pathological findings in atypical teratoid/rhabdoid tumour of the central nervous system. Neuropathol Appl Neurobiol 29:254-261.

Woehrer A, Slavc I, Waldhoer T, Heinzl H, Zielonke N, et al (2010): Incidence of atypical teratoid/rhabdoid tumors in children: A population-based study by the Austrian brain tumor registry, 1996-2006. Cancer 116:5725-5732.

World Medical Association: Declaration of Helsinki - ethical principles for medical research involving human subjects. Available at http://www.wma.net/en/30publications/10policies/b3/index.html. Accessed April, 2011.

Wrensch M, Jenkins RB, Chang JS, Yeh RF, Xiao Y, et al (2009): Variants in the CDKN2B and RTEL1 regions are associated with high-grade glioma susceptibility. Nat Genet 41:905-908.

Wrensch M, Miike R, Lee M, Neuhaus J (2000): Are prior head injuries or diagnostic x-rays associated with glioma in adults? The effects of control selection bias. Neuroepidemiology 19:234-244.

Wrensch M, Minn Y, Chew T, Bondy M, Berger MS (2002): Epidemiology of primary brain tumors: Current concepts and review of the literature. Neuro Oncol 4:278-299.

Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, et al (2009): IDH1 and IDH2 mutations in gliomas. N Engl J Med 360:765-773.

Zankl H, Zang KD (1972): Cytological and cytogenetical studies on brain tumors. 4. Identification of the missing G chromosome in human meningiomas as no. 22 by fluorescence technique. Humangenetik 14:167-169.

Zec N, De Girolami U, Schofield DE, Scott RM, Anthony DC (1996): Giant cell ependymoma of the filum terminale. A report of two cases. Am J Surg Pathol 20:1091-1101.

Paper I

Hanne-Sofie S. Dahlback, Petter Brandal, Torstein R. Meling, Ludmila Gorunova, David Scheie, and Sverre Heim

Genomic Aberrations in 80 Cases of Primary Glioblastoma Multiforme: Pathogenetic Heterogeneity and Putative Cytogenetic Pathways.

Genes, Chromosomes & Cancer. 2009. Oct;48(10):908-24

Paper II

Hanne-Sofie S. Dahlback, Ludmila Gorunova, Petter Brandal, David Scheie, Eirik Helseth, Torstein R. Meling, and Sverre Heim

Genomic Aberrations in Diffuse Low-grade Gliomas.

Genes, Chromosomes & Cancer. 2011. Jun;50(6):409-20

Paper III

Hanne-Sofie S. Dahlback, Ludmila Gorunova, Francesca Micci, David Scheie, Petter Brandal, Torstein R. Meling, and Sverre Heim

Molecular Cytogenetic Analysis of a Gliosarcoma with Osseous Metaplasia

Gytogenetic and Genome Research. 2011;134(2):88-95

Paper IV

Hanne-Sofie S. Dahlback, Petter Brandal, Bård K. Krossnes, Radek Fric, Torstein R. Meling, Leonardo A. Meza-Zepeda, Håvard E. Danielsen, and Sverre Heim

Multiple chromosomal monosomies are characteristic of giant cell ependymoma

Human Pathology, in press

Case study

Multiple chromosomal monosomies are characteristic of giant cell ependymoma

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Summary Giant cell ependymoma (GCE), a rare ependymoma subtype, was recently recognised as a separate diagnostic entity with variations both in malignant potential and course of disease. We analysed the first supratentorial GCE using G-band karyotyping, DNA ploidy analysis, and array comparative genomic hybridisation. The tumour was hypodiploid and the karyotype showed multiple monosomies. This novel cytogenetic pattern seems specific for GCE as the only previous cytogenetic analysis of a GCE found similar monosomies. We were also able to analyse cytogenetically the subsequent recurrent tumour, phenotypically an anaplastic ependymoma, allowing a first insight into the genetic events involved in disease progression.

1. Introduction

Ependymomas are primary tumours of the central nervous system, presumably derived from cells lining the ventricles and the central canal of the spinal cord. They account for 2% to 9% of all neuroepithelial tumours and show remarkable histopathological heterogeneity [1]. The World Health Organisation currently recognises 4 main groups of ependymal tumours: subependymoma, myxopapillary ependymoma, ependymoma (including cellular, papillary, clear cell, and tanycytic subtypes), and anaplastic ependymoma [1]. In 1996, giant cell ependymoma (GCE) was first proposed as a separate morphological subtype of ependymoma of the filum terminale with a likely favourable outcome [2]. Since then, more cases have been described, both in relation to the spinal cord and the brain [3]. The distinguishing pathogenetic and other biological properties of these tumours remain unclear [4-6]. Of the now altogether 13 reported GCE, only 1 has been described cytogenetically [2]. In this study, the use of G-band karyotyping, DNA ploidy analysis and array comparative genomic hybridisation (aCGH) revealed a cytogenetic pattern likely to be characteristic of GCE.

2. Case report

A 38-year-old man presented with a grand-mal seizure and subsequent 2-week history of postictal speech disturbance not responding to steroids. On admission, he had a mild degree of predominantly receptive dysphasia. There were no other neurological symptoms or signs. Magnetic resonance imaging (MRI) revealed a 3.5 x 2 cm expansive, contrast-enhancing mass with evidence of a haemorrhagic component posteriorly in the left temporal lobe. Functional MRI sequences confirmed the presence of a tumour possibly involving Wernicke's area. The tumour was macroscopically totally removed and the histopathology suggested a supratentorial giant cell ependymoma of unknown malignant potential. The

postoperative course was uneventful and the patient showed immediate improvement of speech. No adjuvant therapy was administered, but regular imaging was arranged as follow-up.

A local recurrence was revealed by MRI 18 months after the initial surgery, despite the patient being asymptomatic. The recurrent tumour was macroscopically completely resected and classified as an anaplastic ependymoma. Postoperatively, the patient did well and was treated with radiotherapy (1.8 Gy x 33 to 59.4Gy) given to the postoperative surgical cavity plus a margin of 20 mm. At the latest follow-up (6 months after surgery), there was no sign of recurrence and the patient was clinically doing well.

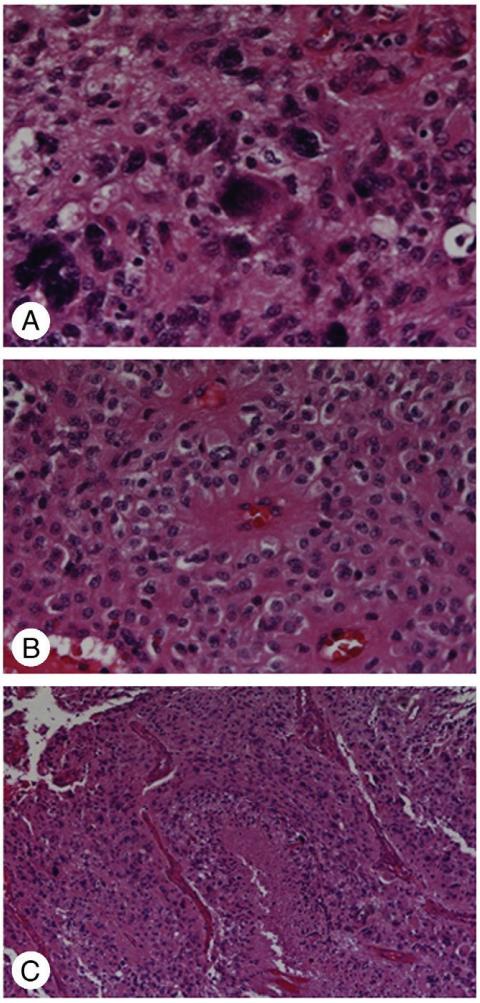

Fig. 1 Pathological features of the 2 ependymomas: In some areas of the initial tumour **(A)**, cells were pleomorphic with mono- and multinucleated tumour giant cells. In other areas, perivascular pseudorosettes and relatively monomorphic tumour cells were found **(B)**. In the recurrent tumour **(C)**, multiple serpentine pseudopalisading necroses and pleomorphic tumour cells reminiscent of glioblastoma were seen.

2.1. Pathological findings

Samples of tumour tissue were fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin (H&E). Immunohistochemistry was performed with standard laboratory detection systems.

The initial tumour was a moderately cellular glial tumour. In some

regions, it was composed of markedly pleomorphic cells, including multinucleated giant cells (Fig. 1A). There were frequent mitotic figures (6 mitoses per 10 high-power fields), including abnormal ones. In other regions, perivascular pseudorosettes were seen, and tumour cells had fairly round monomorphic nuclei (Fig. 1B). No microvascular proliferation was noted. There was evidence of haemorrhage, but no overt necrosis. Most of the neoplastic cells were positive for GFAP, S-100, and vimentin. Approximately 10% of the malignant cells were Ki-67-positive. These features led to a diagnosis of supratentorial giant cell ependymoma of unknown malignant potential.

The recurrent tumour was composed of highly pleomorphic cells with countless mononuclear and multinucleated tumour giant cells. There were numerous mitoses, microvascular proliferation, and large areas of geographical and pseudopalisading necrosis (Fig. 1C). Approximately 20% of the tumour cells were Ki-67-positive. Regions containing pseudorosettes typical for ependymoma were not found. This tumour was classified as an anaplastic ependymoma.

2.2. G-banding and karyotyping

Tumour material from the first and second operation was processed for cytogenetic analysis using standard methods for short-term culture. After an average of 12 days, the cultures were harvested as described by Mandahl et al.[7]. Chromosome preparations were G-banded using Wright stain and karyotyped according to the recommendations of the ISCN [8]. The G-banded karyotype from the initial tumour specimen was hypodiploid with several monosomies (Fig. 2A), i.e. 34~36,XY,-3,-6,-11,-12,-13,-14,-15,-17,-18,-22[cp6]/46XY[10]. In the recurrent tumour sample, only three metaphases were available for analysis and these showed a similar hypodiploid karyotype, i.e. 33~36,XY,-3,-6,-10,-11,-12,-13,-14,-18,-22[cp3] (Fig. 2B).

2.3. DNA ploidy analysis

DNA image cytometry was performed on formalin-fixed, paraffin-embedded tissue from both tumour samples. The procedures for measurement of DNA content and the criteria for ploidy classification have been described [9]. In the first sample, 1281 tumour nuclei were compared with 24 lymphocytes as internal controls. In the recurrent tumour sample, 221 nuclei were compared with 5 lymphocytes. Despite the scarcity of cells, the histograms showed both samples to be aneuploid (Fig. 2C and D). The majority clone in both samples was hypodiploid, but the recurrent sample displayed an additional triploid clone.

2.4. Genome-wide array comparative genomic hybridisation (aCGH)

Genomic DNA was extracted from fresh-frozen tissue using the MagAttract DNA Mini M48 kit (Qiagen Inc., Valencia, CA, USA), labelled according to the manufacturer's instructions, and aCGH was performed using the Agilent SurePrint G3 Human CGH Microarray 4x180K arrays (Agilent Technologies Inc., Palo Alto, California, USA). Data was analysed using Agilent Feature Extraction Software (version 10.5.1.1) and Genomic Workbench software (Agilent Technologies Inc.) as previously described [10].

The original data of the aCGH hybridization can be found in the public database Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE23065). Examination of the initial tumour revealed 9 copy number aberrations (CNAs) with gain of material at 8p, loss of material at 1p, 3q, 7q, 12p, 16q, and 22q, and homozygous deletions at 1q and 22q. No high level amplifications were seen. In the recurrent tumour, 28 CNAs with gain of material at 1q, 2q, 3q, 4p, 5p, 5q, 8q, 9p, 9q, 11q, 13q, 20p, and 21q, and amplifications at 20q and 21q were found. Loss of material was observed at 2q, 4p, 7q, 11q, 14q, 19p, 21p, and 21q, whereas homozygous deletions were seen

only at 8p. No aberrations were shared by both samples.

3. Discussion

The karyotypic features of the GCE presented here and the only other G-banded tumour [2] were markedly hypodiploid with multiple monosomies leading to a stemline midway between 2n and n, and both displayed loss of chromosomes 14 and 22. In this case, the presence of a dominant hypodiploid clone could be confirmed using DNA ploidy analysis. This generally uncommon genetic pattern may, despite the scant data available, be characteristic of this tumour type. The pathogenetic role of these monosomies warrants further investigation, although the classical hypothesis is that loss of tumour suppressor genes on the lost chromosome(s) is the important result.

In general, genomic events involved in ependymoma pathogenesis remain elusive. The 107 ependymomas cytogenetically characterised to date have been near-diploid to near-triploid with loss of chromosome 22 as by far the most common aberration, followed by +7, +12 and/or -17 [11]. Hence, the markedly hypodiploid tumour stemline differentiates GCE from more classical ependymomas, but it is worthy of note that both show loss of chromosome 22. These pathogenetic considerations thus support the histopathological notion that GCE have features resembling ependymomas, while at the same time representing an entity of its own.

From a pathological point of view, GCE can be difficult to differentiate from the much more common glioblastomas [1] that most commonly display near-diploid to hyper-diploid karyotypes with gain of chromosome 7 and loss of chromosomes 10, 13, and 22 as the most frequent aberrations seen by both G-banding and CGH [12]. As GCEs seem to have a very clear-cut cytogenetic pattern, it could be argued that cytogenetic analysis should be included in routine diagnostics.

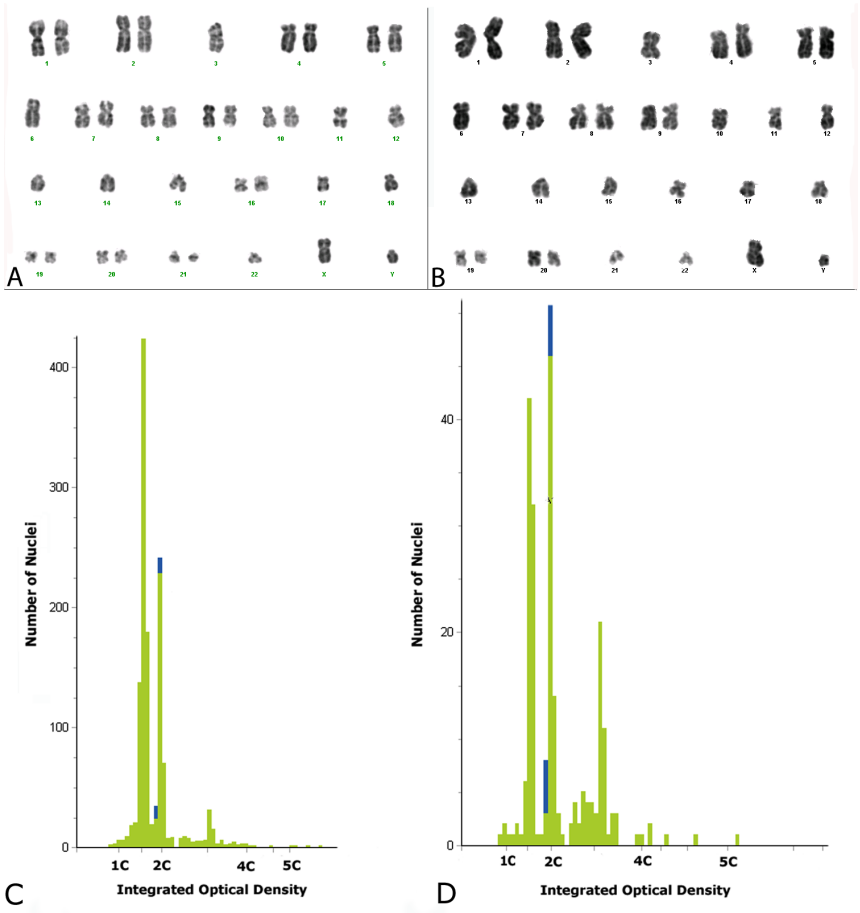

Fig. 2 A, G-banded karyogram of the initial tumour, the giant cell ependymoma, with multiple numerical aberrations giving the karyotype 36,XY,-3,-6,-11,-12,-13,-14,-15,-17,-18,-22. B, G-banded karyogram of the recurrent tumour, the anaplastic ependymoma, with a very similar karyotype 33,XY,-3,-6,-10,-11,-12,-13,-14,-15,-16,-17,-18,-21,-22. C, Ploidy distribution of the giant cell ependymoma. D, Ploidy distribution of the recurrent anaplastic ependymoma. The green area shows the distribution of the tumour cells, whereas the blue areas show the distribution of the internal control cells. The majority of the tumour cells were hypodiploid in both samples, though an additional triploid clone was evident in the recurrent sample only.

The literature has suggested intracerebral GCEs to be more aggressive than those related to the spine [4-6, 13-15]. The remarkable similarity between the cytogenetic profiles of this supratentorial tumour and that of the filum terminale [2] suggests no clear pathogenetic backing for this finding.

In this patient, material from both the initial tumour and the subsequent

relapse was available for analysis, providing an opportunity to assess the genetic events involved in tumour progression. On the chromosomal level, both tumour samples were hypodiploid with multiple monosomies; however, loss of chromosome 10 first occurred in the recurrence and therefore was associated with tumour progression. Little is known about anaplastic ependymomas specifically, so we cannot know to what extent, if at all, anaplastic ependymomas differ cytogenetically from GCEs. Although monosomy 10 was found in the low-grade GCE reported by Zec et al. [2], our findings suggest that loss of this chromosome may be a feature of more aggressive ependymomas, as it is in other gliomas [12].

It was of some concern to us that there was only minimal agreement between findings by G-band karyotyping and aCGH, limited to loss of material from 3q and 22q in the first sample and 11q and 14q in the second sample. In particular, aCGH failed to confirm monosomies detected by karyotyping and instead detected large areas of apparently gained chromosomal material. This discrepancy is most likely attributable to difficulties inherent in the CGH approach when genomic material is lost or gained to the extent that the test sample stemline is (almost) exactly midway between two ploidy levels [16]. Karyotyping, which relies on actually seeing the chromosome complement of individual cells, has no similar problem and is therefore less prone to producing such artefacts. In this setting, DNA ploidy assessment proved useful as it confirmed the karyotypic findings. This also illustrates the usefulness of applying multiple investigative methods to the study of tumour genomes, and that one should be careful interpreting CGH, both conventional and array-based, when used as a stand-alone technique to study the acquired chromosomal abnormalities of neoplastic cells.

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References

[1] Louis D, Ohgaki H, Wiestler O, Cavenee W, Eds. WHO Classification of Tumours of the Central Nervous System, 4th Edition. Ogdensburg: Renouf Publishing Co. Ltd., 2007.

[2] Zec N, De Girolami U, Schofield DE, Scott RM, Anthony DC. Giant cell ependymoma of the filum terminale. A report of two cases. Am J Surg Pathol 1996; 20, 1091-1101.

[3] Barbagallo GM, Caltabiano R, Parisi G, Albanese V, Lanzafame S. Giant cell ependymoma of the cervical spinal cord: case report and review of the literature. Eur Spine J 2009; 18 Suppl 2, 186-190.

[4] Brown DF, Chason DP, Schwartz LF, Coimbra CP, Rushing EJ. Supratentorial giant cell ependymoma: a case report. Mod Pathol 1998; 11, 398-403.

[5] Jeon YK, Jung HW, Park SH. Infratentorial giant cell ependymoma: a rare variant of ependymoma. Pathol Res Pract 2004; 200, 717-725.

[6] Pimentel J, Kepes JJ, Moura Nunes JF, Bentes C, Miguens J, Antunes JL. Supratentorial giant cell ependymoma. Clin Neuropathol 2001; 20, 31-37.

[7] Mandahl N. Methods in solid tumors cytogenetics. In: Rooney DE, Czepulkowski BH, editors. Human Cytogenetics—A Practical Approach, Vol. II: Malignancy and Acquired Abnormalities.: Oxford: IRL Press, 1992.

[8] ISCN. An International System for Human Cytogenetic Nomenclature (2009). Basel: S. Karger, 2009.

[9] Kristensen GB, Kildal W, Abeler VM, Kaern J, Vergote I, Trope CG, Danielsen HE. Large-scale genomic instability predicts long-term outcome for women with invasive stage I ovarian cancer. Ann Oncol 2003; 14, 1494-1500.

[10] Dahlback HS, Gorunova L, Micci F, Scheie D, Brandal P, Meling TR, Heim S. Molecular Cytogenetic Analysis of a Gliosarcoma with Osseous Metaplasia. Cytogenetic and Genome Research (in press) 2010.

[11] Mitelman F, Johansson B, Mertens F, (Eds.). Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer. Available at http://cgap.nci.nih.gov/Chromosomes/Mitelman. Accessed April 2011
2011.

[12] Dahlback HS, Brandal P, Meling TR, Gorunova L, Scheie D, Heim S. Genomic aberrations in 80 cases of primary glioblastoma multiforme: Pathogenetic heterogeneity and putative cytogenetic pathways. Genes Chromosomes Cancer 2009; 48, 908-924.

[13] Adamek D, Dec M, Sobol G, Urbanowicz B, Jaworski M. Giant cell ependymoma: a case report. Clin Neurol Neurosurg 2008; 110, 176-181.

[14] Cooper PB, Katus M, Moores L, Geyer D, Smirniotopoulos JG, Sandberg GD, Rushing EJ. Rare giant cell ependymoma in an octogenarian. Case report and review of the literature. J Neurosurg 2006; 105, 908-911.

[15] Moritani S, Kushima R, Bamba M, Kobayashi TK, Oka H, Fujimoto M, Hattori T, Okabe H. Highly anaplastic extraventricular ependymoma arising in an adult, mimicking metastatic adenocarcinoma with heavy stromal

inflammation and emperiporesis. Pathol Int 2003; 53, 539-546.

[16] Kallioniemi OP, Kallioniemi A, Piper J, Isola J, Waldman FM, Gray JW, Pinkel D. Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Genes Chromosomes Cancer 1994; 10, 231-243.

Paper V

Hanne-Sofie S. Dahlback, Petter Brandal, Ludmila Gorunova, Eva Widing, Torstein R. Meling, Bård Kronen Krossnes, and Sverre Heim

Genomic Aberrations in Paediatric Gliomas and Embryonal Tumours

Genes, Chromosomes & Cancer, in press

Genomic Aberrations in Paediatric Gliomas and Embryonal Tumours

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The pathogenesis of paediatric central nervous system tumours is poorly understood. To increase knowledge about the genetic mechanisms underlying these tumours, we performed genome-wide screening of 17 paediatric gliomas and embryonal tumours combining G-band karyotyping and array comparative genomic hybridisation (aCGH). G-banding revealed abnormal karyotypes in 56% of tumour samples (9 of 16; one failed in culture), whereas aCGH found copy number aberrations in all 13 tumours examined. Pilocytic astrocytomas (n=3) showed normal karyotypes or non-recurrent translocations by karyotyping but the well-established recurrent gain of 7q34 and 19p13.3 by aCGH. Our series included one anaplastic oligoastrocytoma, a tumour type not previously characterised genomically in children, and one anaplastic neuroepithelial tumour (probably an oligoastrocytoma); both showed loss of chromosome 14 by G-banding and structural aberrations of 6q and loss of 14q, 17p, and 22q by aCGH. Three of five supratentorial primitive neuroectodermal tumours showed aberrant karyotypes; two were near-diploid with mainly

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structural changes and one was near-triploid with several trisomies. aCGH confirmed these findings and revealed additional recurrent gains of 1q21-44 and losses of 3p21, 3q26, and 8p23. We describe cytogenetically for the first time a cribriform neuroepithelial tumour, a recently identified variant of atypical teratoid/rhabdoid tumour with a favourable prognosis, which showed loss of 1p33, 4q13.2, 10p12.31, 10q11.22, and 22q by aCGH. This study indicates the existence of distinct cytogenetic patterns in paediatric gliomas and embryonal tumours; however, further studies of these rare tumours using a multi-modal approach are required before their true genomic aberration pattern can be finally established.

INTRODUCTION

In developed countries, tumours of the central nervous system (CNS) represent the largest group of solid cancers (39-51%) in childhood (Parkin et al., 1999; Stiller et al., 2006). Despite steady improvements in prognosis over the past decades, CNS tumours remain the leading cause of cancer mortality in children (U.S. Cancer Statistics Working Group, 2010).

Paediatric CNS malignancies constitute a heterogeneous group of morphological entities that do not replicate the distribution pattern seen in adult patients (Louis et al., 2007). Pilocytic astrocytomas (PAs) are by far the most common tumour entity (24%) followed by medulloblastomas (16%) and ependymomas (10%) (Rickert and Paulus, 2001).

Although more than 450 paediatric CNS tumours have been characterised cytogenetically by G-banding (Mitelman et al., 2011), no clear abberation pattern has yet been demonstrated in the majority of tumour entities. Medulloblastomas are exceptions to this inasmuch as they show i(17q) in 30-40% of cases (Bigner et al., 1988; Griffin et al., 1988), and that gain of 6q and 17q has been suggested to define prognostically adverse groups of patients

(Bigner et al., 1997; Russo et al., 1999; Rossi et al., 2006; Pfister et al., 2009). Similarly, although PAs have mostly shown normal karyotypes or non-recurrent findings by karyotyping (Agamanolis and Malone 1995; Bigner et al., 1997), array comparative genomic analysis (aCGH) has revealed gain of 7q34 in a majority of cerebellar tumours (Deshmukh et al., 2008; Jones et al., 2008).

In an attempt to add to the pathogenetic understanding of some of the common as well as more rare CNS tumours in children, we analysed 17 paediatric CNS tumours by two complementary screening techniques: karyotyping and aCGH. To the best of our knowledge, these are the first paediatric CNS tumours to be examined using this combined approach.

MATERIALS AND METHODS

Tumour Samples

Seventeen tumour samples from 17 children were included (Table 1). Samples were collected as a prospective series between January 2005 and July 2009 at the Department of Neurosurgery, Rikshospitalet, Oslo, Norway. Patients eligible for participation were aged 0-15 years (mean age: 5 years; range: 1-12 years) at the time of surgery. Ten patients were female and seven were male.

All diagnoses were re-evaluated by a neuropathologist and are based on the 4th edition of the World Health Organisation classification (2007). Tumours were considered primary if the diagnosis was made at the time of the first biopsy (16 tumours) and recurrent (one; # 10) if previously biopsied. This latter patient had received adjuvant chemotherapy (vincristine) after the first surgery, i.e., one year prior to the surgery for the recurrent tumour.

All tumours were apparently sporadic. Thirteen tumours were supratentorial and four were infratentorial. Of the ten gliomas, there were three PAs, two low-grade gliomas (one of which was

a likely PA, the other an unclassifiable low-grade astrocytoma), two glioblastomas, one anaplastic oligoastrocytoma, one anaplastic neuroepithelial tumour (most likely an oligoastrocytoma), and one low-grade neuroepithelial tumour (dysembryoplastic neuroepithelial tumour or low-grade astrocytoma). Seven embryonal tumours were also included: one medulloblastoma (classical type), five supratentorial central nervous system primitive neuroectodermal tumours (CNS-PNET), and one cribriform neuroepithelial tumour (CRINET). The study was approved by the Regional Ethics Committee, and samples were retrieved from a biobank established according to national ethical guidelines. Informed consent was obtained from all patients and/or parents/guardians.

G-banding and Karyotyping

Tumour material was disaggregated mechanically and enzymatically using collagenase type II (Worthington, Freehold, NJ). The resulting cells were seeded into tissue culture flasks and cultured in Basal Iscove's medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, 1% non-essential amino acids, 1% ITS+ Premix (Becton Dickinson, Bedford, MA), 0.1% MITO+ Serum Extender (Becton Dickinson), and 0.1% hydrocortisone. After an average of 12 days, the cultures were harvested as described by Mandahl et al. (1992). Chromosome preparations were G-banded using Wright stain and karyotyped according to the ISCN (2009) guidelines. All cases were reviewed independently by two experienced cytogeneticists.

Genome-wide Array Comparative Genomic Hybridisation (aCGH)

Genomic DNA was extracted from fresh-frozen tissue using the MagAttract DNA Mini M48 kit (Qiagen Inc., Valencia, CA), labelled according to the manufacturer's instructions, and aCGH was performed using Agilent SurePrint G3

Human CGH Microarray 4 x 180K arrays (Agilent Technologies Inc., Palo Alto, CA). Based on the Quality Control (QC) metrics, all samples had a derivative log ratio (DLR) spread value of ≤0.2. Data were analysed using Agilent Feature Extraction Software (version 10.5.1.1) and the Genomic Workbench software (Agilent Technologies Inc.) with threshold settings and filters as previously described (Dahlback et al., 2011a). The individual profiles were visually checked. Annotations are based on human genome build 18.

RESULTS

The histopathological classification, karyotypes, and genomic imbalances of the 17 paediatric brain tumours are listed in Table 1. All samples were analysed by at least one method. Cell culturing and subsequent G-banding analysis was informative in 16 samples, nine (56%) of which showed an abnormal karyotype, whereas seven were normal. The remaining one sample failed in cell culture. The nine cases with chromosomal abnormalities included three CNS-PNETs, one PA, one low-grade astrocytoma, one low-grade glioma, one glioblastoma, one anaplastic oligoastrocytoma, and one anaplastic neuroepithelial tumour (probably an anaplastic oligoastrocytoma). Of the samples with abnormal karyotypes, all but one (# 14) had a near-diploid chromosomal constitution. The abnormal karyotypes were simple (≤ four aberrations per clone) in all three low-grade gliomas. The oligoastrocytoma, the neuroepithelial tumour, and the three embryonal tumours showed complex karyotypes with multiple numerical and structural changes, some of which could not be fully identified as they resulted in marker chromosomes and/or additional chromosomal material of unknown origin. No double minutes or ring chromosomes were observed.

Four cases could only be analysed by G-banding, another eight showed normal karyotypes but revealed copy

number alterations by aCGH, whereas the remaining five tumour samples showed aberrations by both techniques. aCGH was informative in all 13 cases where there was sufficient material for both tissue culture and DNA extraction. In most cases, aCGH verified the karyotypic findings but also revealed additional imbalances. The average copy number imbalances per tumour was 8.9 with gains (4.9) being more common than losses (4.0). The original data of the aCGH hybridisations can be found in the public database Gene Expression Omnibus (GEO) at http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE27671.

Of the six low-grade gliomas (#1-6), three displayed abnormal karyotypes with simple changes (≤ 4 aberrations per clone), none of which was recurrent. All three displayed at least one translocation. Two PAs (one supratentorial and one infratentorial) could be analysed by aCGH and both showed gain of 7q34 and 19p13.3. In addition, case 1 showed gain of 12p13.31 and 16q24.2-q24.3, whereas case 3 also showed gain of 13q21.31 by aCGH. The low-grade neuroepithelial tumour (#6) showed gain of 12p13.31 and 19p13.3-13.2, loss of 1q21.1, 8p23.1, and 11p11.2, and homozygous deletions of 4q13.2 and 8p11.23.

The high-grade gliomas included two glioblastomas (#7 and #8), one anaplastic oligoastrocytoma (#10), and an anaplastic neuroepithelial tumour that probably represented another anaplastic oligoastrocytoma (#9). Only one of the glioblastomas (#7) showed an abnormal karyotype by G-banding analysis with gain of chromosomes 7 and 12. By aCGH, this case revealed gain of material at 6q and 7q and loss of 8p, whereas a second glioblastoma (#8) showed copy number aberrations involving 10 chromosomes with gains of 5p, 7q, and 14q, losses of 2p, 4q, 5q, 10q, 13q, and 18q, and homozygous loss from Xp. The anaplastic oligoastrocytoma that had evolved from a previous low-grade glioma (#10) displayed

a complex abnormal karyotype with loss of chromosomes 6, 8, 9, 14, 19, and 22, structural aberrations involving chromosomes 4, 5, 6, 8, 11, and 20, and three marker chromosomes. aCGH verified copy number loss of or from chromosomes 4, 5, 6, 8, 9, 14, and 19, but also identified additional copy number losses of Xp, 15q, and 17p, homozygous loss from 22q, and gains of 4p, 9p, 12p, and 19q. G-banding analysis of the anaplastic neuroepithelial tumour (#9; Fig. 1) revealed a complex karyotype with gain of chromosomes 2 and 8, loss of chromosomes X, 10, 11, 14, 15, 16, and 17, structural rearrangements of 3p, 6p, 6q, 7p, 14p, and 18p, five markers, and a ring chromosome of unknown origin. aCGH verified gain of chromosomes 2 and 6 and loss of chromosomes X, 6, 10, 11, 14, 15, 16, and 17. It also revealed additional gains at 7p, 10q, 11q, 12p, 15q, 16q, 17p, 17q, and 21q, loss from 18q, and homozygous loss from 22q.

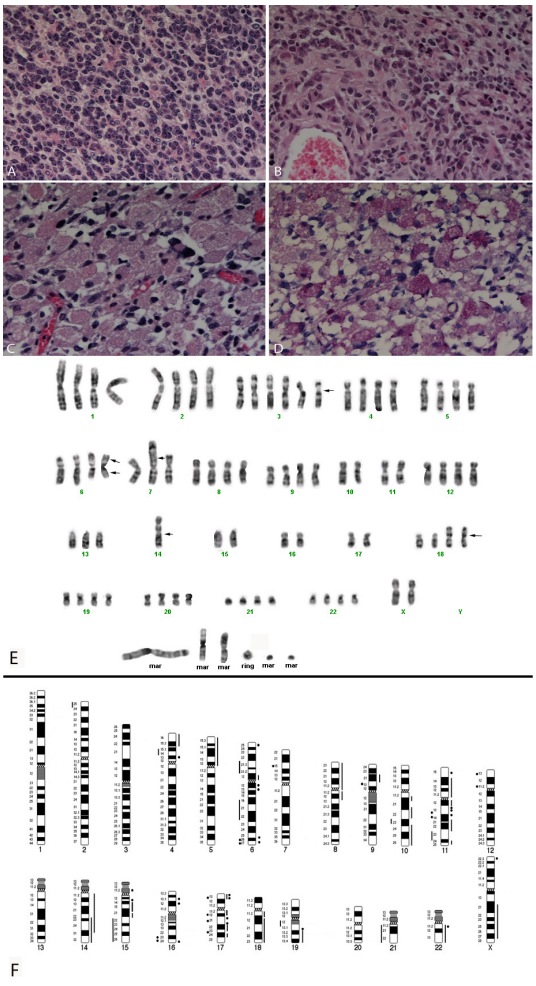

Figure 1. Histopathological and cytogenetic features of case 9 **(A- E)**. The formalin-fixed biopsy specimen showed a cellular neuroepithelial tumour, most likely an anaplastic oligoastrocytoma. **(A)** The majority of tumour cells had relatively round monomorphic nuclei surrounded by a perinuclear halo. **(B)** In small areas, however, the tumour showed astrocytic differentiation. Granular eosinophilic astrocytes **(C)** with Pas-positive cytoplasm **(D)** were also seen. Frequent mitoses were present. No necrosis or microvascular proliferation was found. **(E)** G-banding karyogram of a tetraploid subclone 83,XX,-X,-X,+add(3)(p11)x2,der(6)add(6)(p21)del(6)(q21)x2,-7, add(7)(p22),-10,-10,-11,-11,-13,-14,-14,-14,add(14)(p11), -15,-15,-16,-16,-17,17,add(18)(p11)x2,+r,+5mar (arrows indicate breakpoints). **(F)** Ideogram illustrating the distribution of genomic imbalances observed by aCGH in cases 9 and 10. Gains are seen to the right of each chromosome, whereas losses are seen to the left. Losses of 14q, 17p, and 22q were common to both tumours by aCGH.

The medulloblastoma, one CNS-PNET, and the cribriform neuroectodermal tumour had normal karyotypes by G-banding. aCGH analysis of the medulloblastoma (#11) revealed multiple aberrations with gain of X, 1, 2, 3, 6, 7, 9p, 12p, 14, 15q, 17, 18, 19, 20q, and 22q and loss of 10p, 10q, 11, 16p, 16q, and 21. Three CNS-PNETs displayed near-diploid or near-triploid complex karyotypes with numerical as well as structural aberrations. These karyotypes consisted of only one abnormal clone per case and recurrent changes included gain of chromosome 1 (2 cases), loss of the X chromosome (2 cases), and structural aberrations involving chromosome 21 in all three cases. One CNS-PNET (#14) was near-triploid with multiple numerical aberrations and structural rearrangements of chromosomes 12, 18, and 21. Another was diploid with addition of unknown material to chromosomes 14 and 21, a deleted chromosome 22, as well as a marker chromosome. The third CNS-PNET showed gain of chromosome 1 as well as an unbalanced translocation involving chromosomes 1 and 3, addition of unknown material to chromosome 11, and an i(21q). Four of five CNS-PNETs included in this series could be analysed by aCGH and showed from three to 14 copy number aberrations (Table 1), with losses (5.3) being slightly more common than gains (5.0). Recurrent copy number gains were found at 1q21-44 (#14 and 16) while losses were recurrent at 3p21 (#14 and 16), 3q29 (#13 and 14), and 8p23.1 (#12 and 13).

Case 17 of this series was a two-year-old girl who presented with a large, contrast-enhancing multifocal tumour of the third ventricle, the largest focus measuring 5 cm in all directions. The formalin-fixed biopsy specimen showed regions of epithelial differentiation in a cribriform pattern (Fig. 2A). Other areas showed small cells with cytoplasmic prosesses in a reticular pattern (Fig. 2B). Loss of nuclear INI-1 protein expression

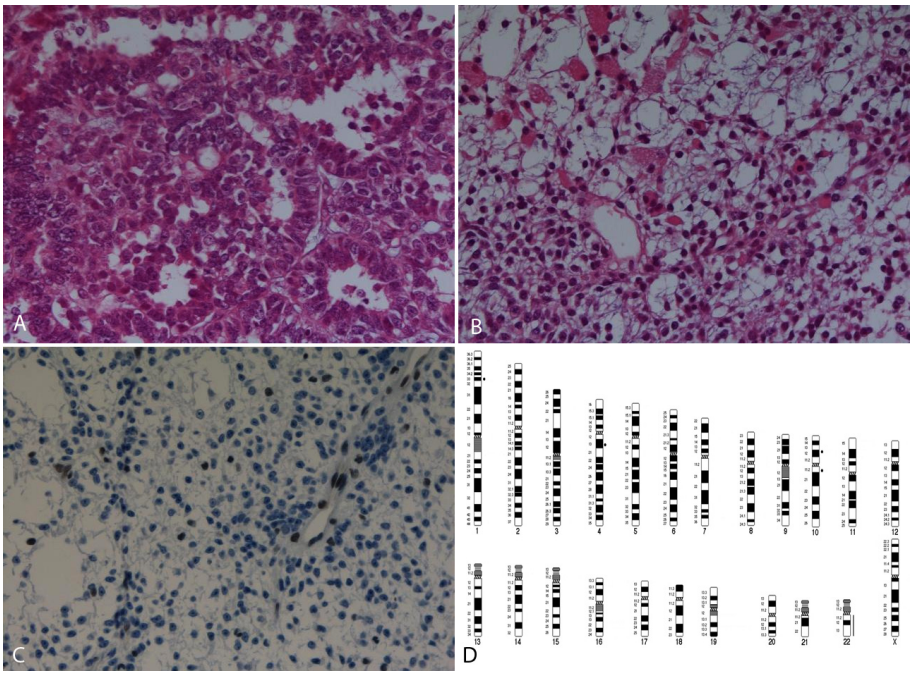

Figure 2. Histopathological and genomic features of case 17. In some areas, the tumour showed epithelial differentiation in a cribriform pattern **(A)**, whereas other areas showed small cells with cytoplasmic prosesses in a reticular pattern **(B)**. The cells were negative for INI1 protein expression **(C)**. This features were highly suggestive of a cribriform neuroectodermal tumour (CRINET). **(D)** Ideogram of aCGH findings. Losses were seen at 1p33, 4q13.2, 10p12.31, 10q11.22, and 22q11.1-13.33.

(BAF47; 1:150, BD Biosciences, San Jose, CA) of tumour cells was observed (Fig. 2C). Widespread positivity was noted for EMA, cytokeratin, GFAP, vimentin, and CD99. Some cells were positive for synaptophysin. The findings were suggestive of AT/RT and the patient was treated accordingly. The histopathological diagnosis was later reevaluated at the University of Texas MD Anderson Cancer Centre, Houston, TX when the patient was referred for proton radiation therapy. It was then thought to represent a cribriform neuroectodermal tumour (CRINET). At the time of writing (18 months after surgery), the patient is alive and in remission. G-banding revealed (# 17; Fig. 2) a normal karyotype, but subsequent aCGH analysis showed losses of 1p, 10p, 10q, and 22q and homozygous loss of 4q.

DISCUSSION

The present series of paediatric CNS tumours illustrates the morphological heterogeneity of these neoplasms, but also that the spectrum of pathological entities differs from the one observed in adults where gliomas, meningiomas, and metastases from extracranial malignancies predominate (Louis et al., 2007). Furthermore, of the 17 examined tumours, one third could not readily be grouped in accordance with the current WHO classification (2007), emphasising the diagnostic challenges neuropathologists and clinicians face. This accentuates the call for pathogenetic classifications, an alternative grouping of tumours that could also hopefully provide information about prognosis as well as key insights into molecular disease mechanisms that may eventually be translated into more effective and individualised therapies (Brandal et al., 2010).

To the best of our knowledge, this is the first series of paediatric CNS tumours to be analysed by both G-banding and aCGH. G-banding is able to visualise small clones, identifies balanced aberrations such as translocations, inversions, and insertions, and detects intratumour heterogeneity, admittedly all at relatively low resolution (5-10Mb). CGH, on the other hand, offers genome screening at a higher resolution level (here approximately 13kb), is independent of cell culture, but is unable to detect the presence of balanced aberrations. The combined use of the two screening techniques thus offers a uniquely unbiased and complementary overview of the whole tumour genome. In this series, all samples could be analysed and were informative by at least one method. Five tumour samples revealed genomic aberrations by both techniques, allowing direct comparison, and the findings were in good overall agreement (Table 1). aCGH provided additional information on genomic copy number changes in all cases where it could be performed, most likely due to the higher

resolution level this technique offers. Additionally, genomic imbalances were seen by aCGH in seven tumour samples with normal karyotypes, probably because the cells of the neoplastic parenchyma could not be induced to divide in vitro in these cases (Table 1).

No tumour samples showed isolated loss of a sex chromosome. Previous cytogenetic analyses of adult CNS tumours and normal brain tissue have frequently shown loss of the Y chromosome as the sole finding (Bigner et al., 1988; Jenkins et al., 1989; Lindström et al., 1991). The rarity of such findings in childhood CNS tumours suggests that they may be related to ageing (Bigner et al., 1997).

In agreement with findings in previous studies (Neumann et al., 1993; Agamanolis and Malone 1995; Roberts et al., 2001; Orr et al., 2002), the PAs and low-grade astrocytomas analysed by G-banding revealed either normal karyotypes or simple aberrations with no recurrent features. The reasons are probably at least two-fold. It was noted that low-grade samples submitted for cytogenetic analysis were very small, probably because the extent of surgical resection is kept to a minimum in order to spare the surrounding developing brain. Secondly, but to some extent related to the point above, the proportion of abnormal cells may have been too low to achieve growth of neoplastic cells in culture. Two pilocytic astrocytomas could be analysed using aCGH and both showed gain of 7q34 and 19p13.3. Gain of 7q34 has been reported in 46-80% of PAs and results in activation of the oncogene *BRAF* which may become a future diagnostic and therapeutic target in these tumours (Deshmukh et al., 2008; Jones et al., 2008; Pfister et al., 2008; Jacob et al., 2009). As it was present in both infratentorial and supratentorial PAs, the gain does not seem to be specific for tumours of a given anatomic location. Gain of 19p13.3 was also found in both PAs (#1 and 3) and the low-grade neuroepithelial

tumour (#6). This is in accordance with findings in previous studies (Pfister et al., 2009). The molecular consequences of the events at 19p13.3 in PAs are unknown but could conceivably, like the *BRAF* activation in 7q34, be of future therapeutic interest. The low-grade neuroepithelial tumour (#6) was difficult to fully classify and could according to the histopathological assessment represent either a low-grade glioma or a dysembryoplastic neuroepithelial tumour (DNET). Although little is known about the cytogenetic features that characterise DNETs (Fujisawa et al., 2002), gains of 12p13.31 and 19p13.3 were shared by both the neuroepithelial tumour and PAs in our series, something that perhaps argues against the DNET diagnosis; however, more cytogenetic data are certainly needed before the karyotypic information can be brought to bear on the differential diagnosis between these two rare subsets of paediatric gliomas in any reliable manner.

Glioblastoma multiforme (GBM) accounts for approximately 7% of all childhood malignancies (Rickert and Paulus, 2001). The genomic pattern of adult GBM has been extensively studied with 513 tumours being analysed by G-banding so far (Mitelman et al., 2011), whereas a mere 32 paediatric GBM (≤5 in each series) have been karyotypically described and have largely failed to reproduce the typical adult pattern of aberrations that includes +7/-10, -13, and -22 (Dahlback et al., 2009). Whereas some GBMs of childhood have had near-diploid karyotypes with only a few chromosomal abnormalities, others had a near-triploid/near-tetraploid chromosome number with much more complex aberrations (Chadduck et al., 1991; Karnes et al., 1992; Sawyer et al., 1992; Vagner-Capodano et al., 1992; Neumann et al., 1993; Fujii et al., 1994; Agamanolis and Malone 1995; Sainati et al., 1996; Bigner et al., 1997; Roberts et al., 2001). Conventional CGH studies limited to 23

paediatric GBMs (Rickert et al., 2001; Warr et al., 2001) have detected gains of or from 1q, 2q, 3q, and 17q and losses of or from 17p and 13q as recurrent copy number changes. In the present series, only one (#7) of the two GBMs showed karyotypic changes with gain of chromosomes 7 and 12 by G-banding and this tumour displayed gains of chromosome material from 6q and 7q and loss from 8p by aCGH. The other (#8) harboured ten copy number aberrations including gain of chromosome material from 7q and loss of 10q by aCGH despite a normal karyotype. Although this is compatible with the existence of two subgroups of childhood GBM based on complexity of acquired aberrations (Bax et al., 2010), existing data is very scant and more multicentre studies are urgently needed to determine to what extent and how the pattern of genomic aberrations is non-random in these tumours.

Oligoastrocytomas are very rare in children but their precise incidence is difficult to determine due to a lack of generally accepted classification criteria (Louis et al., 2007). An incidence of 6% of oligodendroglial tumours in children aged 0-14 years has been reported; however, this includes oligodendrogliomas as well as oligoastrocytomas (Houben et al., 2006). Knowledge about the acquired genomic changes of oligoastrocytomas is limited to CGH (conventional and array) analyses of adult tumours that have revealed frequent losses of 1p/19q, 4q, 9p, 10q, 13q, and 14q as well as gains of 7p, 8q, 10p, and 11q (Kros et al., 1999; Kitange et al., 2005; Dahlback et al., 2011). To the best of our knowledge, no oligoastrocytomas in children have been characterised genomically. The present series included one anaplastic oligoastrocytoma (#10; this was a recurrent anaplastic oligoastrocytoma and the patient had been treated with vincristine one year prior to removal of the recurrence) and an anaplastic neuroepithelial tumour. The latter most probably represented another

anaplastic oligoastrocytoma (#9; Fig. 1) illustrating how difficult it is to firmly decide on a diagnosis of oligoastrocytoma; in this case the alternative CNS-PNET with extensive glial differentiation could not be excluded. Genomically, this tumour showed multiple complex aberrations both by G-banding (Fig. 1E) and aCGH (Fig. 1F), and the finding of trisomies for chromosomes 2 and 8 could point towards a diagnosis of CNS-PNET. On the other hand, none of the other G-banding or aCGH findings fit with those previously described in CNS-PNET (see below). It may be worthy of note that both the anaplastic oligoastrocytoma (#10) and the anaplastic neuroepithelial tumour showed loss of chromosome 14 and structural aberrations of chromosome 6 by G-banding, admittedly as part of complex karyotypes, as well as losses of 14q and 17p and homozygous loss from 22q when analysed by aCGH. The latter argues in favour of a diagnosis of anaplastic oligoastrocytoma also for case 9, but certainly more of these tumours must be characterised genomically before the diagnostic value of cytogenetics in this context can be determined and any non-random role of aberrations of chromosomes 6, 14, 17, and/or 22 is established.

Interestingly, whole-genome screening failed to identify the well-established complete 1p/19q-codeletion that has been reported to be present in 30-50% of adult oligoastrocytomas (Louis et al., 2007). These findings are supported by the fact that such aberrations have not been detected in paediatric oligodendroglial tumours when analysed by fluorescence in situ hybridisation using single probes per chromosome nor by loss of heterozygosity studies (Pollack et al., 2003; Raghavan et al., 2003; Kreiger et al., 2005). Thus, it seems highly likely that the genetic pathways involved in the pathogenesis of paediatric oligodendroglial tumours are different from those of their adult counterpart.

Medulloblastomas and CNS-PNETs are embryonal tumours that occur most commonly in the cerebellum and in supratentorial sites, respectively. The former account for 25-30% of childhood CNS tumours and the aCGH findings of the presently included case are in accordance with those of previous studies with multiple copy number aberrations including gain of 17q (Rossi et al., 2006). CNS-PNETs, on the other hand, make up a mere 1.9-2.5% of paediatric CNS tumours (Gaffney et al., 1985; Pollack 1994; Rickert and Paulus 2001). These tumours carry a particularly poor prognosis compared with the morphologically similar medulloblastomas and usually fail to respond to standard therapy, especially in childhood when cranial irradiation is best avoided because of the pronounced neurocognitive deficits that usually follow (Timmermann et al., 2002; Larouche et al., 2006). Due to the rarity of CNS-PNETs, few such tumours have been investigated cytogenetically.

In this series, three cases showed abnormal karyotypes. Two were near-diploid with mainly structural changes whereas one was near-triploid with several trisomies including three copies of chromosomes 1, 2, and 7. This pattern is by and large similar to that of the five previously reported abnormal cases, two of which showed hyperdiploid to near-triploid karyotypes with multiple trisomies including gain of chromosome 1, while the remaining three were near-diploid with various structural changes (Bhattacharjee et al., 1997; Roberts et al., 2001; Batanian et al., 2003). In our series, the patient who had the CNS-PNET with a near-triploid karyotype (#14) died 7 months after surgery, whilst the remaining two have no sign of tumour progression after 3 and 4 years, respectively. Though our findings suggest that CNS-PNETs may consist of separate subgroups based on ploidy status, this must be replicated in larger studies that should also take into account clinical parameters and survival. To date, only 33

CNS-PNETs have been studied for genomic aberrations by conventional or array-based CGH (Russo et al., 1999; Inda et al., 2005; McCabe et al., 2006; Pfister et al., 2007). In line with findings in previous studies, all our cases analysed by aCGH showed multiple copy number aberrations. Recurrent gains of 1q were seen in two cases, specifically of bands 1q21-44, something that has formerly been described in 10 tumours (Russo et al., 1999; Inda et al., 2005; Pfister et al., 2007). What gene-level change this might correspond to is unknown. Recurrent losses were found at 3p21, 3q26, and 8p23. In contrast to previous studies, no tumour showed loss of 9p or chromosomes 13 and 16 (Russo et al., 1999; McCabe et al., 2006). Gain of 4q and losses of 19q and 20q, which have all been seen recurrently in previous studies (Russo et al., 1999; Inda et al., 2005; Pfister et al., 2008), were observed in one tumour each. Also in agreement with previous reports, no CNS-PNETs showed gain of 17q. This may explain some of the biological differences between the morphologically similar medulloblastomas and CNS-PNETs, but further studies are required to corroborate these conclusions.

Atypical teratoid/rhabdoid tumours (AT/RT) are rare malignant embryonal tumours that contain rhabdoid cells and carry a particularly poor prognosis. Cytogenetically, only 16 such cases have been described and the only common aberration has been monosomy 22 (Biegel et al., 1990; Biegel 2006; Mitelman et al., 2011). Only 10 cases have been described using conventional CGH (Wharton et al., 2003; Rickert and Paulus 2004); they showed recurrent losses of chromosomes and chromosome arms 1p, 3p, 17q, 19, and 22q. Jackson et al. (2009) recently showed inactivation of the *INI1/SMARCB1* gene, which maps to chromosome band 22q11.2, in a clear majority of cases. Inactivation of both copies of the gene leads to loss of protein expression in the nucleus which is detectable by immunohistochemistry in

almost all cases (Judkins et al., 2004). Routine screening of malignant paediatric CNS tumours has been advocated and is particularly helpful in small biopsy specimens where rhabdoid cells may be hard to find. Additionally, AT/RT are morphologically diverse tumours and are often difficult to distinguish from other embryonal tumours such as medulloblastomas and CNS-PNETs (Louis et al., 2007).

CRINET was only recently suggested as a separate tumour entity by Hasselblatt et al. (2009) who described two cases of non-rhabdoid ventricular tumours with loss of INI1 protein and a relatively favourable prognosis compared to AT/RT. The same investigators have also reported a homozygous duplication as well as deletions affecting the *INI1* locus in another two yet unpublished cases (Pfister et al., 2010). Our case is the first CRINET to be analysed genomically, and although G-banding revealed a normal karyotype, aCGH showed loss of the entire 22q, 1p33, 10p12.31, and 10q11.22 as well as homozygous loss of 4q13.2 (Fig. 2D). The absence of data on more cases makes firm conclusions impossible. However, losses of 1p and 22q have been described in two and nine of the ten AT/RT analysed by conventional CGH, respectively (Wharton et al., 2003; Rickert and Paulus 2004). This suggests a pathogenetic link between the two tumour entities. It could be speculated that loss of 10q and homozygous loss of 4q might represent changes specific to the CRINET variant, but this must be confirmed in future studies of this prognostically distinct tumour type.

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REFERENCES

Agamanolis DP, Malone JM. 1995. Chromosomal abnormalities in 47 pediatric brain tumors. Cancer Genet Cytogenet 81:125-134.

Batanian JR, Havlioglu N, Huang Y, Gadre B. 2003. Unusual aberration involving the short arm of chromosome 11 in an 8-month-old patient with a supratentorial primitive neuroectodermal tumor. Cancer Genet Cytogenet 141:143-147.

Bax DA, Mackay A, Little SE, Carvalho D, Viana-Pereira M, Tamber N, Grigoriadis AE, Ashworth A, Reis RM, Ellison DW, Al-Sarraj S, Hargrave D, Jones C. 2010. A distinct spectrum of copy number aberrations in pediatric high-grade gliomas. Clin Cancer Res 16:3368-3377.

Bhattacharjee MB, Armstrong DD, Vogel H, Cooley LD. 1997. Cytogenetic analysis of 120 primary pediatric brain tumors and literature review. Cancer Genet Cytogenet 97:39-53.

Biegel JA, Rorke LB, Packer RJ, Emanuel BS. 1990. Monosomy 22 in rhabdoid or atypical tumors of the brain. J Neurosurg 73:710-714.

Biegel JA. 2006. Molecular genetics of atypical teratoid/rhabdoid tumor. Neurosurg Focus 20:E11.

Bigner SH, Mark J, Burger PC, Mahaley MS, Jr., Bullard DE, Muhlbaier LH, Bigner DD. 1988. Specific chromosomal abnormalities in malignant human gliomas. Cancer Res 48:405-411.

Bigner SH, McLendon RE, Fuchs H, McKeever PE, Friedman HS. 1997. Chromosomal characteristics of childhood brain tumors. Cancer Genet Cytogenet 97:125-134.

Brandal P, Teixeira MR, Heim S. 2010. Genotypic and phenotypic classification of cancer: How should the impact of the two diagnostic approaches best be balanced? Genes Chromosomes Cancer 49:763-774.

Chadduck WM, Boop FA, Sawyer JR. 1991. Cytogenetic studies of pediatric brain and spinal cord tumors. Pediatr Neurosurg 17:57-65.

Dahlback HS, Brandal P, Meling TR, Gorunova L, Scheie D, Heim S. 2009. Genomic aberrations in 80 cases of primary glioblastoma multiforme: Pathogenetic heterogeneity and putative cytogenetic pathways. Genes Chromosomes Cancer 48:908-924.

Dahlback HSS, Gorunova L, Brandal P, Scheie D, Helseth E, Meling TR, Heim S. 2011. Genomic Aberrations in Diffuse Low-grade Gliomas. Genes Chromosomes Cancer. 50:409-420.

Deshmukh H, Yeh TH, Yu J, Sharma MK, Perry A, Leonard JR, Watson MA, Gutmann DH, Nagarajan R. 2008. High-resolution, dual-platform aCGH analysis reveals frequent HIPK2 amplification and increased expression in pilocytic astrocytomas. Oncogene 27:4745-4751.

Fujii Y, Hongo T, Hayashi Y. 1994. Chromosome analysis of brain tumors in childhood. Genes Chromosomes Cancer 11:205-215.

Fujisawa H, Marukawa K, Hasegawa M, Tohma Y, Hayashi Y, Uchiyama N, Tachibana O, Yamashita J. 2002. Genetic differences between neurocytoma and dysembryoplastic neuroepithelial tumor and oligodendroglial tumors. J Neurosurg 97:1350-1355.

Gaffney CC, Sloane JP, Bradley NJ, Bloom HJ. 1985. Primitive neuroectodermal tumours of the cerebrum. Pathology and treatment. J Neurooncol 3:23-33.

Griffin CA, Hawkins AL, Packer RJ, Rorke LB, Emanuel BS. 1988. Chromosome abnormalities in pediatric brain tumors. Cancer Res 48:175-180.

United States Cancer Statistics Working Group. 2010. United States Cancer Statistics: 1999–2007. Incidence and Mortality Web-based Report. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute.

Hasselblatt M, Oyen F, Gesk S, Kordes U, Wrede B, Bergmann M, Schmid H, Fruhwald MC, Schneppenheim R, Siebert R, Paulus W. 2009. Cribriform neuroepithelial tumor (CRINET): a nonrhabdoid ventricular tumor with INI1 loss and relatively favorable prognosis. J Neuropathol Exp Neurol 68:1249-1255.

Houben MP, Aben KK, Teepen JL, Schouten-Van Meeteren AY, Tijssen CC, Van Duijn CM, Coebergh JW. 2006. Stable incidence of childhood and adult glioma in The Netherlands, 1989-2003. Acta Oncol 45:272-279.

Inda MM, Perot C, Guillaud-Bataille M, Danglot G, Rey JA, Bello MJ, Fan X, Eberhart C, Zazpe I, Portillo E, Tunon T, Martinez-Penuela JM,

Bernheim A, Castresana JS. 2005. Genetic heterogeneity in supratentorial and infratentorial primitive neuroectodermal tumours of the central nervous system. Histopathology 47:631-637.

ISCN. 2009. An International System for Human Cytogenetic Nomenclature. Schaffer LG, Slovak ML, Campbell LJ, editors. Basel: S. Karger.

Jackson EM, Sievert AJ, Gai X, Hakonarson H, Judkins AR, Tooke L, Perin JC, Xie H, Shaikh TH, Biegel JA. 2009. Genomic analysis using high-density single nucleotide polymorphism-based oligonucleotide arrays and multiplex ligation-dependent probe amplification provides a comprehensive analysis of INI1/SMARCB1 in malignant rhabdoid tumors. Clin Cancer Res 15:1923-1930.

Jacob K, Albrecht S, Sollier C, Faury D, Sader E, Montpetit A, Serre D, Hauser P, Garami M, Bognar L, Hanzely Z, Montes JL, Atkinson J, Farmer JP, Bouffet E, Hawkins C, Tabori U, Jabado N. 2009. Duplication of 7q34 is specific to juvenile pilocytic astrocytomas and a hallmark of cerebellar and optic pathway tumours. Br J Cancer 101:722-733.

Jenkins RB, Kimmel DW, Moertel CA, Schultz CG, Scheithauer BW, Kelly PJ, Dewald GW. 1989. A cytogenetic study of 53 human gliomas. Cancer Genet Cytogenet 39:253-279.

Jones DT, Kocialkowski S, Liu L, Pearson DM, Backlund LM, Ichimura K, Collins VP. 2008. Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. Cancer Res 68:8673-8677.

Judkins AR, Mauger J, Ht A, Rorke LB, Biegel JA. 2004. Immunohistochemical analysis of hSNF5/INI1 in pediatric CNS neoplasms. Am J Surg Pathol 28:644-650.

Karnes PS, Tran TN, Cui MY, Raffel C, Gilles FH, Barranger JA, Ying KL. 1992. Cytogenetic analysis of 39 pediatric central nervous system tumors. Cancer Genet Cytogenet 59:12-19.

Kitange G, Misra A, Law M, Passe S, Kollmeyer TM, Maurer M, Ballman K, Feuerstein BG, Jenkins RB. 2005. Chromosomal imbalances detected by array comparative genomic hybridization in human oligodendrogliomas and mixed oligoastrocytomas. Genes Chromosomes Cancer 42:68-77.

Kreiger PA, Okada Y, Simon S, Rorke LB, Louis DN, Golden JA. 2005. Losses of chromosomes 1p and 19q are rare in pediatric oligodendrogliomas. Acta Neuropathol 109:387-392.

Kros JM, van Run PR, Alers JC, Beverloo HB, van den Bent MJ, Avezaat CJ, van Dekken H. 1999. Genetic aberrations in oligodendroglial tumours: an analysis using comparative genomic hybridization (CGH). J Pathol 188:282-288.

Larouche V, Capra M, Huang A, Bartels U, Bouffet E. 2006. Supratentorial primitive neuroectodermal tumors in young children. J Clin Oncol 24:5609; author reply 5609-5610.

Lindström E, Salford LG, Heim S, Mandahl N, Strömblad S, Brun A, Mitelman F. 1991. Trisomy 7 and sex chromosome loss need not be representative of tumor parenchyma cells in malignant glioma. Genes Chromosomes Cancer 3:474-479.

Louis D, Ohgaki H, Wiestler O, Cavenee W, editors. 2007. WHO Classification of Tumours of the Central Nervous System, 4th Edition. Ogdensburg: Renouf Publishing Co. Ltd., pp. 14-65

Mandahl N. 1992. Methods in solid tumors cytogenetics. In: Rooney DE, Czepulkowski BH, editors. Human Cytogenetics - A Practical Approach, Vol. II: Malignancy and Acquired Abnormalities. Oxford: IRL Press, pp. 155-187

McCabe MG, Ichimura K, Liu L, Plant K, Backlund LM, Pearson DM, Collins VP. 2006. High-resolution array-based comparative genomic hybridization of medulloblastomas and supratentorial primitive neuroectodermal tumors. J Neuropathol Exp Neurol 65:549-561.

Mitelman F, Johansson B, Mertens F. 2011. Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer. Available at http://cgap.nci.nih.gov/Chromosomes/Mitelman. Accessed April 2011

Neumann E, Kalousek DK, Norman MG, Steinbok P, Cochrane DD, Goddard K. 1993. Cytogenetic analysis of 109 pediatric central nervous system tumors. Cancer Genet Cytogenet 71:40-49.

Orr LC, Fleitz J, McGavran L, Wyatt-Ashmead J, Handler M, Foreman NK. 2002. Cytogenetics in pediatric low-grade astrocytomas. Med Pediatr Oncol 38:173-177.

Parkin D, Kramarova E, Draper G, Masuyer E, Michaelis J, Neglia J, Qureshi S, Stiller C, editors. 1999. International Incidence of Childhood Cancer. Lyon: International Agency for Research on Cancer.

Pfister S, Remke M, Toedt G, Werft W, Benner A, Mendrzyk F, Wittmann A, Devens F, von Hoff K, Rutkowski S, Kulozik A, Radlwimmer B, Scheurlen W, Lichter P, Korshunov A. 2007. Supratentorial primitive neuroectodermal tumors of the central nervous system frequently harbor deletions of the CDKN2A locus and other genomic aberrations distinct from medulloblastomas. Genes Chromosomes Cancer 46:839-851.

Pfister S, Janzarik WG, Remke M, Ernst A, Werft W, Becker N, Toedt G, Wittmann A, Kratz C, Olbrich H, Ahmadi R, Thieme B, Joos S,

Radlwimmer B, Kulozik A, Pietsch T, Herold-Mende C, Gnekow A, Reifenberger G, Korshunov A, Scheurlen W, Omran H, Lichter P. 2008. BRAF gene duplication constitutes a mechanism of MAPK pathway activation in low-grade astrocytomas. J Clin Invest 118:1739-1749.

Pfister S, Remke M, Benner A, Mendrzyk F, Toedt G, Felsberg J, Wittmann A, Devens F, Gerber NU, Joos S, Kulozik A, Reifenberger G, Rutkowski S, Wiestler OD, Radlwimmer B, Scheurlen W, Lichter P, Korshunov A. 2009. Outcome prediction in pediatric medulloblastoma based on DNA copy-number aberrations of chromosomes 6q and 17q and the MYC and MYCN loci. J Clin Oncol 27:1627-1636.

Pfister SM, Korshunov A, Kool M, Hasselblatt M, Eberhart C, Taylor MD. 2010. Molecular diagnostics of CNS embryonal tumors. Acta Neuropathol 120:553-566.

Pollack IF. 1994. Brain tumors in children. N Engl J Med 331:1500-1507.

Pollack IF, Finkelstein SD, Burnham J, Hamilton RL, Yates AJ, Holmes EJ, Boyett JM, Finlay JL. 2003. Association between chromosome 1p and 19q loss and outcome in pediatric malignant gliomas: results from the CCG-945 cohort. Pediatr Neurosurg 39:114-121.

Raghavan R, Balani J, Perry A, Margraf L, Vono MB, Cai DX, Wyatt RE, Rushing EJ, Bowers DC, Hynan LS, White CL. 2003. Pediatric oligodendrogliomas: a study of molecular alterations on 1p and 19q using fluorescence in situ hybridization. J Neuropathol Exp Neurol 62:530-537.

Rickert CH, Paulus W. 2001. Epidemiology of central nervous system tumors in childhood and adolescence based on the new WHO classification. Childs Nerv Syst 17:503-511.

Rickert CH, Strater R, Kaatsch P, Wassmann H, Jurgens H, Dockhorn-Dworniczak B, Paulus W. 2001. Pediatric high-grade astrocytomas show chromosomal imbalances distinct from adult cases. Am J Pathol 158:1525-1532.

Rickert CH, Paulus W. 2004. Chromosomal imbalances detected by comparative genomic hybridisation in atypical teratoid/rhabdoid tumours. Childs Nerv Syst 20:221-224.

Roberts P, Chumas PD, Picton S, Bridges L, Livingstone JH, Sheridan E. 2001. A review of the cytogenetics of 58 pediatric brain tumors. Cancer, Genetics and Cytogenetics 131:1-12.

Rossi MR, Conroy J, McQuaid D, Nowak NJ, Rutka JT, Cowell JK. 2006. Array CGH analysis of pediatric medulloblastomas. Genes Chromosomes Cancer 45:290-303.

Russo C, Pellarin M, Tingby O, Bollen AW, Lamborn KR, Mohapatra G, Collins VP, Feuerstein BG. 1999. Comparative genomic hybridization in patients with supratentorial and infratentorial primitive neuroectodermal tumors. Cancer 86:331-339.

Sainati L, Bolcato S, Montaldi A, Celli P, Stella M, Leszl A, Silvestro L, Perilongo G, Cordero di Montezemolo L, Basso G. 1996. Cytogenetics of pediatric central nervous system tumors. Cancer Genet Cytogenet 91:13-27.

Sawyer JR, Swanson CM, Roloson GJ, Longee DC, Chadduck WM. 1992. Cytogenetic findings in a case of pediatric glioblastoma. Cancer Genet Cytogenet 64:75-79.

Stiller CA, Marcos-Gragera R, Ardanaz E, Pannelli F, Almar Marques E, Canada Martinez A, Steliarova-Foucher E. 2006. Geographical patterns of childhood cancer incidence in Europe, 1988-1997. Report from the Automated Childhood Cancer Information System project. Eur J Cancer 42:1952-1960.

Timmermann B, Kortmann RD, Kuhl J, Meisner C, Dieckmann K, Pietsch T, Bamberg M, Rutkowski S, Deinlein F, Urban C, Warmuth-Metz M. 2002. Role of radiotherapy in the treatment of supratentorial primitive neuroectodermal tumors in childhood: results of the prospective German brain tumor trials HIT 88/89 and 91

Role of radiotherapy in supratentorial primitive neuroectodermal tumor in young children: results of the German HIT-SKK87 and HIT-SKK92 trials. J Clin Oncol 20:842-849.

Vagner-Capodano AM, Gentet JC, Gambarelli D, Pellissier JF, Gouzien M, Lena G, Genitori L, Choux M, Raybaud C. 1992. Cytogenetic studies in 45 pediatric brain tumors. Pediatr Hematol Oncol 9:223-235.

Warr T, Ward S, Burrows J, Harding B, Wilkins P, Harkness W, Hayward R, Darling J, Thomas D. 2001. Identification of extensive genomic loss and gain by comparative genomic hybridisation in malignant astrocytoma in children and young adults. Genes Chromosomes Cancer 31:15-22.

Wharton SB, Wardle C, Ironside JW, Wallace WH, Royds JA, Hammond DW. 2003. Comparative genomic hybridization and pathological findings in atypical teratoid/rhabdoid tumour of the central nervous system. Neuropathol Appl Neurobiol 29:254-261.

TABLE 1: Clinical, Histopathological, and Cytogenetic Findings in 17 Paediatric Intracranial Neoplasms

Case No.	Sex/Age (yrs)	Survival[a]	Histological diagnosis	G-band karyotype	aCGH gain	aCGH loss
1	M/14	A	Pilocytic astrocytoma[b]	46,XY[25]	7q34,12p13.31,16q24.2-q24.3,19p13.3	
2	M/3	A	Pilocytic astrocytoma[b]	46,Y,t(X;19)(p10;q10)[2]/46,XY[cp48]	–	
3	F/1	A	Pilocytic astrocytoma	46,XX[25]	7q34,13q21.31,19p13.3	
4	M/3	D	Low-grade glioma, likely pilocytic astrocytoma[b]	45~46,XY,t(15;17)(p11;q11)[3]/47,XY,idem,+der(17)t(15;17)[4]/46,XY,t(1;6)(p22;q15)[4]/46,XY,t(4;16)(p16;q22)[4]/46,XY,der(1)inv(1)(p32;q21)t(1;4)(p32;q21),t(6;15)(q23;q22),t(7;20)(q22;q11)[2]/46,XY[25]	–	
5	F/5	A	Low-grade astrocytoma, unclassifiable	46,XX,der(6)t(6;12)(q27;q12~q13)[2]/46,XX[23]	–	
6	F/8	A	Low-grade neuroepithelial tumour (DNET or low-grade astrocytoma)	46,XX[25]	12p13.31,19p13.3-13.2	1q21.1,*4q13.2*,8p23.1,*8p11.23*,11p11.2
7	M/11	D	Glioblastoma	47~48,XY,**+7**,+12[cp3]/46,XY[25]	6q26,**7q22.1**	8p23.1
8	M/12	D	Glioblastoma	46,XY[25]	5p15.33-p13.3,7q36.1,14q11.2,14q32.33	*Xp11.23*,2p25.3-p11.2,4q12-q35.3,5q11.1-q35.3,10q11.21-q26.3,13q14.12-q34,18q12.3-q23
9	F/3	D	Anaplastic neuroepithelial tumour (most likely an anaplastic oligoastrocytoma)	41~46,X,**-X**,**+2**,**der(6)add(6)(p21)del(6)(q21),**+8,**-10**,**-11,-14**,add(14)(p11),**-15**,**-16**,**-17**,add(18)(p11),+r,+5mar[cp15]/81~85,XX,**-X,-X**,+add(3)(p11)x2,**der(6)x2**,add(7)(p22),**-14,-14,-14**,add(14),**-15,-15,-16,-16,-17,-17**,add(18)x2,+r,+5mar [cp13]	**2p25.3-p24.3**,**6p21.31-p21.1,6q16.2-q22.31,6q26-q27,6q27**,7p15.3,10q22.3-q23.2,11q13.2-q13.3,11q14.1,11q23.1-q24.1,12p11.21,15q22.1-q26.3,16q23.1,16q24.1,17p13.3-p13.2,17q12,17q21.2-q21.31,17q21.32,17q23.2-q24.1,21q11.2-q22.3	**Xq21.2-q27.3,6p25.3-p25.2,6p12.2-p11.2,6q25.3-q26,6q27,10q11.22-q21.1,10q21.3-q22.3,10q23.33-q26.3,11p15.5-p15.4,11p14.3-p11.12,11q12.1-q13.1,11q13.1,11q13.2,11q13.5-q14.1,11q14.1-q23.1,11q24.1-q25,14q22.3-q32.33,15q13.2-q13.3,15q13.3-q21.1,15q21.3-q22.1,16p13.12,16p13.11,16p12.3-p12.2,16q24.2,17p13.3,17p13.2-p13.1,17q11.2-q12,17q21.1,17q21.31,17q22-q23.2,17q24.1-q24.2,17q24.3**,18q11.1-q12.1,18q12.2-q23,*22q11.23*
10	F/6	D	Anaplastic oligoastrocytoma – recurrence[c]	41~44,XX,**der(4)t(4;6)(p16;q13),del(5)(p11),-6,-8,-9**,der(11)t(8;11)(q13;q21),**-14,-19**,add(20)(q13),**-22**,+3mar[cp5]/46,XX[25]	4p15.1-p14,9p13.3,12p13.31,19q12-q13.11	Xp22.3-p22.32,**4p16.3-p15.2,*4q13.2*,5p15.33-p11,6q12,6q13,8p23.3-q11.1,8q11.1-q13.1,9p21.3-p13.3,*9p21.3*,14q11.1-q31.1**,15q11.2,17p13.3-p13.1,**19q13.11-q13.12,19q13.12-q13.43,22q11.21-q13.33,*22q11.23***

Case	Sex/Age	Survival[a]	Histological diagnosis	G-band karyotype	aCGH gain	aCGH loss

No.	(yrs)					
11	M/3	A	Medulloblastoma (classical type)[b]	46,XY[25]	Xp22.33-q11.1,Xq11.1-q28,1p36.33-p11.2,1q21.1-q44,2p25.3-q11.1,2q11.1-q21.2,2q21.2-q37.3,3p26.3-p11.1,3q11.2-q29,6p25.3-q11.1,6q11.1-q27,7p22.3-p11.1,7q11.21-q36.3,9p24.3,12p13.31,14q11.2-q32.33,15q22.31,17p13.3-q11.1,17q11.1-q25.3,18p11.32-q11.1,18q11.1-q23,19p13.3-p12,19q12-q13.43,20q11.21-q11.22,22q11.23	10p15.3-p11.21,10q11.1-q26.3,11p15.5-q11,11q11-q25 16p13.3-p11.1,16q11.2-q22.1,16q22.2-q24.3, 21q11.2-q22.3
12	F/2	A	CNS-PNET (with extensive glial differentiation)	46,XX[25]	10q11.22	2p24.3,8p23.1
13	M/3	P	CNS-PNET	Cell culture failure	8p11.23-p11.22,15q12,22q11.23	1q42.13,3q29,7p22.1,7q22.1,7q36.1,8p23.1,9q32, 11q13.1,14q32.31,17q21.31,19p13.3,19p13.12, 19q13.33,19q13.42,19q13.43,20q13.31
14	F/5	D	CNS-PNET	55~59<3n>,XX,-X,**+1,+2,-3**,-4,-5,-6,**+7**,-8,-9,-10,-11,?der(12)t(12;21)(q13;q11)x2,-13,-14,-15,-17,**-18**,add(18)(q23)x2,-21,-21,-21,-22[cp4]/46,XX[17]	**1p36.33-p11.2,1q12-q44,1q21.3-q22, 2p25.3-q11.1,2q11.1-q37.3**,4q31.3-q32.1,4q35.2,6p25.3,**7p22.3-p11.1, 7q11.21-q36.3**,9p21.1-p13.3,11p15.5-q11,11q11-q25,12p13.33-p11.1,12q12, *12q12-13.11*,16p13.3-p11.1,16q11.2-q24.3, 17q21.31,20p13-q11.1,20q11.21-q13.33	***3p21.1.3q26.1***,**3q29**,*8p11.23*,***18p11.32***
15	F/6	A	CNS-PNET (with extensive glial and limited neuronal differentiation)	45~46,X,-X,add(14)(p11),add(21)(p11), del(22)(q11),+mar[cp13]/46,XX[2]	–	
16	F/5	A	CNS-PNET	45,XX,**+1,der(1;3)(q10;q10),add(11)(q23), i(21)(q10)**,+mar[cp2]/46,XX[5]	**1q21.1-q44**,3q26.33,5p15.33-p11,8q24.21,17q22-q25.3,**21q11.2-q22.3**	**3p26.3-p12.3,3p12.3-p12.1**,9p23,**11q22.3-q25**, 15q26.1,20p13
17	F/2	A	Cribriform nevroepithelial tumor (CRINET)	46,XX[7]		1p33,***4q13.2***,10p12.31,10q11.22,22q11.11-q13.33

[a]A, Alive, D, Tumour related cause of death, P,Tumour progression radiologically, patient receiving radiotherapy.
[b]Infratentorial tumour, all others are supratentorial tumours.
[c]Patient received vincristine following previous surgery.
In italics and underscored are gained areas that represent amplifications and diminished areas that represent homozygous deletions.
In bold are findings seen by both G-banding and aCGH.