High-resolution karyotyping by oligonucleotide microarrays : the next revolution in cytogenetics

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Chapter 1

Introduction

1. Clinical Cytogenetics

1.1 Cytogenetics

Cytogenetics is a branch of genetics concerned with the study of the structure of chromosomes and their role in heredity. Conventional chromosome analysis using G-banding is widely used for clinical diagnostics and genomic research. However, over the past 30 years the development of new techniques with increasingly higher resolution has led to the new field of molecular cytogenetics.

1.2 History of Cytogenetics

The field of human cytogenetics emerged in 1879 when Walther Flemming described the first human chromosomes and coined the term mitosis (Flemming, 1879). In 1888, Waldeyer introduced the word chromosome which comes from the Greek words chroma for colour and soma for body (Waldeyer, 1888). The term cytogenetics (from cytology and genetics) was formulated by Sutton in 1903 (Sutton, 1903). In the years that followed there were several publications on the human chromosome number (Von Winiwarter, 1912; Painter, 1923; Hsu, 1952). For a long time the chromosome number of humans was considered to be 48. Finally, in 1956 using improved tissue culture techniques, Tjio and Levan showed the correct human chromosome number in lung fibroblasts to be 46 (Tjio and Levan, 1956). This was soon confirmed on meiotic chromosomes from human testicular tissue (Ford and Hamerton, 1956). Three years later the first chromosomal aberration was discovered by Lejeune and his colleagues (1959) who identified an extra small chromosome in fibroblast cultures from patients with Down syndrome. Soon thereafter more chromosomal abnormalities were described involving both sex chromosomes and the autosomes: 45,X in Turner syndrome (Ford et al., 1959), 47,XXY in Klinefelter syndrome (Jacobs and Strong, 1959), 47,XXX (Jacobs et al., 1959), trisomy 13 in Patau syndrome (Patau et al., 1960), trisomy 18 in Edwards syndrome (Edwards et al., 1960), and deletion of the short arm of chromosome 5 in Cri-du-chat syndrome (Lejeune et al., 1963).

1.3 Chromosome banding and nomenclature

At first the chromosome pairs were classified into seven different groups (A-G), based on morphology (Patau, 1960). A system of nomenclature was proposed at a conference in Denver (Denver Conference, 1960). This classification was officially approved at a conference in London (London Conference, 1963). However, although certain chromosomes could be identified by their size and centromere position, it was not possible to identify individual chromosomes as we know them today. Furthermore, many structural abnormalities, such as inversions, that were suspected could not be proven.

In 1968, it was demonstrated that plant chromosomes, stained with fluorescent quinacrine compounds (Q-banding), showed a distinct staining whereby each chromosome could be identified by its unique banding pattern (Caspersson et al., 1968). In 1970 the first human banded chromosomes were produced by this method (Caspersson et al., 1970). Q-banding proved to be very useful in identifying many chromosome abnormalities. However, as it requires a relatively expensive fluorescence microscope, and the fluorescence fades rapidly, Q-banding was largely replaced by G-banding.

For G-banding, which gives permanent staining, the chromosomes are treated with trypsin followed by staining with a Giemsa dye (Seabright, 1971). This method

is now the most commonly used banding technique in most laboratories worldwide, except in France. In France and many French speaking countries R-banding, which gives a banding pattern that is reverse to that of G-banding, is used as a standard technique (Dutrillaux and Lejeune, 1971). G-banding, a simple and an inexpensive technique, enabled rapid identification of many new chromosomal abnormalities, including deletions, duplications, translocations, inversions and insertions. In 1971, at the Paris Conference, a new system for the classification of chromosomes, based on Q, G, and R banding patterns, was introduced to identify individual chromosomes and chromosome regions (Paris Conference, 1971). This was followed in 1978 by a new document entitled "An International System for Human Cytogenetic Nomenclature" which included the major decisions of the Denver, London and Paris Conferences (ISCN 1978). This nomenclature for human chromosomes became widely used and is regularly updated (most recently in 2009, ISCN 2009). G-banding of long pro-metaphase chromosomes gave high resolution banding (Yunis et al, 1980) and enabled the identification of subtle chromosome alterations.

2. Chromosome morphology

2.1 Chromosome variants

Chromosomes consist of chromatin which is a combination of DNA and proteins. There are two types of chromatin, euchromatin and heterochromatin, which show different degrees of condensation. Euchromatin is the less condensed form, is generally rich in genes and is actively transcribed whereas heterochromatin is normally more condensed, poor in gene content, and rich in repetitive DNA. Even before the advent of banding it was known that the size and position of the heterochromatic segments could vary between individuals without a phenotypic effect. They were called heterochromatic variants. In recent years, variants of certain euchromatic chromosome segments have also been described.

2.2 Heterochromatic variants

Already in 1960 it was evident that the Y chromosome could vary considerably between individuals (Patau, 1960). At the London Conference on 'The normal human karyotype' in 1963, it became apparent that also the secondary constrictions near the centromeres of chromosomes 1, 9 and 16 could vary in size. Although these so-called heteromorphisms had been widely reported, it was unclear whether or not they were associated with clinical abnormalities (Cooper and Hernits, 1963; Yunis and Gorlin, 1963; Palmer and Schroder, 1971; Lobitz et al., 1972). It was only after large studies on consecutive newborns that it became clear that most of the heterochromatic variants were not disease-related (Sergovich et al., 1969; Lubs and Ruddle, 1970; Friedrich and Nielsen, 1973; Jacobs et al., 1974; Nielsen and Sillesen, 1975; Hamerton et al., 1975). Eventually, large scale population studies based on banded chromosomes revealed heteromorphisms on chromosomes 1, 3, 4, 6, 9, 13-16, 21, 22 and the Y (Geraedts and Pearson, 1974; Madan and Bobrow, 1974; Müller et al., 1975; McKenzie and Lubs, 1975; Madan and Bruinsma, 1979) and demonstrated that these were heritable (reviewed by Wyandt, 2004). The heritability of heteromorphisms was used in various ways, such as to demonstrate maternal contamination in prenatal samples (Olson et al., 1987), to determine paternity (Olson et al., 1983; Olson et al., 1986) and to determine the parental origin of chromosome abnormalities (Magenis et al., 1977; Mikkelsen et al., 1980; Jacobs et al., 1982; Juberg and Mowrey, 1983).

2.3 Euchromatic variants

Deletions and duplications of euchromatic segments are usually pathogenic. However, several microscopically visible euchromatic deletions and duplications without any phenotypic consequences have been described. These euchromatic variants reflect copy number variation of chromosomal segments containing genes and pseudogenes. They can be polymorphic in the normal population and only reach a cytogenetically detectable level when the multiple copies are long enough to be observed under a microscope as constitutional cytogenetic amplifications. Euchromatic variants segregate in families without apparent phenotypic consequences. The first euchromatic variant was reported on the short arm of chromosome 9 in a G-banded chromosome study of live-born infants (Buckton et al., 1980). Other examples of regions with known euchromatic variants are 8p23.1, 9p12, 9q12, 9q13, 15q11.2 and 16p11.2 (reviewed by Wyandt, 2004; Barber et al., 2005; Hansson et al., 2007). In many cases it is difficult to decide whether a euchromatic deletion or a duplication is pathogenic or benign. Since most of the aberrations are initially detected in phenotypically abnormal individuals, their benign or pathogenic status can be established only after family studies.

3. Molecular Cytogenetics

3.1 Overview

Although G-banding has improved substantially since its initial discovery in 1971, it enables the detection of deletions or duplications only in the order of 5-10 Mb. For the identification of smaller aberrations new techniques are needed. Fluorescence *in Situ* Hybridization (FISH) was the first of a series of methods that led to the emergence of the field of molecular cytogenetics, a combined application of cytogenetics and molecular biology. Some of the new methods are used only for the confirmation or further characterization of previously identified chromosomal aberrations (FISH, Multiplex Ligation-dependent Probe Amplification, MLPA and Quantitative Fluorescence-Polymerase Chain Reaction, QF-PCR) whereas others are used for whole genome screening with different resolutions (array Comparative Genome Hybridization, aCGH and Single Nucleotide Polymorphism array, SNP array). Table 1.1 gives an overview of the (molecular) cytogenetic techniques and their possible resolutions. The techniques most commonly used in cytogenetic diagnostics are described below.

3.2 Fluorescence *in Situ* Hybridization

In Situ Hybridization (ISH) was developed to detect specific DNA sequences on chromosomes and was initially based on radioactively labelled probes (Pardue and Gall, 1969). This technique was used for the localization of genes to chromosomes (Harper et al., 1981; Gerhard et al., 1981). FISH, a locus-specific technique, was introduced in the 1980s (Prooijen-Knegt et al., 1982; Landegent et al., 1986). It allows the detection of chromosomal abnormalities directly on metaphase chromosomes and in interphase nuclei. A fluorescent labelled DNA probe hybridizes to its complementary sequence in chromosomes and is visualized with a fluorescent microscope. Probes used for FISH can be made from Bacterial Artificial Chromosome (BAC), P1 Artificial Chromosome (PAC), cosmid or fosmid clones, or from PCR products. The resolution depends on the size of the probe (>50 kb - 2 Mb). This method can be used to examine those regions of chromosomes that are suspected of carrying a specific abnormality based on the clinical picture of the patient, for instance microdeletion syndromes (Dauwerse et al., 1990). FISH is a powerful technique that can be used to detect specific structural

Table 1.1 Overview of cytogenetic techniques

	Conventional karyotyping	Locus specific metaphase FISH	Locus specific interphase FISH	Fibre FISH	SKY/M-FISH	MLPA	QF-PCR	arrayCGH (BAC)	arrayCGH (oligo's)	SNP array
Whole genome screening	+	-	-	-	+	-	-	+	+	+
Resolution	5-10Mb	50 kb-2 Mb*	50 kb-2 Mb*	5 kb -500 kb	3-5 Mb	50-70 bp	50-70 bp	100-300 kb*	10 kb⁺	10 kb⁺
Deletion	+	+	+	+	±	+	+	+	+	+
Duplication	+	-	±	+	±	+	+	+	+	+
Triplication	+	-	±	+	±	±	+	+	+	+
Trisomy	+	+	+	-	+	+	+	+	+	+
Triploidy	+	+	+	-	+	-	+	-	-	±
Monosomy	+	+	+	-	+	+	+	+	+	+
Inversion	+	++	-	+	-	-	-	-	-	-
Insertion	+	++	-	+	++	-	-	-	-	-
Balanced translocation	+	++	-	-	++	-	-	-	-	-
Unbalanced translocation	+	++	-	-	++	±	-	+	+	+
Complex rearrangement	+	++	-	+	++	±	-	±	±	±
Identification marker chromosomes	+	+	+	-	++	-	-	±	±	±
UPD	-	-	-	-	-	-	+	-	-	+
LOH	-	-	-	-	-	-	+	-	-	+
Genotyping	-	-	-	-	-	-	-	-	-	+

Table is obtained and adapted from Schoumans and Ruivenkamp (2010) *Resolution depending on the size of the probes; ⁺ resolution depending on the number of probes on the array

chromosome abnormalities. However, it is relatively labour intensive and its use in diagnostics is limited because of its low resolution.

Fiber FISH allows the detection of deletions and duplications of even just a few kilobases (Heng et al., 1992; Wiegant et al., 1992). This technique uses extended chromatin fibers and is able to resolve complex chromosomal rearrangements (Florijn et al., 1995; Giles et al., 1997). The preparation of fiber FISH samples, however, requires a highly skilled technician, and the technique is used only in specialized laboratories. Furthermore it is only used for a more detailed characterization of complex chromosomal aberrations that have been identified already.

Other FISH applications use telomere-specific probes and whole-chromosome painting probes. Spectral karyotyping (SKY) and multicolour-FISH (M-FISH) are methods whereby in one experiment a whole karyotype can be produced in which each chromosome has a different fluorescent colour (Schröck et al., 1996; Speicher et al., 1996). These techniques allow the detection of interchromosomal aberrations such as translocations, insertions, complex chromosome rearrangements and the origin of marker chromosomes. The major disadvantage of SKY and M-FISH is that inversions, deletions and small duplications are not detectable.

While using different types of FISH for increasingly detailed analysis of chromosomes, it became apparent that techniques used for DNA analysis essentially also give information on chromosome morphology, albeit at another level of resolution. It was soon realized that these high throughput DNA techniques could also be used for studying chromosome morphology.

3.3 Array-CGH using BAC clones

Array comparative genome hybridization (aCGH) is a technique which compares DNA of a test sample with DNA of a reference sample and is generally seen as the first cytogenetic application of genomic (DNA-based) array technology. These arrays contained BAC and PAC clones covering the whole genome (BAC arrays) and were mostly in-house made by spotting (Solinas-Toldo et al., 1997; Pinkel et al., 1998). aCGH using BAC clones allows the detection of copy number variants (deletions and duplications) that are approximately 100 times smaller than those identifiable with conventional karyotyping. The resolution of these arrays depends on the distance between the probes as well as on the sizes of the probes. Initially, the BAC arrays contained approximately one clone per Mb (i.e. about 3500 BAC clones for the coverage of the full human genome) followed by a tiling path array with a resolution that is 10 fold higher (including 33000 BACs). The technique is based on competitive hybridization of test and reference DNA labelled with different fluorochromes (e.g. red for test and green for reference) on the spotted BAC clones. The array is imaged by a scanner and the relative fluorescence intensities are calculated. The chromosomal regions that are equally represented in the test and reference samples appear yellow, but those that are deleted in the test sample appear more green and those that are amplified appear more red. This results in intensity ratios for each mapped clone and reflects the copy number difference which can be visualized by a number of software packages.

3.4 Array-CGH using oligonucleotides

The procedure for oligonucleotide CGH arrays is similar to that for BAC arrays, using differentially labelled test and reference DNA. However, these arrays are commercially manufactured by lithography, and consist of 45- to 85- mer oligonucleotide probes.

The major manufacturers are Agilent and Nimblegen. Their first arrays contained 44.000 and 72.000 oligonucleotides respectively, but the latest released oligonucleotide CGH arrays contain 1 and 2.1 million probes respectively.

3.5 Single Nucleotide Polymorphism (SNP) array

A different type of oligonucleotide array is the SNP array which was initially developed for genome-wide linkage and association studies. This method allows the detection of SNP-genotype as well as the presence of deletions and amplifications. The major manufacturers of SNP arrays are Affymetrix and Illumina. These two companies use different technologies for the discrimination of alleles and their sensitivity for the detection of CNVs is different. A major advantage of this type of array is that it can be used both for genotyping studies and for copy number screening.

Affymetrix

The first SNP array produced by Affymetrix contained 10.000 (10K) SNP probes on a single array slide. This was followed by a 100K and 500K, the probes being distributed in the latter case over two array slides of 250K each. Later the 5.0 (500K) array combined the 500.000 probes on a single slide and the newer arrays 6.0 and 2.7M contain 1.8 and 2.7 million probes respectively on a single slide. These arrays include SNP probes and additional probes specific for the detection of copy number variation.

The Affymetrix method is a single color assay and is composed of multiple overlapping allele-specific hybridization probes that are complementary to SNP regions present in the reduced fraction of the genome amplified in the assay. Since the 500K assay is now used for routine cytogenetic diagnostics in a number of laboratories, the method (adapted from www.affymetrix.com) is explained here in detail. The probes are 25-mer oligonucleotides with the variable SNP located at the 13th nucleotide. The probes present are for both possible alleles of each SNP, so-called 'perfect match' (PM). Besides these PM probes, mismatch (MM) probes are included for each allele to allow discrimination between signal and noise. Four additional probe quartets are present for each SNP where the probe sequences are different at four other positions. Additionally, all the quartets are present in both the forward and the reverse orientations. As a consequence, each SNP is represented by 40 distinct probe sequences. The probes are scattered over the array to diminish any effects of array variation and each probe has a fixed location on the array.

Approximately 250 ng of genomic DNA is digested with restriction enzymes and ligated to adaptors recognizing the overhangs (Figure 1.1). A universal primer, which recognizes the adaptor sequence, is used to amplify ligated DNA fragments and PCR conditions are optimized to preferentially amplify fragments in the 200-1000 bp size range. These products are purified and fragmented to 50-200 bp products. The amplified and fragmented DNA is incorporated with biotine labeled nucleotides and hybridized to GeneChip arrays. After hybridization of 16-18 hours, the arrays are washed, fluorochromes are labeled to the biotine on the GeneChip fluidics station, and scanned on a GeneChip Scanner 3000. Signals from the allele-specific probes are analyzed to determine whether a SNP is AA, AB, or BB. The signal intensity is quantified and compared to signal intensities of normal individuals to determine SNP copy number. Several software packages have been developed to analyze SNP genotypes and to determine copy number.

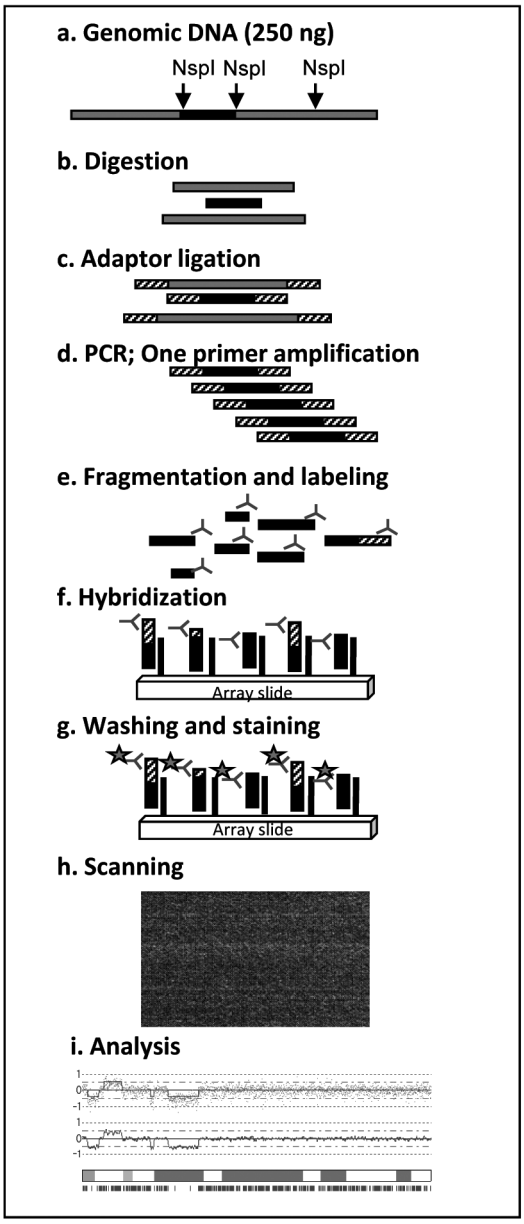

Figure 1.1 Schematic overview of the Affymetrix platform procedure

Illumina

The first SNP arrays from Illumina contained 317.000 probes (300K) on a single slide. The next generation contained twice the same 317.000 probes on a single slide, thus enabling hybridization of DNA from two individuals (317K-duo). This was followed by a 370K-duo and a 550K-duo. The latest SNP arrays from Illumina contain 650.000 and 1 million probes.

The concept of the Illumina assay is based on direct hybridization of whole genome-amplified genomic DNA to a bead array of 50-mer locus-specific probes. These probes end one nucleotide before the SNP. After hybridization each SNP is scored by a single base extension assay using different labeled nucleotides. These labels are visualized by staining with an immunohistochemistry assay. The A and T nucleotides are stained in one color and C and G in another. The signal intensity is used to determine copy number. On each array the beads are randomly assembled, therefore every array is provided with a file with the exact probe locations. A brief description of

the protocol (adapted from www.illumina.com) is described here. The first step in the Illumina assay is a whole genome amplification of 750 ng of genomic DNA (Figure 1.2). The amplified genomic DNA is fragmented to an average size of approximately 300 bp using an endpoint enzymatic fragmentation protocol. These fragments are precipitated and re-suspended in a hybridization buffer. The precipitated DNA is hybridized to a BeadChip in a humidified chamber. After 16-18 hours the hybridized arrays are washed and the next step is a single base extension with differentially labeled nucleotides. The BeadChips are scanned with a two-color confocal Illumina BeadArrayTM Reader. Image intensities are extracted and genotypes and copy number are determined using Illumina's BeadStudio software. Several other software packages have been developed to analyze Illumina data.

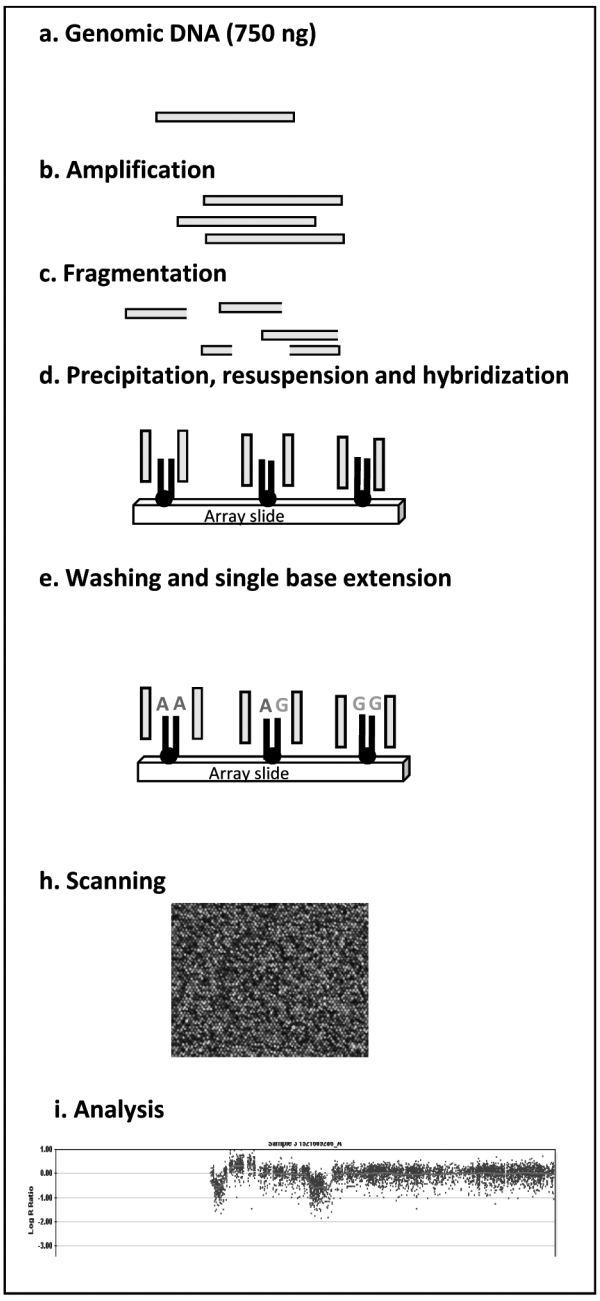

Figure 1.2 Schematic overview of the Illumina platform procedure

4. Copy number variants

As mentioned earlier, variation in chromosome morphology has been known from the earliest days of cytogenetics. It has now become possible to study variation in the composition of chromosomes right down to the nucleotide base level. Variation in the human genome takes many forms, ranging from the heterochromatic and euchromatic variants that have been described above to SNPs. With the advent of high-resolution whole-genome array technologies it has become evident that many submicroscopic copy number variants (CNVs), varying in size from kilobases to megabases, are present in all humans (Iafrate et al., 2004; Sebat et al., 2004; Sharp et al., 2005; Tuzun et al., 2005; Hinds et al., 2006; McCarroll et al., 2006; Locke et al., 2006; Feuk et al., 2006; Friedman et al., 2006; Redon et al., 2006; Conrad and Hurles, 2006). The identification of disease causing CNVs is hampered by our inability to distinguish between normal and causative variants.

New recurrent deletions and duplications for which the clinical significance is not directly evident have been reported. In 2007, a deletion of approximately 600 kb on 16p11.2 was reported in 1% of individuals with autistic features (Weiss et al., 2008). This was supported by two additional studies (Kumar et al., 2008; Marshall et al., 2008). However, in one of our studies we identified the same CNV in patients with mental retardation (MR) as well as in healthy individuals, both groups without autism (**Chapter 3.1**). This indicates that the recurrent deletion of 16p11.2 gives rise to a broad and highly variable phenotype, including a normal phenotype. Another aberration which is detected in MR patients as well as in healthy individuals is a 253 kb deletion in 15q11.2 (Murthy et al., 2007; Doornbos et al., 2009). This deletion was first reported in a boy with MR and dysmorphic features. He inherited it from his father, who had a history of developmental delay (Murthy et al., 2007). Doornbos and colleagues studied nine patients with the same deletion. In seven cases it was inherited from one of the parents. Only one of these parents showed an affected phenotype (the same developmental and behavioural problems as in the child). They concluded that a deletion in this region was associated with variable phenotypes, some of which could be explained by other genetic or environmental modifiers.

One of the consequences of the continuous increase in the resolution of the whole-genome arrays is that also the number of detected pathogenic, benign and potentially pathogenic CNVs has increased. Estimates indicate that approximately 12% of the human genome may involve CNVs (Redon et al., 2006). The detected CNVs may be difficult to interpret in both clinical and research settings. Different workflows have been developed and are used to categorize CNVs into the different groups (Lee et al., 2007; Koolen et al., 2009; **Chapter 2**). In general, CNVs that overlap with known microdeletion or microduplication syndromes are likely to be pathogenic. Benign CNVs are mostly determined by comparing them with healthy or normal reference sets from one's own laboratory or with the data in the Database of Genomic Variants (DGV). The DGV documents structural variants of 1 kb or more that were detected in apparently healthy individuals (http://projects.tcag.ca/variation). This database is still growing and laboratories that use high-resolution whole-genome arrays use it to screen for genomic deletions and amplifications to exclude the benign CNVs. Caution should be exercised when using this database as DGV is not always reliable. This is because many CNVs have been reported in single individuals, different platforms have been used in determining the CNVs and data submitted to this database is not subject to curation or an editorial screening. It is possible that 'normal' healthy individuals reported in the

DGV have phenotypic abnormalities which would have been noticed if the person had been seen by a clinical geneticist. Another example illustrating the need for caution is a case where a heterozygous deletion of 15q15.3 reported in the DGV turned out to be pathogenic in the homozygous state (Knijnenburg et al., 2009). Some heterozygous deletions could be pathogenic if there is a mutation on the other allele. Furthermore, there are CNVs such as 22q11.2 deletion that may show incomplete penetrance.

CNVs that do not overlap with known microdeletion or microduplication syndromes and are not reported as benign variants are categorized as potentially pathogenic. Databases have been developed to collect cases of potentially pathogenic CNVs found in individuals seen in genetic clinics. DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER) collects clinical information about patients with microdeletions, microduplications, insertions, translocations and inversions (https://decipher.sanger.ac.uk). Another database with similar aims is the European Cytogenetics Association Register of Unbalanced Chromosome Aberrations (ECARUCA, http://www.ecaruca.net). CNVs that are not found in the DGV, should be compared with and added to one of these databases to create a platform of molecular cytogenetic and clinical data. This would enable collaboration between clinical genetic centres that have found (approximately) the same CNV in phenotypically similar patients and should make it possible to unravel new microdeletion and microduplication syndromes. The gene content, the size, and the inheritance pattern of potentially pathogenic CNVs are important factors that influence pathogenicity.

The implementation of high-resolution whole-genome arrays in cohorts with unexplained MR patients has resulted in the identification of new microdeletion and microduplication syndromes. These new syndromes were determined by screening large groups of patients with a similar clinical phenotype, mostly unexplained MR (reviewed by Slavotinek 2008; Vissers et al., 2009).

Chapter 2 describes the implementation of the SNP arrays in the diagnosis of MR. An increase from approximately 5% chromosomal aberrations found by conventional karyotyping to 22.6% aberrations by SNP array was noticed. However, most of the detected CNVs are neither described in the DGV nor are they known to be associated with microdeletion or microduplication syndromes. At present we do not know their clinical significance, so most of them have been classified as potentially pathogenic for the time being. More patients or healthy individuals with the same CNV are needed before we can understand their clinical significance.

We are seeing history repeat itself. Similar problems arose with the heterochromatic variants when karyotyping and banding were first introduced. The same was true for euchromatic variants identified by high resolution banding. In a few years one can expect that most of the potentially pathogenic CNVs will have been classified into either pathogenic or benign CNVs. We will then be faced with the same challenge at the base pair level with the introduction of the next-generation whole genome sequencing.

5. Clinical cytogenetic diagnostics

Patients are referred for chromosome analysis for various reasons which can be divided in three groups. 1) Postnatal diagnosis: this includes patients with multiple congenital abnormalities with or without mental retardation (MCA/MR), individuals with infertility and abnormal sexual development and couples with recurrent miscarriages.

2) Prenatal diagnosis: increased maternal age, ultrasound abnormalities in the foetus and chromosome abnormalities in the family. 3) Tumorcytogenetics: acquired chromosome abnormalities in tumor cells.

Identification of chromosomal abnormalities is particularly important for a child with MCA/MR, for diagnosis and prognosis and for estimating the recurrence risk for the parents and other family members. Mental retardation is a highly diverse neurologic disorder with an incidence of 1-3% in the general population (Roeleveld et al., 1997; Leonard and Wen 2002). It is a lifelong disability characterized by impairment of cognitive and adaptive skills, with or without dysmorphic features. MR presents most often during infancy or in the first years of school. The cause of MR can be identified in only about 50% of all patients and is therefore one of the major unsolved problems in modern medicine (Battaglia and Carey, 2003).

The underlying causes of MR are extremely heterogeneous. There are non-genetic factors that can act prenatally, perinatally or during early infancy to cause brain injury. These include infectious diseases (such as rubella, toxoplasmosis and cytomegalovirus during pregnancy and postnatal meningitis), oxygen deprivation (perinatal event), very premature birth and fetal alcohol syndrome, which is caused by excessive maternal alcohol consumption during pregnancy. Genetic factors include (1) single-gene disorders, such as Rubinstein-Taybi syndrome (*CREBBP*-gene) (Petrij et al., 1995) and Pitt-Hopkins syndrome (*TCF4*-gene) (Amiel et al., 2007), (2) chromosomal abnormalities, including presence of an extra chromosome, as in Down syndrome (Lejeune et al., 1959), or a deletion or a duplication of a chromosome segment, for example 5p- in Cri-du-chat syndrome (Lejeune et al., 1963) and microdeletion in 22q11.2 in DiGeorge syndrome (de la Chapelle et al., 1981; Kelley et al., 1982), (3) multifactorial disorders, due to a combination of multiple genetic as well as environmental causes and (4) mitochondrial disorders, caused by alterations in the small cytoplasmic mitochondrial chromosome (e.g. Leigh syndrome OMIM # 256000).

Patients with unexplained MR/MCA, who are referred to genetic laboratories, were screened initially with conventional karyotyping and if necessary with targeted FISH or MLPA analysis. The combined diagnostic yield of these analyses is approximately 5-10% (de Vries et al., 2005). Consequently, a clinical diagnosis is lacking in the majority of these patients and this impedes development of treatment strategies and adequate genetic counseling. Therefore, new high-resolution whole-genome technologies facilitating an increased detection rate of subtle chromosome imbalances have been developed to improve diagnosis of MR/MCA patients.

6. Scope of this thesis

The main aim of this thesis is to detect genome-wide submicroscopic CNVs at high resolution in a cohort of idiopathic MR patients to identify genomic regions or loci involved in developmental disorders. SNP arrays, from two manufacturers (Affymetrix and Illumina) were used to explore the possibility of identifying these abnormalities. The different arrays were directly compared for detection rate, accuracy, software, and costs. By applying these arrays, new regions involved in the etiology of MR were identified, resulting in an increase in the number of patients with a known cause for their developmental disorder. These methods have a significantly improved sensitivity as compared to the conventional karyotyping and FISH analyses. The Affymetrix array was finally chosen and implemented as a first-tier routine diagnostic analysis for MR patients.

Chapter 2 describes the platform comparison and implementation of the SNP array in the diagnosis of MR. **Chapter 3** demonstrates the difficulty in differentiating between CNVs that are possibly implicated in the etiology of MR and those that are not. **Chapter 4** presents two case reports. The first shows that the SNP arrays can be used to further characterize a complex chromosomal abnormality detected with conventional karyotyping. The second shows that not every CNV, in this case a large CNV, is pathogenic. **Chapter 5** is comprised of two studies using patients with phenotypes other than MR. The first report is about patients with a BPES-like phenotype and the second is about patients with a SOTOS-like phenotype. Both patient groups were screened for CNVs. **Chapter 6** includes the conclusions and a discussion on the future applications of the SNP array technology.

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