4 Cytogenetic Testing and Chromosomal Disorders

Joris Robert Vermeesch · Karen Buysse

Cytogenetic Testing

It took until 1956 before the correct number of human chromosomes of 46/cell was determined by Tjio and Levan. The discovery of the correct number of human chromosomes lead to the subsequent discovery that trisomy 21 is the cause of Down syndrome. Soon thereafter, a series of associations of different birth defects with specific chromosomal imbalances became apparent. First, the chromosomes 13 trisomy (Patau syndrome), trisomy 18 (Edwards syndrome), and monosomy and trisomy X syndromes were identified. Subsequently, smaller segmental chromosomal imbalances such as 5p- and 4p- were proven to cause birth defects. These associations launched cytogenetic genetic testing as a routine diagnostic tool and resulted in systematic screening for children with birth defects. These screenings, in turn, resulted in the identification of thousands of chromosomal imbalances associated with specific syndromic features.

Initially, chromosome studies were performed using simple staining techniques that only allowed the detection of entire groups of chromosomes. The degree of precision was increased in the 1970s with the introduction of chromosome banding techniques. These techniques enabled the detection of individual chromosomes and segments (bands) within chromosomes. Although chromosomal karyotyping allows a genome-wide detection of large chromosomal abnormalities and translocations, it has a number of inherent limitations: (1) it takes 4–10 days to culture the cells, visualize the chromosomes and perform the analysis; (2) the resolution is limited to 5–10 Mb depending on (a) the location in the genome, (b) the quality of the chromosome preparation, and (c) the skill and experience of the cytogeneticist; (3) it requires skilled technicians to perform a Giemsa-banded karyotype analysis, which increases employment costs and can lead to organizational difficulties in small laboratories.

With the introduction of fluorescence in situ hybridization (FISH), the detection of submicroscopic chromosomal imbalances (imbalances not visible by conventional karyotyping because they are too small) became possible. In FISH, labeled DNA probes are hybridized to nuclei or metaphase chromosomes to detect the presence, number, and location of small (submicroscopic) regions of chromosomes. FISH is routinely applied to confirm the clinical suspicion of known microdeletion syndromes. Some common examples are the detection of the velocardiofacial (VCFS, 22q11 deletion, OMIM 192430), William's (7q11.23 deletion, OMIM 194050) and Prader-Willi (15q11.2–13 deletion, OMIM 176270) syndromes. FISH also detects deletions in the gene-rich subtelomeres, which are involved in mental retardation and a number of syndromes, such as the Wolf–Hirschhorn (deletion 4p, OMIM 194190) and chromosome 1p36 deletion (OMIM 607872) syndromes.

Unfortunately, FISH can only detect individual DNA targets rather than the entire genome. To overcome this problem, multicolor FISH–based karyotyping (SKY, MFISH, and COBRA FISH) was developed, which enables simultaneous detection of all chromosomes. Another technology allowing the genome-wide detection of copy number aberrations was introduced in 1992 and termed comparative genomic hybridization (CGH). In CGH, test and reference genomic DNAs are differentially labeled with fluorochromes and then co-hybridized onto normal metaphase chromosomes. Following hybridization, the chromosomes are scanned to measure the fluorescence intensities along the length of the normal chromosomes to detect intensity ratio differences that subsequently pinpoint to genomic imbalances. Overall, the resolution at which copy number changes can be detected using these techniques are only slightly higher as compared to conventional karyotyping (>3 Mb) and experiments are labor intensive and time consuming.

By replacing metaphase chromosomes with mapped DNA sequences or oligonucleotides arrayed onto glass slides as individual hybridization targets, the resolution could be tremendously increased. Following hybridization of differentially labeled test and reference genomic DNAs to the target sequences on the microarray, the slide is scanned to measure the fluorescence intensities at each

Abdelaziz Y. Elzouki (ed.), *Textbook of Clinical Pediatrics*, DOI 10.1007/978-3-642-02202-9_4,

target on the array. The normalized fluorescent ratio for the test and reference DNAs is then plotted against the position of the sequence along the chromosomes. Gains or losses across the genome are identified by values increased or decreased from a 1:1 ratio ($\log_2$ value of 0), and now the detection resolution only depends on the size and the number of targets on an array and the position of these targets (their distribution) on the genome. A schematic overview of the technique is provided in *Fig. 4.1*. This methodology was first described in 1997 and is termed "matrix CGH" or "array CGH". Array CGH has initially been employed to analyze copy number changes in tumors with the aim to identify genes involved in the pathogenesis of cancers. More recently however, this methodology has been optimized and applied to detect unbalanced constitutional human rearrangements. With improved protocols, it rapidly became clear that not only larger insert BAC clones were appropriate targets for array CGH, but also smaller-sized cDNA fragments, PCR products, and oligonucleotides. In addition to comparative hybridization using two differentially labeled DNA samples, single sample hybridization can also be compared versus different reference arrays. This approach is the basis of the so-called SNP arrays.

Genome-wide array CGH has been called molecular karyotyping in analogy with conventional karyotyping. Because many cytogeneticists object to this term, most recently, the term "cytogenomic array" has been put forward to refer to high-resolution array-based whole genome testing for genomic copy number (recommendation of the consortium of International Standards on Cytogenomic Arrays (ISCA), https://isca.genetics.emory.

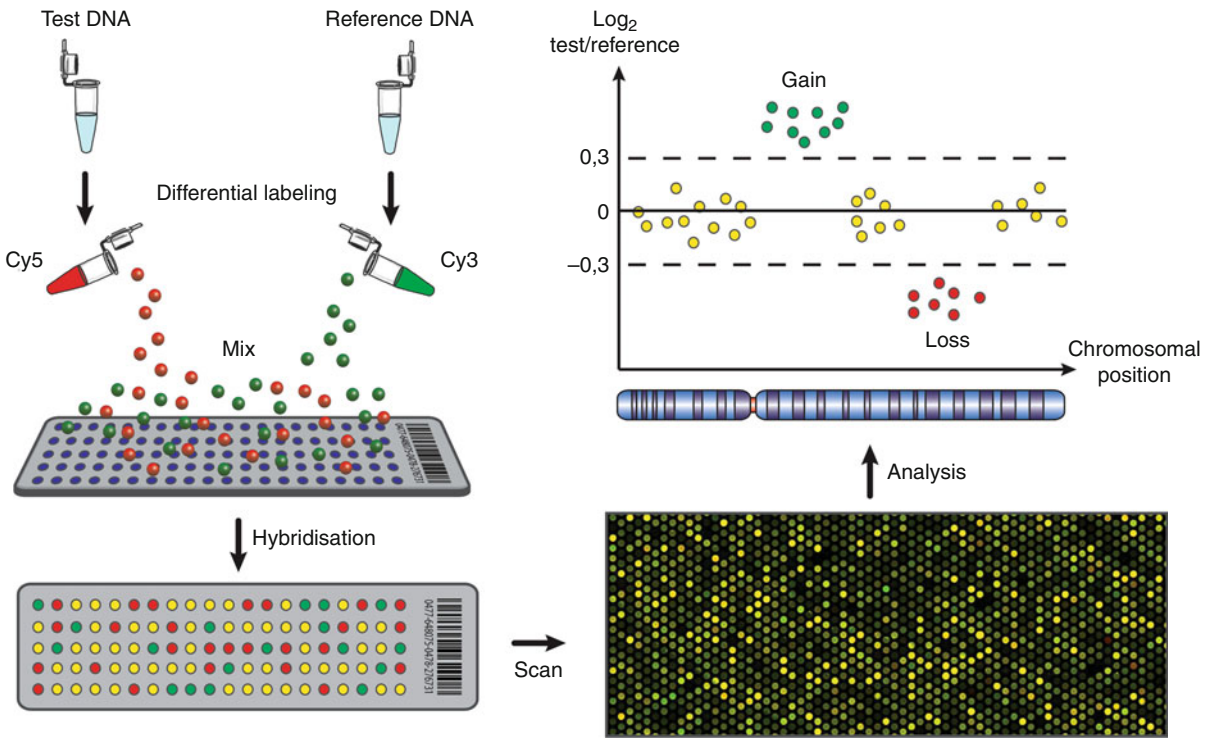

Figure 4.1

Principle of array CGH. Equal amounts of test and reference DNA are differentially labeled with fluorochromes (e.g., Cy5 and Cy3), mixed and when necessary supplemented with Cot-1 DNA to block repetitive sequences. This mixture is denatured and hybridized on a microarray slide on which DNA probes (e.g., BACs or oligonucleotides) are immobilized. Slides are scanned and fluorescent intensities quantified from the image. Signal intensity ratios are plotted corresponding to the genomic position of the DNA probe and represent the relative DNA copy number of the test DNA in comparison with the reference DNA

edu/iscaBrowser/learnabout.jsp). The implementation of genome-wide arrays as a tool to screen children with developmental anomalies has increased the diagnostic yield significantly. A series of early studies showed diagnostic yields between 9% and 25%. A meta-analysis of patients with congenital and mental anomalies on 13,926 subjects reported an overall diagnostic rate of 10% for causal anomalies and a retrospective analysis of 36,325 patients revealed abnormalities in about 19% of the patients. While conventional G-banding has a diagnostic yield of about 3% in similar patient populations, it can be concluded that molecular karyotyping is outperforming conventional karyotyping for the detection of causative chromosomal imbalances in patients with birth defects. Therefore, the technology is currently complementing traditional cytogenetic testing and is recommended as a first-tier diagnostic test for children with developmental disorders.

Types and Incidences of Chromosomal Abnormalities

Microscopically Visible Chromosomal Imbalances

All chromosomal imbalances that can be detected by conventional karyotyping are microscopically visible. These aberrations are either numerical (abnormal chromosome number) or structural (altered structure).

Numerical Chromosome Aberrations

Normal humans are diploid, meaning they have 22 pairs of autosomes and one pair of sex chromosomes. The presence of three sets (triploidy) or four sets (tetraploidy) can occasionally occur; however, these are not viable.

Numerical aberrations result from the loss (monosomy) or gain (trisomy) of an individual chromosome. Autosomal monosomies are inviable, while the absence of one X chromosome may result in a liveborn girl with Turner syndrome (45,X). A few autosomal trisomies are compatible with life. Fetuses with trisomy 13 (Patau syndrome) and trisomy 18 (Edwards syndrome) can survive to term but usually die shortly after birth due to severe congenital anomalies. Individuals with trisomy 21 (Down syndrome) can stay alive longer, with an average life span reaching up to 55 years. In addition, sex chromosomal trisomies and tetrasomies are often encountered; the best known is Klinefelter syndrome (47,XXY).

Structural Chromosome Aberrations

Structural chromosomal aberrations result from double-strand breaks and inappropriate DNA repair leading to translocations, deletions, duplications, inversions, isochromosomes, and ring chromosomes (*Fig. 4.2*). They may involve single or multiple chromosomes. One can distinguish rearrangements without and with loss or gain of chromosomal segments. The former are most often not associated with an abnormal phenotype, while the latter most often cause developmental disorders. Carriers of apparently balanced rearrangements, however, are at risk for having children with chromosomal imbalances.

In translocations, chromosomal segments between two or more chromosomes are exchanged. Robertsonian translocations are translocations between two acrocentric chromosomes (chromosomes 13, 14, 15, 21, and 22). There is no loss of euchromatin and the carriers are normal. In reciprocal translocations, segments between two chromosomes are exchanged. The translocation is termed balanced if no chromosomal material has been lost or gained. Inversions represent a special type of apparently balanced rearrangement. In an inversion, the rearrangements occur intrachromosomally and a chromosomal segment is inverted. If the inversion occurs within one chromosomal arm it is called "paracentric" (not including the centromere); if it occurs in two chromosomal arms it is termed "pericentric" (spanning the centromere). Carriers of balanced translocations and inversions are usually normal, but developmental anomalies are detected in 6% of de novo translocation carriers. The presence of a developmental disorder can be due to (1) the breakage of a gene resulting in a dominant disorder or in a recessive disorder if the second allele is also mutated, (2) a position effect on a gene flanking the breakpoint, or (3) the gain of function via the creation of a fusion gene. Recently, it was shown that 40% of the apparently balanced translocation carriers with developmental disorders have submicroscopic imbalances at the breakpoints or elsewhere in the genome that may be disease causing.

If genetic material is gained or lost, the abnormality is called unbalanced. If there is loss of a chromosomal segment it is called a deletion, or if there is a gain, a duplication. The presence of both a large deletion and duplication suggests the presence of an unbalanced translocation. Those usually result from the transmission of the unbalanced products during the meiosis of a balanced translocation-carrying parent. Occasionally unbalanced

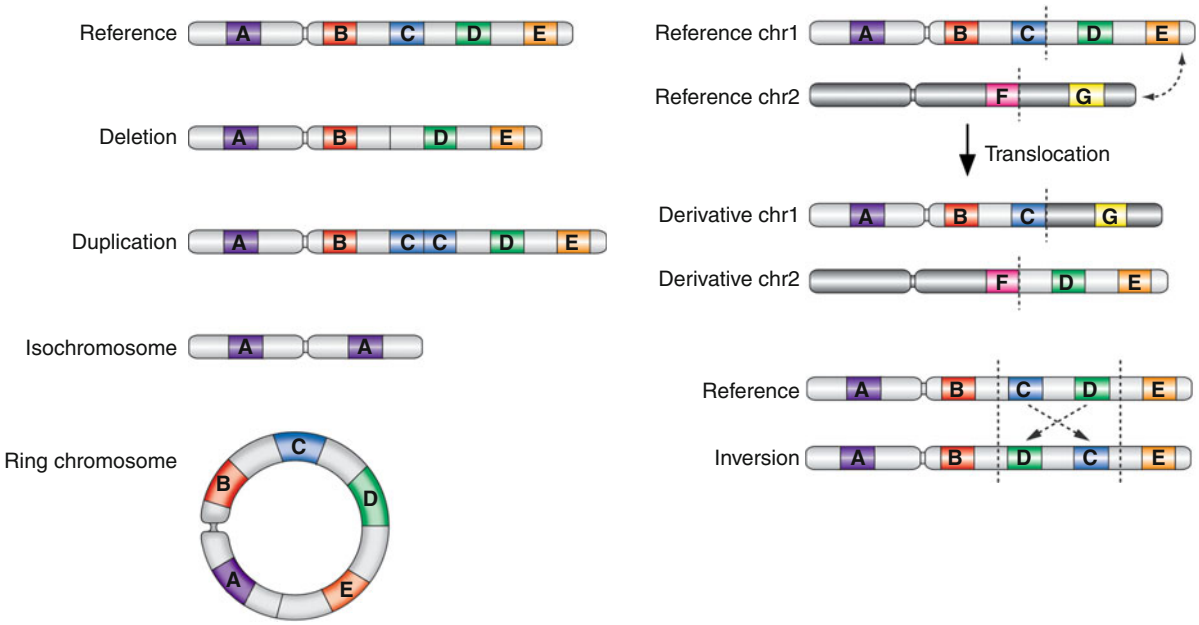

Figure 4.2

Schematic overview of structural chromosomal rearrangements. A deletion results in the loss of chromosomal material (segment C), while there is a gain of material (two copies of part C) in a duplication. Duplication of one arm and deletion of the other arm gives rise to an isochromosome, while fusion of the short and long arms of a chromosome leads to a ring chromosome. In a translocation, part of one chromosome is transferred to another chromosome. In this example, there is an exchange between segments D and E of chromosome 1 and segment G of chromosome 2. An inversion results in a reversed orientation of genetic material (inversion of segments C and D)

translocations arise de novo. The deletion or duplication of one or more dosage-sensitive genes usually results in developmental disorders.

Incidence of Chromosomal Abnormalities

Studies performed in the late 1960s and early 1970s (i.e., before the widespread use of prenatal diagnosis and pregnancy intervention) provide estimates for the frequencies of chromosomal abnormalities at birth. A combined survey of 68,159 livebirths and of 34,910 liveborns found that 0.65–0.84% of newborns or 1 in 119–154 livebirths had a major chromosomal abnormality (*Table 4.1*). Trisomy 21 (Down syndrome) was shown to be the most frequent chromosomal anomaly, with an incidence of 1.2–1.7/1,000 liveborns. Sex chromosome aneuploidies were the next most common, with approximately one XYY and one XXY in every 900–1,000 male and one XXX in every 900–1,000 female livebirths. Structural balanced rearrangements had a frequency of approximately 2/1,000 livebirths.

Structural rearrangements can occur de novo or be the consequence of the unbalanced transmission of a parent carrying a chromosomal rearrangement. However, all arose de novo at one point. It is estimated that de novo balanced reciprocal translocations arise at birth with a frequency of 1.6×10^{-4} and unbalanced rearrangements with a frequency of 2.9×10^{-4}.

Submicroscopic Imbalances

The first methodology to enable the visualization of imbalances below the resolution of regular light microscopes was FISH. Recurrent syndromes were proven to be caused by recurrent submicroscopic imbalances. Once the imbalance was characterized, metaphase spreads or interphase nuclei of patients with similar phenotypes could be screened with locus specific probes for the loss or gain of a specific locus. This methodology requires careful clinical examinations in order to instigate appropriate genetic testing.

Table 4.1
Incidence of chromosomal abnormalities in newborns

Type of abnormality	Rate per 1,000 Benn and Hsu (2004)	Rate per 1,000 Nielsen and Wohlert (1991)
Autosomal trisomies		
+13	0.04	0.09
+18	0.13	0.29
+21	1.2	1.69
Sex chromosomes males		
47,XYY	1.03	1.18
47,XXY	1.03	1.57
Other	0.73	0.17
Sex chromosomes females		
45,X	0.24	0.53
47,XXX	1.09	1.06
Other	0.36	0.06
Structural balanced		
Robertsonian	0.9	1.23
Reciprocal and insertional	1.21	1.74
Structural unbalanced		
Deletions & duplications	0.4	0.34
Marker chromosomes	0.2	0.66
Total	6.24	8.42

With the advent of cytogenomic arrays, a true revolution in the analysis of genomes in general and especially the analysis of the genomes of patients with mental retardation and developmental anomalies is taking place for two reasons: (1) It has now become possible to screen the genome at very high resolution for copy number changes and (2) no *a priori* clinical identification is required to enable correct cytogenetic testing. In the last 5 years, more pathogenic copy number changes have been linked to developmental disorders than in the 50 years before.

Recurrent Submicroscopic Rearrangements

Recurrent imbalances often result from nonallelic homologous recombination (NAHR) between low-copy repeats (LCRs) flanking the commonly deleted or duplicated region (see Recurrent Submicroscopic Imbalances). Many of such recurrent imbalances, also known as *genomic disorders*, were identified before the array era and were often known as clinically well-delineated syndromes and are typically screened for by FISH. The first recurrent imbalance identified was the imbalance at 17p12 associated with Charcot-Marie-Tooth disease type 1A (CMT1A, OMIM 118220). A list of well-known recurrent submicroscopic imbalance syndromes is shown in *Table 4.2*. With the advent of molecular karyotyping, a series of novel recurrent imbalances causal for or associated with MR/MCA (mental retardation/multiple congenital anomalies) have been identified and these are listed in *Table 4.3*.

Nonrecurrent Submicroscopic Rearrangements

For several genomic regions, overlapping rearrangements have been identified that show variable breakpoints in each patient. Despite the different sizes, these nonrecurrent imbalances share a shortest region of overlap (SRO) for which a copy number change may lead to similar phenotypes in different patients. Two pertinent examples are the *MECP2* gene duplications at Xq28 and the 12q14 microdeletion syndrome.

Table 4.2

Microdeletion/duplication syndromes associated with developmental disorders identified before the advent of array CGH

Syndrome	Chromosome location	Deletion incidence	Parental origin	Deletion size (Mb)	Gene (incidence)
Sotos	5q35	ND	Paternal (90%)	2.2	*NSD1* (10%)
Williams	7q11.23	1/20,000–1/50,000	Equal	1.6	CGS
8p deletion	8p23.1	ND	Maternal	5	CGS
Prader–Willi	15q11.2–13	1/20,000	Paternal	3.5	CGS
Angelman	15q11.2–13	1/20,000	Maternal	3.5	*UBE3A* (10–15%)
Smith–Magenis	17p11.2	1/25,000	Equal	4	*RAI1* (ND)
Neurofibromatosis 1	17q11.2	1/40,000–1/80,000	Maternal	1.5	*NF1* (90–95%)
Velocardiofacial	22q11.2	1/4,000	Equal	3 (1.5)	CGS

CGS contiguous gen syndrome, *ND* not determined

Table 4.3

Newly recognized interstitial microdeletion/duplication syndromes identified by array CGH and associated with developmental disorders

Name	Size (Mb)	OMIM	Clinical features	Reference
1q21 microdeletion (TAR syndrome)	0.5	27400	Hypomegakaryocytic thrombocytopenia and bilateral radial aplasia	(Klopocki et al. 2007)
1q21 microdeletion & microduplication	1.35	612474 and 612475	Asymptomatic to severe developmental delay and multiple congenital anomalies, susceptibility locus for neuropsychiatric disorders	(Brunetti-Pierri et al. 2008; Mefford et al. 2008)
3q29 microdeletion	1.6	609425	MR, mild FD including high nasal bridge and short philtrum	(Ballif et al. 2008; Willatt et al. 2005)
3q29 microduplication	1.6	611936	Mild/moderate MR, MC, obesity	(Ballif et al. 2008; Lisi et al. 2008)
7q11.23 microduplication	1.5	609757	MR, speech and language delay, autism spectrum disorders	(Somerville et al. 2005)
15q13.3 microdeletion	1.5	612001	MR, epilepsy, FD, digital dysmorphisms	(Sharp et al. 2008)
15q24 microdeletion	1.7		MR, growth retardation, MC, digital abnormalities, genital abnormalities	(Sharp et al. 2007)
16p13.11 microdeletion	1.7		MR, MC, seizures	(Hannes et al. 2009; Ullmann et al. 2007)
17p11.2 microduplication	3.7	610883	MR, infantile hypotonia, autistic features	(Potocki et al. 2007)
17q21.31 microdeletion	0.5	610443	MR, hypotonia, typical face	(Koolen et al. 2006; Sharp et al. 2006; Shaw-Smith et al. 2006)
22q11.2 distal microdeletion	1.4–2.1	611867	MR, growth delay, mild skeletal abnormalities, FD.	(Ben-Shachar et al. 2008)

FD facial dysmorphism, *MC* microcephaly, *MR* mental retardation

Mechanisms Causing Genomic Disorders

Mutations causing chromosomal rearrangements can occur during both meiosis and mitosis. Classically, meiosis has been considered the main period during which chromosomal rearrangements occur. Chromosomes are very active during meiosis, because the homologues pair, synapse, and crossover. During this process, multiple DNA nicks are generated and it is likely that some of the rearrangements originate as a result of these processes. The recent discovery of large-scale chromosomal rearrangements in the cleavage stage embryo makes it likely that many chromosomal rearrangements originate at this time. How the chromosomal breaks originate remains unclear.

Aneuploidies

The majority of aneuploidies arise via meiotic nondisjunction events, though mitotic nondisjunction events are also a frequent cause of constitutional aneuploidies. Nondisjunction is defined as the failure of homologous chromosomes to segregate symmetrically at cell division. If the pair of homologues comprising a bivalent at meiosis I fail to separate, one daughter cell will have two of the chromosomes while the other will have none (*Fig. 4.3a*). Nondisjunction may also occur in meiosis II when the chromatids fail to separate (*Fig. 4.3b*). In both meiotic errors, the conception ends up trisomic or monosomic. The majority of the nondisjunction events appear to occur at meiosis I. An alternative mechanism for nondisjunction is premature separation of the chromatids. First, homologues fail to pair during meiosis I. These univalents are prone to predivide, that is, separation of the two chromatids, and subsequently these chromatids segregate independently (*Fig. 4.3c*). Since the frequency of meiotic errors increases with advanced maternal age, not surprisingly the overwhelming majority of aneuploidies are of maternal origin.

Recurrent Submicroscopic Imbalances

Recurrent rearrangements are often flanked by low-copy repeats (LCRs). LCRs or segmental duplications (SDs) are segments of DNA that map to two or more genomic locations, are >1 kb in size and share a sequence identity of at least 90%. Segmental duplications account for about 5% of the human genome. Due to their high degree of sequence homology, these segmental duplications provide substrates for nonallelic homologous recombination (NAHR) in which crossing over occurs between two similar sequences at nonallelic positions that erroneously align in mitosis or meiosis (*Fig. 4.4*). Depending on their location and orientation, they give rise to various types of rearrangements. Misalignment and subsequent recombination between two LCRs that are in direct orientation on the same chromosome cause deletions and duplications, while inversions are driven by LCRs that are in

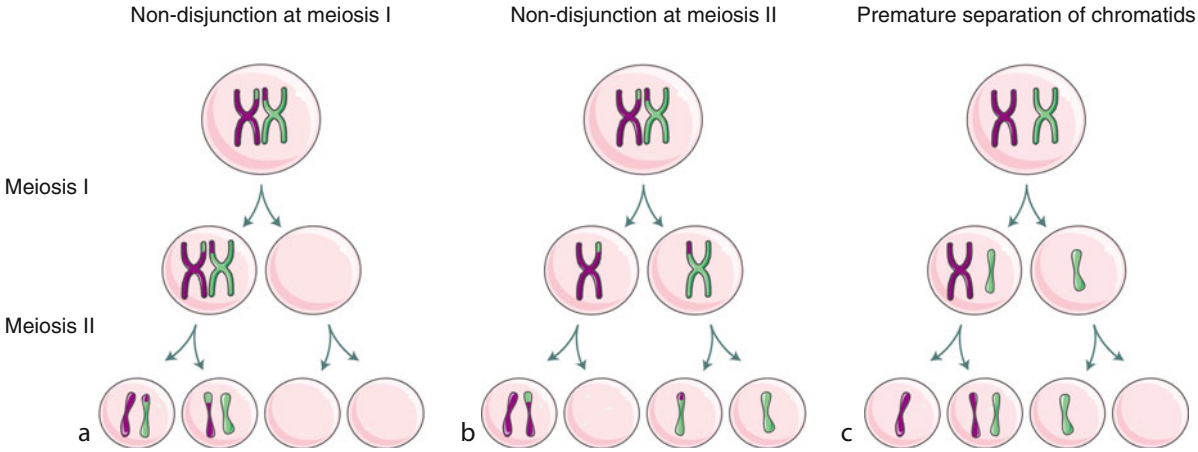

Figure 4.3

Different possibilities for nondisjunction during meiosis leading to trisomic or monosomic conceptions. (a) Nondisjunction at meiosis I; (b) nondisjunction at meiosis II; (c) premature separation of the chromatids of one of the homologous chromosomes at meiosis I and subsequent random migration of the chromatid to either pole at meiosis II

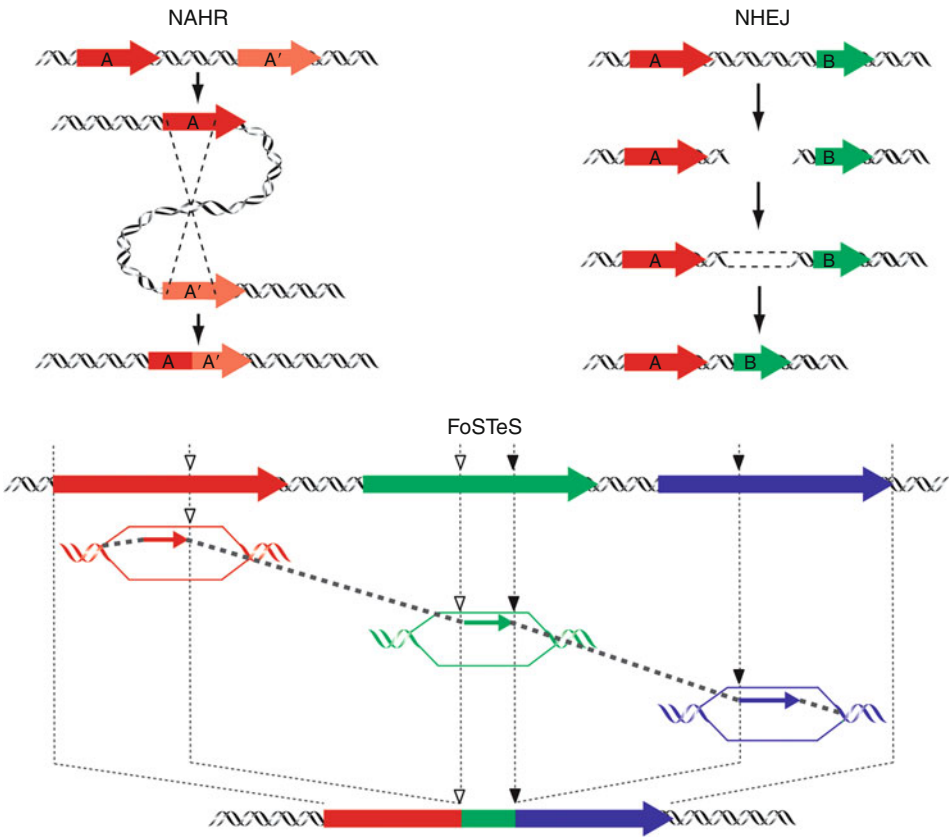

Figure 4.4

Schematic representation of nonallelic homologous recombination (NAHR), nonhomologous end-joining (NHEJ), and Fork Stalling and Template Switching (FoSTeS) mechanisms that lead to chromosomal rearrangements. The examples shown here lead to genomic deletion. *Upper left panel*: an intrachromatid NAHR event. The arrows A and A′ depict two highly homologous low-copy repeats (LCRs) that are in direct orientation. The LCRs align at nonallelic positions and subsequent recombination results in deletion of part of the two LCRs and the segment in between them. *Upper right panel*: a NHEJ event. Double-strand breaks (DSB) occur between two sequences that share no homology, represented as differently sized arrows (A and B). The NHEJ system modifies and rejoins the two ends, resulting in the deletion of the segment between the two DSBs. *Lower panel*: a FoSTeS × 2 event. The arrows depict three substrate sequences that do not share extensive homology. However, the small open and filled triangles depict a site of microhomology between the respective sequences. The leading strand of the first fork invades the second fork via the site of microhomology and primes its own further synthesis using the second fork as template. This event happens again between the second and third fork, leading to the deletion of the two fragments flanked by each pair of microhomology sites. This results in the juxtaposition of genomic sequences from multiple distinct regions yielding a complex deletion (Adapted from Gu W, Zhang F, Lupski JR (2008) Mechanisms for human genomic rearrangements. Pathogenetics 1:4)

opposite orientation on the same chromosome. NAHR between LCRs on different (nonhomologous) chromosomes leads to translocations. Recombination may occur between LCRs on the same chromatid (intrachromatid), on sister chromatids (intrachromosomal or interchromatid) or on homologous chromosomes (interchromosomal). The efficiency of NAHR is influenced by the distance, size, and degree of homology between two LCRs. Larger genomic rearrangements tend to correlate with larger LCRs and most genomic disorders result from NAHR between LCRs that are 10–400 kb in length and have >96% sequence identity.

NAHR can also be mediated by highly homologous repetitive sequences such as *Alu*'s (a class of SINEs, short interspersed nuclear elements) and LINEs (long interspersed nuclear elements) or LTRs (long terminal repeats), thus accounting for some of the nonrecurrent rearrangements.

The incidence of those recurrent genomic disorders varies and their estimated incidence for well-established recurrent disorders is indicated in *Table 4.2*.

Nonrecurrent Submicroscopic Imbalances

Nonhomologous End-Joining (NHEJ)

NHEJ is one of the two major repair mechanisms (the other being homologous recombination) for double-strand breaks (DSB) in mammals. After detection of the DSB and molecular bridging of the broken DNA ends, modifications are made to the ends to make them compatible for the final ligation step (*Fig. 4.4*). This process implies two important characteristics of NHEJ: it does not require sequence homology at the breakpoints and it leaves an "information scar" at the rejoining site due to the addition or deletion of several nucleotides. Interestingly, breakpoints of nonrecurrent rearrangements that are apparently caused by NHEJ are often located within LCRs or repetitive elements such as LTR, LINE, *Alu,* and MER2 DNA elements. This indicates that NHEJ may be stimulated and regulated by specific genomic features.

Fork Stalling and Template Switching (FoSTeS)

By breakpoint sequence analysis of nonrecurrent *PLP1* duplications associated with Pelizaeus-Merzbacher disease, Lee et al. discovered an unexpected complexity that is inconsistent with a simple recombination model. Within the duplicated sequence, they found interspersed stretches of DNA that were triplicated or of normal copy number and additional sequence complexity at the junctions. They proposed a model of replication Fork Stalling and Template Switching to explain these complex duplication and deletion rearrangements. During DNA replication, the replication fork stalls or pauses at a DNA lesion and the leading or the lagging strand disengages and switches to another replication fork where it anneals on the invaded site by virtue of microhomology and restarts DNA synthesis (*Fig. 4.4*). The replication forks are in physical proximity, but may be separated by sizeable linear distances, even megabases away. This procedure of disengaging, invading/annealing and synthesis/extension could occur multiple times in series (that is FoSTeS × 2, FoSTeS × 3 and so on), causing the observed complex rearrangements. Depending on whether the invaded fork is located downstream or upstream, this will result in a deletion or a duplication event, respectively.

Interestingly, the genomic positions at which FoSTeS occurs show a very complex genomic architecture including multiple LCRs, cruciforms, and palindromes that may stimulate and facilitate the FoSTeS mechanism. As opposed to NAHR and NHEJ, a single-strand DNA lesion is the initiating damage rather than a double-strand break.

Microhomology-Mediated Break-Induced Replication (MMBIR)

As an alternative to FoSTeS, the MMBIR model has been proposed in which the rearrangement is initiated by a single-end double-strand DNA break resulting from a collapsed replication fork. This model is based on the break-induced replication model observed in yeast. The single-strand 3′ tails from the broken replication fork will anneal with microhomology on any single-stranded DNA nearby, where it forms a new replication fork. The replication in this new fork is of low processivity and the extended end will dissociate and invade different templates. Multiple template switches generate complex rearrangements until there is reestablishment of processive replication (*Fig. 4.5*). Again, complex genomic architecture may play a role in this process by generating secondary DNA structures such as cruciforms and hairpin loops that expose single-stranded sequence.

Molecular Mechanisms Leading to Phenotypes

There are several ways in which chromosomal rearrangements can lead to a clinical phenotype. The most obvious mechanism is altering the copy number of dosage-sensitive genes that are encompassed within the rearrangement (*Fig. 4.6a*). When the breakpoint is located within a gene, it will be disrupted, leading to loss-of-function. The disruption can occur either through deletion (*Fig. 4.6b*), duplication, or translocation, as well as inversion (*Fig. 4.6c*). Alternatively, new transcripts can be created at the breakpoint through gene fusion (*Fig. 4.6d*) or exon shuffling. This leads to gain-of-function mutations, a mechanism that is

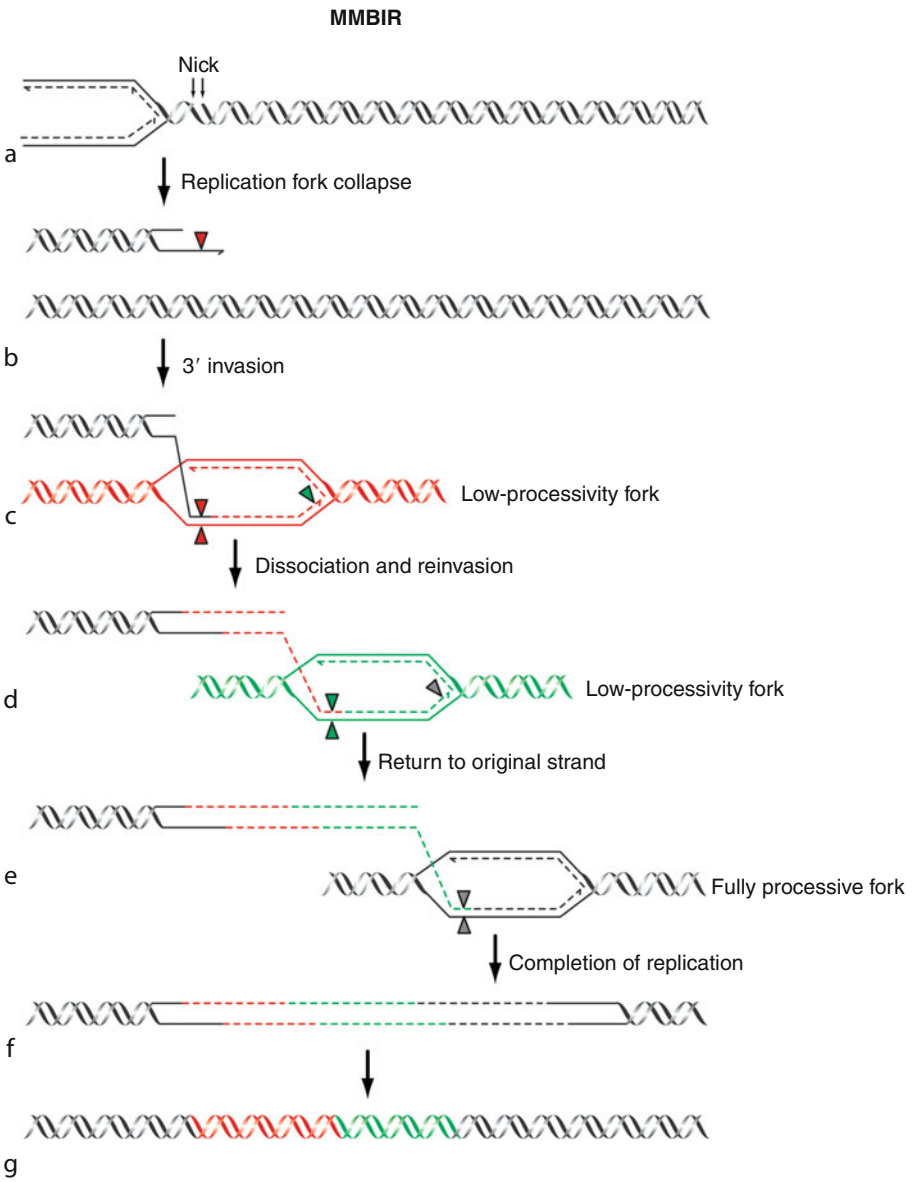

Figure 4.5

Schematic representation of microhomology-mediated break-induced replication (MMBIR). Successive switches to different genomic positions forming microhomology junctions (filled triangles) are shown. When a replication fork encounters a nick in a template strand (a), one arm of the fork breaks off, producing a collapsed fork (b). The single-stranded 3′ end will invade a site of microhomology (filled triangle) and form a new low-processivity fork (c). The extended end will dissociate repeatedly and reform the fork on different templates, using sites of microhomology (d). When the switch returns to the original sister chromatid (e) it will form a processive replication fork that completes replication (f). The final product contains sequences from different regions (g). Whether the return to the sister chromatid occurs in front of or behind the position of the original collapse determines if there is a deletion or duplication (Adapted from Hastings PJ, Ira G, Lupski JR (2009) A microhomology-mediated break-induced replication model for the origin of human copy number variation. PLoS Genet 5:e1000327)

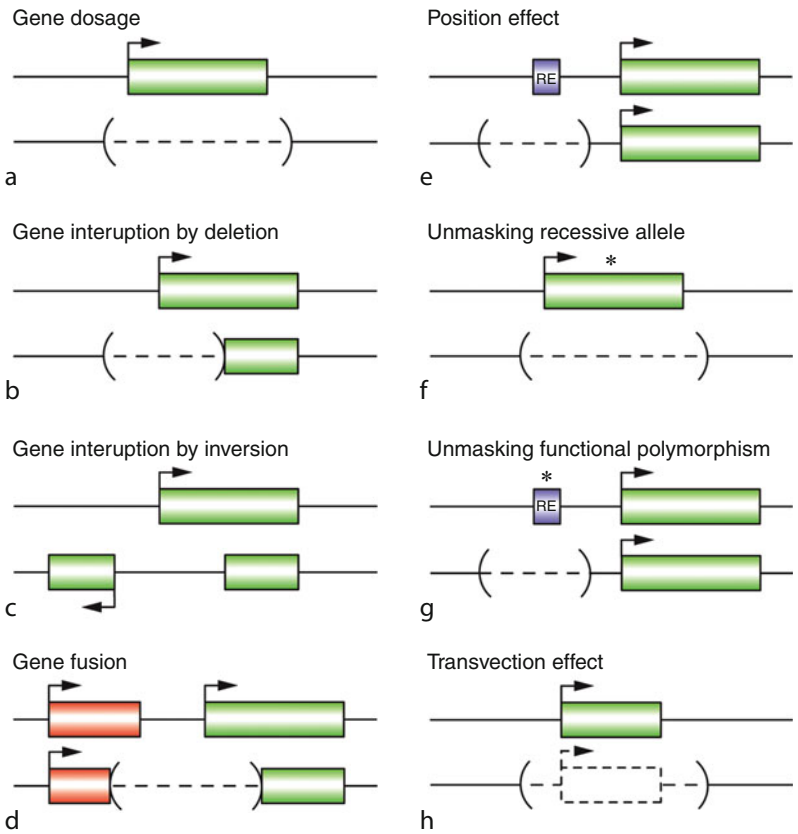

Figure 4.6

Molecular mechanisms by which chromosomal rearrangements can influence phenotypes. The rearrangement can encompass a dosage-sensitive gene that causes disease (a); disrupt a dosage sensitive gene through deletion (b); duplication, translocation or inversion (c); create a fusion gene (d); exert a position effect by affecting a regulatory element (e); unmask a recessive allele (f) or functional polymorphism (g) on the homologous chromosome; and interrupt effects of transvection (h) where the deletion of a gene affects communication between alleles. Genes are depicted as rectangles, regulatory elements as RE and an asterisk (*) indicates a point mutation (Adapted from Lupski JR, Stankiewicz P (2005) Genomic disorders: molecular mechanisms for rearrangements and conveyed phenotypes. PLoS Genet 1:e49 and Feuk L, Carson AR, Scherer SW (2006) Structural variation in the human genome. Nat Rev Genet 7:85–97)

prominent amongst cancers associated with specific chromosomal translocations. The rearrangement can also influence the regulation of a nearby gene by position effects (*Fig. 4.6e*). Deletion, duplication, or translocation of important regulatory elements may alter gene expression at distances as far as ~1 Mb from the target gene. Dosage-insensitive genes can also cause disease if a deletion of the gene unmasks a recessive mutation or a hypomorphic allele on the homologous chromosome (*Fig. 4.6f*) or when the deletion unmasks a functional polymorphism in a regulatory element of the remaining allele (*Fig. 4.6g*). Another way in which deletions can convey a phenotype is by interrupting transvection, where communication and interaction between two alleles on homologous chromosomes is disturbed (*Fig. 4.6h*).

Indications for Cytogenetic Testing

Indications in Children

Intellectual disability and developmental disorders affect up to 3% of the population and remains to this day an enormous etiological challenge. The finding of the cause is of great importance not only to the individual, his or her parents, and family but also to the treating physician. For

the individual, it adds to the identification of appropriate medical and related therapies, indicates medical interventions/referrals, presymptomatic screening for associated complications, educational planning, and elimination of further unnecessary evaluations. For the family, it forms a step toward the acceptance of the disability and the basis to understanding the cause, the reason and the recurrence risks. Carrier testing and reproductive options become a reality. It also allows social support and contact with other similarly affected families. The ongoing etiological evaluation in a bid to attain a diagnosis does thus have a significant role to play in the all-round care of the intellectually disabled individual and the family.

The etiology of intellectual disability is extensive and ranges from acquired/environmental (sequelae of prematurity, pre- and postinfections, trauma, and neurotoxicity – alcohol, metals), to chromosomal (aneuploidy, genomic imbalances – microdeletions and duplications), and to monogenic disorders. The rate of etiological diagnosis is influenced by the level of the intellectual disability – the more severe, the higher the diagnostic success. A systematic literature review of the usefulness of classical karyotyping, subtelomere screening, and molecular genetics investigations in institutionalized individuals with mental retardation indicated 0% etiological detection in borderline to mildly retarded individuals as opposed to 6.5% (range 0.8–13.0%) in those moderately to severely/profoundly retarded. Also, the differences in setting, the patient selection criteria, study protocols, technological advances, definition of a positive diagnosis, method of classification, and expertise of the clinician have been factors resulting in the varying rate of diagnosis.

In a systematic etiological study of 471 institutionalized individuals with mild to profound intellectual disability, 92.6% of which were males, Van Buggenhout et al. reported 49.5% without known cause. Chromosomal anomalies accounted for 21.2% (87 or 18.5% of the 471 individuals had Down syndrome), monogenic disorders 13%, and acquired causes 14.6%.

This was, however, before the era of array comparative genomic hybridization (array CGH). The initial studies using this new technology on selected cohorts of individuals with an intellectual disability and dysmorphism made use of around 3,500 BAC clones, resulting in an average resolution of 1 Mb. The rate of genomic imbalance detection was between 9% and 25%. The few studies at higher, 100 kb resolution, have also detected about 10% of pathogenic submicroscopic aberrations. The chromosome imbalances occur throughout the genome. Once the validity of the technique to detect chromosomal constitutional imbalances was demonstrated it was rapidly introduced into the genetic diagnostic laboratories as a routine technique in the genetic diagnostic workup of patients with learning disabilities and/or multiple congenital anomalies.

In addition to the identification of pathogenic imbalances in patients with intellectual disabilities, several studies have proven associations of copy number variants (CNVs) with several other conditions or specific patient groups: Lu et al. reported an incidence of 17.1% imbalances in neonates with various birth defects. Thienpont et al. report a frequency of 17% causal imbalances in patients with heart diseases. Finally, CNVs are now believed to be an important cause of neuropsychiatric conditions such as autism spectrum disorders and psychiatric diseases such as schizophrenia. Hence, also for these indications it is or will be warranted to perform cytogenetic testing.

Indications in Parents

Balanced translocations are relatively common in the population. The translocation heterozygote (carrier) may have a risk to have a child with developmental disorders because of a segmental aneusomy. Typically, the imbalance in the child is due to a segment of one of the participating chromosomes being duplicated, and a segment of the other chromosome being deleted. This confers a partial trisomy and a concomitant partial monosomy. In families where more than one child is born with developmental disorders and/or families with recurrent miscarriages, a chromosomal investigation is warranted.

In addition, when an imbalance is identified in a child, it is common practise to determine whether or not the imbalance is derived de novo or was inherited. When terminal imbalances are identified, the presence of a balanced translocation in one of the parents should be investigated. In addition, for submicroscopic interstitial imbalances, the presence of a balanced insertional translocation in one of the parents can be present.

Interpretation Issues

Chromosomal Polymorphisms

Chromosomal polymorphisms or heteromorphisms are structural chromosome variants that are widespread in human populations and have no effect on the phenotype. These variants are most often found at the centromeric regions of chromosomes 1, 9, and 16, the distal part of the long arm of the Y chromosome and the short arms of the

acrocentric chromosomes. In addition to these recurrent imbalances, many more cytogenetically visible but apparently benign imbalances have been described. An excellent overview on this topic is provided in the article by Barber , *Directly Transmitted Unbalanced Chromosome Abnormalities and Euchromatic Variants*, and the collected data is online available at the "Chromosome Anomaly Collection" at http://www.ngrl.org.uk/Wessex/collection.

Submicroscopic Chromosomal Polymorphisms (The Blurred Boundary Between Benign and Pathogenic CNVs)

Besides the identification of disease-associated CNVs, molecular karyotyping has also uncovered large numbers of copy number variants between normal individuals. Thus far, single-nucleotide polymorphisms have been considered the main source of genetic variation; hence the discovery of an unexpected large number (12% of the genome) of apparently benign copy number variants, regions of 1–1,000 kb that are present in different copy numbers in different individuals, was rightly called the discovery of the year 2007, according to *Science* magazine.

A number of array CGH studies had demonstrated the presence of polymorphic copy number variants. In a first large systematic study, Redon et al. mapped all CNVs using both array CGH and single nucleotide polymorphism (SNP) genotyping arrays on the 270 individuals of the HapMap collection from ancestry in Europe, Africa, and Asia. In the human genome, 1,447 submicroscopic copy variable regions were uncovered. This involves about 12% of the genome and includes hundreds of genes in deletions, duplications, insertions and complex multisite variants. These nonpathogenic variations are scattered throughout the human genome and contain also 12% of the genes, including a large number of genes known to be involved in genetic disorders and registered in OMIM. Recent fine mapping studies have revealed that those CNVs can result in intragenic variation resulting in different splice variants, the use of different exons and even new gene products. The most comprehensive population-based CNV map so far consists of 11,700 CNVs and is estimated to include about 80–90% of common CNVs greater than 1 kb in length. Although the authors indicate that those common CNVs are highly unlikely to account for much of the missing heritability for complex traits, they suggest that CNVs might contribute appreciably to rare variants involved in common and rare diseases.

The consequence of the detection of large numbers of benign CNVs is that, at present, the clinical significance of a novel CNV remains often unclear. The traditional rules of thumb used when analyzing genomes by conventional karyotyping are not applicable anymore. The identification of a large de novo cytogenetically visible imbalance was usually sufficient to confidently associate it with the disease phenotype. However, it is obvious that smaller imbalances carrying few or no genes may not at all be associated with a disease phenotype. Equally, it is becoming clear that de novo copy number variation arises frequently. Van Ommen estimated that copy number changes arise every one in eight births. Hence, not all de novo copy number changes would be pathogenic.

To determine which, if any, CNVs might be associated with the disease phenotype, the collection of large numbers of patient genotypes and phenotypes is required. Several efforts are currently ongoing to collect both large numbers of phenotypes and genotypes. These efforts will eventually allow pinpointing highly penetrant CNVs, revealing which imbalances are causal and which imbalances are spurious. The best-known open source examples are the DatabasE of Chromosomal Imbalances and Phenotype in Humans using Ensembl Resources with acronym DECIPHER which is organized at the Sanger institute (https://decipher.sanger.ac.uk/) and the European Cytogenetics Association Register of Unbalanced Chromosome Aberrations, ECARUCA, a register with a basis in Nijmegen, The Netherlands (http://www.ecaruca.net).

In addition, several large-scale collaborative efforts are underway to map population-embedded, apparently benign CNVs. These data are collected in the database of genomic variants (DGV, http://projects.tcag.ca/variation/). To fine map those imbalances, increasingly higher resolution arrays are being used. Those efforts aim to identify CNVs with likely minor or no developmental consequences.

While the mapping of apparently benign and pathogenic CNVs is an important endeavor, it is not sufficient to predict whether an imbalance will cause an abnormal phenotype. Apparently benign CNVs can cause autosomal recessive, autosomal dominant, and X-linked disorders, and imprinted regions may only cause disease dependent on the parental origin. In addition, variable expressivity and penetrance may obscure the pathogenic relevance of CNVs. It is not only becoming clear that interindividual phenotypic variation is caused by benign copy number variations, but more and more it is realized that even well-known disease-causing copy number variations may occasionally be tolerated and be part of the normal human phenotypic spectrum. For example, the 22q11 deletion as well as the duplication can cause both heart anomalies and midline defects such as cleft palate. However, both the

familial inherited 22q11 deletion and duplication have now recurrently been reported. The parent carrying the 22q11 duplication is phenotypically normal. Similarly, subtelomeric imbalances are known to be a major cause of birth defects and mental retardation. In contrast to the view that these imbalances are always causal and result in phenotypic anomalies, several reports indicate that several subtelomeric imbalances, up to 10 Mb in size, may not result in obvious phenotypic anomalies.

More recently identified recurrent imbalances with variable penetrance are the 16p13.1 region and the 1q21 region. During the screening of patients with mental handicap and developmental anomalies, reciprocal deletions, and duplications of the 16p13.1 region were recurrently observed. This 1.65 Mb rearrangement involves 15 genes. At first, it was unclear whether these imbalances were causing the developmental problems in patients because of two reasons: First, the imbalance, be it deletion or duplication, was often observed to be inherited from an apparently normal parent. Second, the phenotypes associated with either the deletion or duplication are quite variable. An association study showed that the deletion is a risk factor for mental handicap while the duplication is more likely to be a benign variant. Interestingly, Law et al. reported the prenatal diagnosis of a de novo 16p13.11 microdeletion by array CGH. Because of the unclear clinical significance, the pregnancy was not terminated and an apparently healthy baby was born. Chromosome 1q21 harbors two flanking regions, where, recently, recurrent reciprocal rearrangements were detected in patients with MR/MCA. The deletions and duplications are mediated by nonallelic homologous recombination of flanking low-copy repeats. All 30 investigated patients with thrombocytopenia absent radius (TAR) syndrome carry a 200 kb deletion on chromosome 1q21.1. Analysis of the parents revealed that this deletion occurred de novo in 25% of affected individuals. Intriguingly, inheritance of the deletion along the maternal line as well as the paternal line was observed in the other patients. The absence of this deletion in a cohort of control individuals argues for a specific role played by the microdeletion in the pathogenesis of TAR syndrome. It is hypothesized that TAR syndrome is associated with a deletion on chromosome 1q21.1 but that the phenotype develops only in the presence of an additional as-yet-unknown modifier (mTAR). Recently, the first prenatal diagnosis of TAR by array CGH was reported.

Mefford and colleagues identified 20 individuals with a recurrent 1.35 Mb deletion distal from the TAR region from a screen of about 5,000 patients ascertained with mental retardation and/or associated congenital anomalies (MR/MCA). The microdeletions arose de novo in six patients, were inherited from a mildly affected parent in three patients, and were inherited from an apparently unaffected parent in five patients. The absence of the deletion in about 5,000 control individuals represents a significant association with disease. In addition, the reciprocal duplication was also enriched in children with mental retardation or autism spectrum disorder although very few cases have been observed to conclude statistical significance.

It seems likely that those recurrent rearrangements with variable penetrance and expressivity are only the tip of an iceberg of a large number of structural variants with diverse and complex phenotypes that will elude both traditional syndromic classifications as well as evade traditional Mendelian inheritance patterns. The elucidation of their association with disease will require genotyping and phenotyping large numbers of patients and controls. These imbalances pose challenges to the clinician upon interpreting array CGH data. It seems likely that, in the future, the interpretation will be aided by computerized expert systems to aid the interpretation of a genomic profile.

Technical Issues in Array CGH

Quality Parameters

In a clinical setting, it is of utmost importance to detect all chromosomal abnormalities (i.e., to avoid false negatives) without calling false positives. In other words, both the sensitivity, which is the ability to detect a true positive result, and the specificity, which is the correct assessment of true negatives, should be as high as possible. This is dependent on the resolution of the platform, but also on the quality of the hybridization experiment. Therefore, strict quality parameters such as a maximum allowable standard deviation, appropriate thresholds, and algorithms for CNV calling and a minimum number of flagged reporters (i.e., those that are excluded from analysis due to technical artifacts) need to be maintained.

Chromosomal Rearrangements Missed by Array CGH

Array CGH is often touted to be able to replace conventional karyotyping in a diagnostic analysis of pediatric disorders. However, it should be realized that some chromosomal anomalies would be missed.

Inherent to the technique, balanced chromosomal rearrangements (inversions and balanced translocations) are not detected. When balanced rearrangements are detected prenatally on karyotypes, parents are usually tested and if a "normal" parent carries the same rearrangement, the translocation is considered benign. If the rearrangement is de novo, counseling is very difficult and the risk for developmental defects is estimated to be 6%. Array CGH analysis of patients with developmental anomalies and de novo translocations has revealed that about 45% of these are actually imbalanced. Considering that de novo translocations occur in about 1/1,000 births with 6% pathogenic and half of these detectable by array CGH, this would leave 0.003% pathogenic translocations undetected if no karyotype is performed.

Also neither triploidies, 69,XXX and 69,XXY, nor tetraploidies are readily detected. The use of DNA from a patient with Klinefelter (47,XXY) as a control does result in aberrant X and Y chromosome ratios, enabling the detection of XXX triploidies and all tetraploidies.

It should also be borne in mind that array CGH results represent the additive and not the allele-specific copy number. In this way, the true inheritance pattern can be masked and what looks like a de novo event may actually be the inheritance of a copy number variant in one of the parents. Carelle-Calmels and colleagues have recently reported a striking example. FISH analysis of the parents of a girl carrying a deletion at 22q11.2 revealed an unexpected rearrangement of both 22q11.2 regions in the phenotypically normal father. He carried a 22q11.2 deletion on one copy of chromosome 22 and the reciprocal duplication on the other copy of chromosome 22. Quantitative expression analysis of the genes located in the critical DiGeorge/VCFS region showed genomic compensation, consistent with the normal phenotype of the father. As the total copy number in the father equals the reference copy number, this would not have been detected by array CGH and the rearrangement would have been classified as de novo. The finding of the mirror rearrangement in the father has tremendous clinical consequences for genetic counseling, as there is a 100% risk of an unbalanced outcome.

Technical Standards for Cytogenetic Laboratories

A broad range of platforms including BAC, oligonucleotide, and SNP arrays has become commercially available, greatly facilitating the introduction of molecular karyotyping in the diagnostic setting. For the clinical implementation of array CGH in cytogenetic laboratories, the following technical standards should be achieved. The chosen methodology has to be validated with known aberrations, the performance of the arrays evaluated by internal and external quality controls, standard protocols have to be established, and the effective resolution of the platform has to be determined, as this differs from the theoretical resolution of the array as provided by the manufacturer. When reporting array CGH results, referral should be made to the platform, effective resolution, procedures, and quality parameters used. The detected aberrations should be defined according to the ISCN 2009 nomenclature with reference to the appropriate genome build in order to guide standardization across different cytogenetic laboratories.

Mosaicism

Chromosomal mosaicism can be defined as the coexistence, within one conceptus, of two or more distinct cell lines that are genetically identical except for the chromosomal difference between them, these cell lines having been established by the time that embryonic development is complete (the point at which the embryo becomes a fetus). Thus, the different cell lines are fixed in the individual and are a part of his or her chromosome constitution.

The phenotype associated with any particular type of mosaicism can be expected to be highly variable, reflecting the differences in the proportions of normal and abnormal cells. Mosaicism has been detected for all different chromosome abnormalities described in the section Types and Incidences of Chromosomal Abnormalities. Mosaicism is especially common for (small) marker chromosomes. Clinically, mosaicism can be suspected when a patient shows nonsymmetrical features, pigmentation lines, or specific syndromic features known to be associated with certain forms of mosaicism.

In order to detect mosaicism, sufficiently many cells must be analyzed. In most cytogenetic laboratories, 15–20 karyotypes are analyzed. This will allow the detection of a chromosomal abnormality with a certainty of 95% when present in 22–28% of the cells. With arrays it is equally possible to detect low-grade mosaicism. With the BAC arrays mosaicism as low as 7% could be detected. The degree of mosaicism that can be detected depends on the standard deviation of the array as well as on the size of the imbalance. In general, mosaicism down to 30% should readily be detected.

Current State of the Art and Future of Cytogenetic Testing

Current State of the Art

Any cytogenetic laboratory should be skilled in all conventional and molecular cytogenetic techniques. These include G-banded karyotyping, fluorescent in situ hybridizations, and array CGH. G-banded karyotyping has been available for more than 40 years and has the advantage that there is a widely accepted and uniform technique with an international system of cytogenetic nomenclature (ISCN). By contrast, cytogenomic arrays are much newer. Because of this novelty, there is still discussion about the best platforms to use, there is not yet a comprehensive knowledge base about the clinical consequences of all CNVs, the language to describe CNVs is still evolving. Currently, some recommendations are provided by the International Standard Cytogenomic Array (ISCA) Consortium:

- Cytogenomic array testing standards should not be specific to a particular array platform. Arrays based on BAC, oligonucleotide, or SNP probes can achieve the recommended coverage and level of resolution.
- In order to perform the same intended purpose as a karyotype, cytogenomic arrays must have uniform coverage to detect all areas of imbalance greater than or equal to 400 kb throughout the genome.
- Cytogenomic array testing can be prioritized over G-banded karyotyping. Cytogenomic arrays will detect many more submicroscopic genomic CNVs than the number of balanced rearrangements it would miss.
- G-banded karyotyping should always be available to patients with a family history of a rearrangement or a history of multiple miscarriages. In addition, G-banded karyotyping should still be offered in settings where both tests will be covered by insurance.

Decision Tree(s) for Array CGH Interpretation

Cytogenomic arrays have moved from bench to bedside for the genetic screening of patients with mental retardation and/or congenital anomalies. The advent of commercially available microarrays has facilitated the implementation of this technique in clinical diagnostic laboratories. As described earlier, the incomplete understanding of structural polymorphism and the appreciation that many disorders show a high degree of clinical variation and incomplete penetrance is blurring clear-cut genotype–phenotype correlations. As a consequence, the causality of many smaller CNVs often remains to be elucidated and the clinical interpretation of the detected CNVs has become a major challenge for diagnostic laboratories. To aid in the assessment of the clinical significance of a CNV, several decision trees that can be used as a guideline have been proposed. These decision trees all include three major steps as outlined in *Fig. 4.7*. The first step is to identify known causal CNVs, which include: (1) CNVs that overlap with well-established as well as recently recognized microdeletion and microduplication syndromes, (2) CNVs that overlap with pathogenic CNVs detected by other (microarray) studies in patients with similar phenotypes, and (3) CNVs that encompass known OMIM genes that have been associated with the phenotype observed in the affected patient. In this way, the pitfall of unintentionally disregarding a causal CNV as a benign variant is avoided because the fact is that some CNVs have been described as benign variants but reside in regions that are known to be associated with disease or are at recessive loci that are only pathogenic in the homozygous state. The second step is to remove normal benign variants (also known as common CNVs) that have been detected in healthy individuals and are thus less likely to account for the patient's phenotype. The third step is to determine the inheritance for the remaining CNVs. Aberrations that occur de novo in the patient are more likely to be pathogenic, especially when they are relatively large and/or contain several genes. For inherited CNVs and CNVs of unknown inheritance, the clinical interpretation is more complicated and these CNVs are currently classified as of unknown clinical significance. However, as international efforts are underway to map both pathogenic and benign CNVs (see Submicroscopic Chromosomal Polymorphisms (The Blurred Boundary Between Benign and Pathogenic CNVs)), it can be expected that a significant proportion of these CNVs will turn out to be causal, thus increasing the diagnostic yield in patients with mental retardation and/or congenital anomalies.

Future

Conventional and molecular cytogenetic testing will remain important since it provides information about the location of the abnormality. With the advent of arrays, the connection between the visible localization of the abnormality is somewhat lost. This trend is likely to

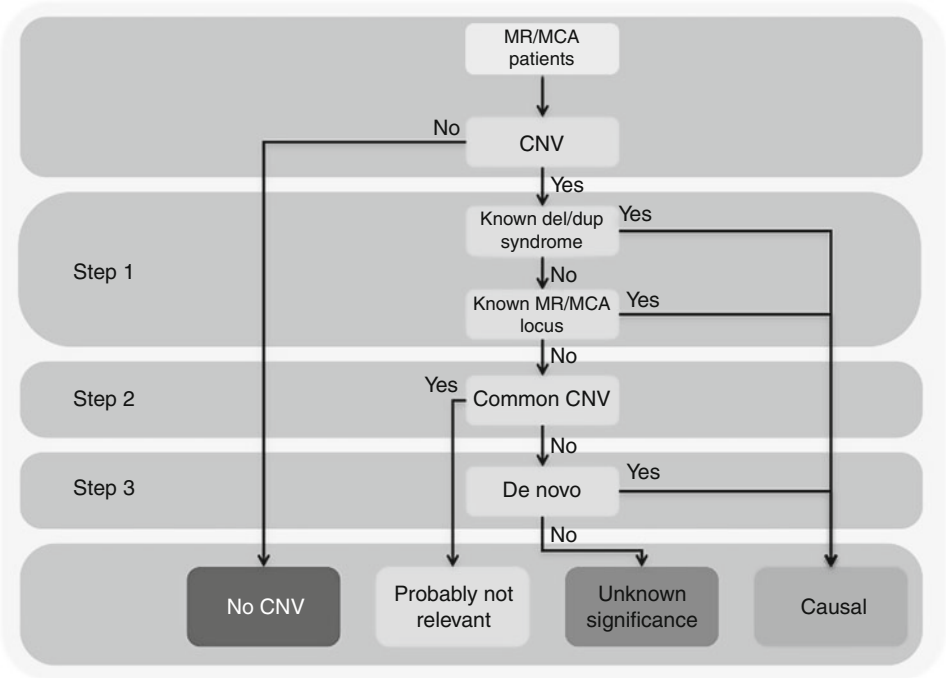

Figure 4.7
Decision tree for the assessment of clinical relevance for a particular CNV. Step 1: Identify known causal CNVs. Step 2: Discard common CNVs. Step 3: Determine the inheritance. Details are described in the text (Adapted from Buysse K, Delle Chiaie B, Van Coster R et al. (2009a) Challenges for CNV interpretation in clinical molecular karyotyping: lessons learned from a 1001 sample experience. Eur J Med Genet 52:398–403)

continue with the advent of full genome sequencing techniques. The latest technical revolution in human genetics is next-generation sequencing (NGS). Its strength lies in the ability to process millions of sequence reads in parallel rather than 96 at a time. Several platforms using different techniques are commercially available (Roche's 454 sequencing, Illumina's Solexa Genome Analyzer technology, and the SOLiD platform from Applied Biosystems), but they all rely on cyclic-array sequencing, which involves the sequencing of thousands to millions of immobilized DNA features by iterative cycles of enzymatic manipulation and imaging-based data acquisition. Depending on the platform, NGS generates hundreds of megabases to gigabases of nucleotide-sequence output in a single instrument run.

It is expected that costs will drop and that genome sequencing of individuals will be commonplace in the foreseeable future. If it will become feasible to assemble complete genomes as well as accurately determine copy numbers, full genome sequencing may ultimately replace cytogenetic as well as molecular genetic testing.

Concluding Remarks

In this chapter, the aim was to touch upon the important aspects of cytogenetic testing and provide a basic text on the topic for pediatricians new to the field. Considering that several books and numerous articles have been written about this topic, it is realized that the resume presented here is incomplete and biased. For those interested, a number of excellent books on the topics touched upon here are referred to. To help pediatricians in the interpretation of cytogenetic results and counseling of those results with patients, the books "Chromosome abnormalities and genetic counselling" as well as "The principles of clinical cytogenetics" are recommended. Phenotypic information about chromosomal imbalances has been collected by Schinzel in "Catalogue of unbalanced chromosome aberrations in man". Clinicians closely interacting with obstetricians and involved in prenatal diagnosis can consult "Genetic disorders and the fetus". Those interested to know more about the mechanisms underpinning genomic disorders

are referred to "Genomic disorders: the genomic basis of disease".

Over the last 50 years, cytogenetics has become a cornerstone of genetic testing of children with birth defects and developmental anomalies. Conventional karyotyping is rapidly replaced or at least complemented by array screening. In the future possibly full genome sequencing will enable both mutation and copy number detection in all individuals with developmental disorders. Certainly, knowledge about the organization and location of chromosomal aberrations is important for counseling and family planning, and therefore cytogenetics is here to stay, perhaps under a new name – "cytogenomics?"

References

Albertson DG, Pinkel D (2003) Genomic microarrays in human genetic disease and cancer. Hum Mol Genet 12(Spec No 2):R145–R152

Angell R (1997) First-meiotic-division nondisjunction in human oocytes. Am J Hum Genet 61:23–32

Bailey JA, Yavor AM, Massa HF et al (2001) Segmental duplications: organization and impact within the current human genome project assembly. Genome Res 11:1005–1017

Bailey JA, Gu Z, Clark RA et al (2002) Recent segmental duplications in the human genome. Science 297:1003–1007

Balikova I, Menten B, de Ravel T et al (2007) Subtelomeric imbalances in phenotypically normal individuals. Hum Mutat 28:958–967

Balikova I, Martens K, Melotte C et al (2008) Autosomal-dominant microtia linked to five tandem copies of a copy-number-variable region at chromosome 4p16. Am J Hum Genet 82:181–187

Balikova I, Lehesjoki AE, de Ravel TJ et al (2009) Deletions in the VPS13B (COH1) gene as a cause of Cohen syndrome. Hum Mutat 30:E845–E854

Ballif BC, Kashork CD, Saleki R et al (2006a) Detecting sex chromosome anomalies and common triploidies in products of conception by array-based comparative genomic hybridization. Prenat Diagn 26:333–339

Ballif BC, Rorem EA, Sundin K et al (2006b) Detection of low-level mosaicism by array CGH in routine diagnostic specimens. Am J Med Genet A 140:2757–2767

Ballif BC, Hornor SA, Sulpizio SG et al (2007) Development of a high-density pericentromeric region BAC clone set for the detection and characterization of small supernumerary marker chromosomes by array CGH. Genet Med 9:150–162

Ballif BC, Theisen A, Coppinger J et al (2008) Expanding the clinical phenotype of the 3q29 microdeletion syndrome and characterization of the reciprocal microduplication. Mol Cytogenet 1:8

Baptista J, Mercer C, Prigmore E et al (2008) Breakpoint mapping and array CGH in translocations:comparison of a phenotypically normal and an abnormal cohort. Am J Hum Genet 82:927–936

Barber JC (2005) Directly transmitted unbalanced chromosome abnormalities and euchromatic variants. J Med Genet 42:609–629

Bauters M, Van Esch H, Friez MJ et al (2008) Nonrecurrent MECP2 duplications mediated by genomic architecture-driven DNA breaks and break-induced replication repair. Genome Res 18: 847–858

Benn PA, Hsu LYF (2004) Prenatal diagnosis of chromosomal abnormalities through amniocentesis. In: Milunsky A (ed) Genetic disorders and the fetus: diagnosis, prevention, and treatment, 5th edn. Johns Hopkins University Press, Baltimore, p 214

Ben-Shachar S, Ou Z, Shaw CA et al (2008) 22q11.2 distal deletion: a recurrent genomic disorder distinct from DiGeorge syndrome and velocardiofacial syndrome. Am J Hum Genet 82:214–221

Breckpot J, Takiyama Y, Thienpont B et al (2008) A novel genomic disorder: a deletion of the SACS gene leading to spastic ataxia of Charlevoix-Saguenay. Eur J Hum Genet 16:1050–1054

Browne CE, Dennis NR, Maher E et al (1997) Inherited interstitial duplications of proximal 15q: genotype-phenotype correlations. Am J Hum Genet 61:1342–1352

Brunetti-Pierri N, Berg JS, Scaglia F et al (2008) Recurrent reciprocal 1q21.1 deletions and duplications associated with microcephaly or macrocephaly and developmental and behavioral abnormalities. Nat Genet 40:1466–1471

Buysse K, Crepel A, Menten B et al (2008) Mapping of 5q35 chromosomal rearrangements within a genomically unstable region. J Med Genet 45:672–678

Buysse K, Delle Chiaie B, Van Coster R et al (2009a) Challenges for CNV interpretation in clinical molecular karyotyping: lessons learned from a 1001 sample experience. Eur J Med Genet 52:398–403

Buysse K, Reardon W, Mehta L et al (2009b) The 12q14 microdeletion syndrome: additional patients and further evidence that HMGA2 is an important genetic determinant for human height. Eur J Med Genet 52:101–107

Cai WW, Mao JH, Chow CW et al (2002) Genome-wide detection of chromosomal imbalances in tumors using BAC microarrays. Nat Biotechnol 20:393–396

Carelle-Calmels N, Saugier-Veber P, Girard-Lemaire F et al (2009) Genetic compensation in a human genomic disorder. N Engl J Med 360:1211–1216

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

Caspersson T, Zech L, Johansson C (1970) Differential binding of alkylating fluorochromes in human chromosomes. Exp Cell Res 60:315–319

Chelly J, Khelfaoui M, Francis F et al (2006) Genetics and pathophysiology of mental retardation. Eur J Hum Genet 14:701–713

Cheung J, Estivill X, Khaja R et al (2003) Genome-wide detection of segmental duplications and potential assembly errors in the human genome sequence. Genome Biol 4:R25

Conrad DF, Pinto D, Redon R et al (2010) Origins and functional impact of copy number variation in the human genome. Nature 464: 704–712

Consortium IS (2008) Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature 455:237–241

Cook EH Jr, Scherer SW (2008) Copy-number variations associated with neuropsychiatric conditions. Nature 455:919–923

Curry CJ, Stevenson RE, Aughton D et al (1997) Evaluation of mental retardation: recommendations of a Consensus Conference: American College of Medical Genetics. Am J Med Genet 72:468–477

De Gregori M, Ciccone R, Magini P et al (2007) Cryptic deletions are a common finding in "balanced" reciprocal and complex chromosome rearrangements: a study of 59 patients. J Med Genet 44:750–762

de Ravel TJ, Balikova I, Thienpont B et al (2006) Molecular karyotyping of patients with MCA/MR: the blurred boundary between normal and pathogenic variation. Cytogenet Genome Res 115:225–230

de Vries BB, Pfundt R, Leisink M et al (2005) Diagnostic genome profiling in mental retardation. Am J Hum Genet 77:606–616

Dhami P, Coffey AJ, Abbs S et al (2005) Exon array CGH: detection of copy-number changes at the resolution of individual exons in the human genome. Am J Hum Genet 76:750–762

Duncan IW (2002) Transvection effects in Drosophila. Annu Rev Genet 36:521–556

Feenstra I, Fang J, Koolen DA et al (2006) European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (ECARUCA); an online database for rare chromosome abnormalities. Eur J Med Genet 49:279–291

Feuk L, Carson AR, Scherer SW (2006) Structural variation in the human genome. Nat Rev Genet 7:85–97

Freeman JL, Perry GH, Feuk L et al (2006) Copy number variation: new insights in genome diversity. Genome Res 16:949–961

Friedman JM, Baross A, Delaney AD et al (2006) Oligonucleotide microarray analysis of genomic imbalance in children with mental retardation. Am J Hum Genet 79:500–513

Gardner RJM, Sutherland GR (2004) Chromosome abnormalities and genetic counseling, 3rd edn. Oxford University Press, New York

Gersen GL, Keagle MB (2005) The principles of clinical cytogenetics. Humana, Totowa

Gijsbers AC, Lew JY, Bosch CA et al (2009) A new diagnostic workflow for patients with mental retardation and/or multiple congenital abnormalities: test arrays first. Eur J Hum Genet 17:1394–1402

Glasson EJ, Sullivan SG, Hussain R et al (2002) The changing survival profile of people with Down's syndrome: implications for genetic counselling. Clin Genet 62:390–393

Gribble SM, Prigmore E, Burford DC et al (2005) The complex nature of constitutional de novo apparently balanced translocations in patients presenting with abnormal phenotypes. J Med Genet 42:8–16

Gu W, Zhang F, Lupski JR (2008) Mechanisms for human genomic rearrangements. Pathogenetics 1:4

Hannes FD, Sharp AJ, Mefford HC et al (2009) Recurrent reciprocal deletions and duplications of 16p13.11: the deletion is a risk factor for MR/MCA while the duplication may be a rare benign variant. J Med Genet 46:223–232

Hassold T, Hall H, Hunt P (2007) The origin of human aneuploidy: where we have been, where we are going. Hum Mol Genet 16(Spec No. 2): R203–R208

Hastings PJ, Ira G, Lupski JR (2009) A microhomology-mediated break-induced replication model for the origin of human copy number variation. PLoS Genet 5:e1000327

Hinds DA, Kloek AP, Jen M et al (2006) Common deletions and SNPs are in linkage disequilibrium in the human genome. Nat Genet 38:82–85

Hochstenbach R, van Binsbergen E, Engelen J et al (2009) Array analysis and karyotyping: workflow consequences based on a retrospective study of 36, 325 patients with idiopathic developmental delay in the Netherlands. Eur J Med Genet 52:161–169

Hoyer J, Dreweke A, Becker C et al (2007) Molecular karyotyping in patients with mental retardation using 100 K single-nucleotide polymorphism arrays. J Med Genet 44:629–636

Iafrate AJ, Feuk L, Rivera MN et al (2004) Detection of large-scale variation in the human genome. Nat Genet 36:949–951

Inoue K, Osaka H, Thurston VC et al (2002) Genomic rearrangements resulting in PLP1 deletion occur by nonhomologous end joining and cause different dysmyelinating phenotypes in males and females. Am J Hum Genet 71:838–853

Jacobs PA (1981) Mutation rates of structural chromosome rearrangements in man. Am J Hum Genet 33:44–54

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

Kidd JM, Cooper GM, Donahue WF et al (2008) Mapping and sequencing of structural variation from eight human genomes. Nature 453:56–64

Kleinjan DA, van Heyningen V (2005) Long-range control of gene expression: emerging mechanisms and disruption in disease. Am J Hum Genet 76:8–32

Klopocki E, Schulze H, Strauss G et al (2007) Complex inheritance pattern resembling autosomal recessive inheritance involving a microdeletion in thrombocytopenia-absent radius syndrome. Am J Hum Genet 80:232–240

Knijnenburg J, Oberstein SA, Frei K et al (2009) A homozygous deletion of a normal variation locus in a patient with hearing loss from non-consanguineous parents. J Med Genet 46:412–417

Komura D, Shen F, Ishikawa S et al (2006) Genome-wide detection of human copy number variations using high-density DNA oligonucleotide arrays. Genome Res 16:1575–1584

Koolen DA, Vissers LE, Pfundt R et al (2006) A new chromosome 17q21.31 microdeletion syndrome associated with a common inversion polymorphism. Nat Genet 38:999–1001

Koolen DA, Pfundt R, de Leeuw N et al (2009) Genomic microarrays in mental retardation: a practical workflow for diagnostic applications. Hum Mutat 30:283–292

Korbel JO, Urban AE, Affourtit JP et al (2007) Paired-end mapping reveals extensive structural variation in the human genome. Science 318:420–426

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

Law LW, Lau TK, Fung TY et al (2009) De novo 16p13.11 microdeletion identified by high-resolution array CGH in a fetus with increased nuchal translucency. BJOG 116:339–343

Lee JA, Madrid RE, Sperle K et al (2006) Spastic paraplegia type 2 associated with axonal neuropathy and apparent PLP1 position effect. Ann Neurol 59:398–403

Lee JA, Carvalho CM, Lupski JR (2007) A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. Cell 131:1235–1247

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

Lieber MR (2008) The mechanism of human nonhomologous DNA end joining. J Biol Chem 283:1–5

Lieber MR, Ma Y, Pannicke U et al (2003) Mechanism and regulation of human non-homologous DNA end-joining. Nat Rev Mol Cell Biol 4:712–720

Liehr T, Claussen U, Starke H (2004) Small supernumerary marker chromosomes (sSMC) in humans. Cytogenet Genome Res 107: 55–67

Lisi EC, Hamosh A, Doheny KF et al (2008) 3q29 interstitial microduplication: a new syndrome in a three-generation family. Am J Med Genet A 146A:601–609

Locke DP, Sharp AJ, McCarroll SA et al (2006) Linkage disequilibrium and heritability of copy-number polymorphisms within duplicated regions of the human genome. Am J Hum Genet 79:275–290

Lu XY, Phung MT, Shaw CA et al (2008) Genomic imbalances in neonates with birth defects: high detection rates by using chromosomal microarray analysis. Pediatrics 122:1310–1318

Lupski JR (1998) Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. Trends Genet 14:417–422

Lupski JR, Stankiewicz P (2005) Genomic disorders: molecular mechanisms for rearrangements and conveyed phenotypes. PLoS Genet 1:e49

Lupski JR, Stankiewicz P (2006) Genomic disorders: the genomic basis of disease. Humana, Totowa

Lupski JR, de Oca-Luna RM, Slaugenhaupt S et al (1991) DNA duplication associated with Charcot-Marie-Tooth disease type 1A. Cell 66:219–232

Marshall CR, Noor A, Vincent JB et al (2008) Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet 82:477–488

Mefford HC, Sharp AJ, Baker C et al (2008) Recurrent rearrangements of chromosome 1q21.1 and variable pediatric phenotypes. N Engl J Med 359:1685–1699

Menten B, Maas N, Thienpont B et al (2006) Emerging patterns of cryptic chromosomal imbalance in patients with idiopathic mental retardation and multiple congenital anomalies: a new series of 140 patients and review of published reports. J Med Genet 43:625–633

Menten B, Buysse K, Zahir F et al (2007) Osteopoikilosis, short stature and mental retardation as key features of a new microdeletion syndrome on 12q14. J Med Genet 44:264–268

Miller DT, Adam MP, Aradhya S et al (2010) Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet 86:749–764

Milunsky A (2004) Genetic disorders and the fetus: diagnosis, prevention and treatment. The John Hopkins University Press, Baltimore

Moeschler JB, Shevell M (2006) Clinical genetic evaluation of the child with mental retardation or developmental delays. Pediatrics 117:2304–2316

Morrow DM, Connelly C, Hieter P (1997) "Break copy" duplication: a model for chromosome fragment formation in Saccharomyces cerevisiae. Genetics 147:371–382

Nielsen J, Wohlert M (1991) Chromosome abnormalities found among 34, 910 newborn children: results from a 13-year incidence study in Arhus, Denmark. Hum Genet 87:81–83

Nobile C, Toffolatti L, Rizzi F, Simionati B et al (2002) Analysis of 22 deletion breakpoints in dystrophin intron 49. Hum Genet 110: 418–421

Pentao L, Wise CA, Chinault AC et al (1992) Charcot-Marie-Tooth type 1A duplication appears to arise from recombination at repeat sequences flanking the 1.5 Mb monomer unit. Nat Genet 2:292–300

Pinkel D, Segraves R, Sudar D et al (1998) High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 20:207–211

Portnoi MF, Lebas F, Gruchy N et al (2005) 22q11.2 duplication syndrome: two new familial cases with some overlapping features with DiGeorge/velocardiofacial syndromes. Am J Med Genet A 137: 47–51

Potocki L, Bi W, Treadwell-Deering D et al (2007) Characterization of Potocki-Lupski syndrome (dup(17)(p11.2p11.2)) and delineation of a dosage-sensitive critical interval that can convey an autism phenotype. Am J Hum Genet 80:633–649

Rauch A, Ruschendorf F, Huang J et al (2004) Molecular karyotyping using an SNP array for genomewide genotyping. J Med Genet 41:916–922

Redon R, Ishikawa S, Fitch KR et al (2006) Global variation in copy number in the human genome. Nature 444:444–454

Rosenberg C, Knijnenburg J, Bakker E et al (2006) Array-CGH detection of micro rearrangements in mentally retarded individuals: clinical significance of imbalances present both in affected children and normal parents. J Med Genet 43:180–186

Sagoo GS, Butterworth AS, Sanderson S et al (2009) Array CGH in patients with learning disability (mental retardation) and congenital anomalies: updated systematic review and meta-analysis of 19 studies and 13, 926 subjects. Genet Med 11:139–146

Schena M, Shalon D, Davis RW et al (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270:467–470

Schinzel A (2001) Catalogue of unbalanced chromosome aberrations in man. Walter de Gruyter, Berlin

Schoumans J, Ruivenkamp C, Holmberg E et al (2005) Detection of chromosomal imbalances in children with idiopathic mental retardation by array based comparative genomic hybridisation (array-CGH). J Med Genet 42:699–705

Sebat J, Lakshmi B, Troge J et al (2004) Large-scale copy number polymorphism in the human genome. Science 305:525–528

Sebat J, Lakshmi B, Malhotra D et al (2007) Strong association of de novo copy number mutations with autism. Science 316:445–449

Shaffer LG, Slovak ML, Campbell LJ (2009) ISCN 2009 an international system for human cytogenetic nomenclature. Karger, Basel

Sharp AJ, Locke DP, McGrath SD et al (2005) Segmental duplications and copy-number variation in the human genome. Am J Hum Genet 77:78–88

Sharp AJ, Hansen S, Selzer RR et al (2006) Discovery of previously unidentified genomic disorders from the duplication architecture of the human genome. Nat Genet 38:1038–1042

Sharp AJ, Selzer RR, Veltman JA E et al (2007) Characterization of a recurrent 15q24 microdeletion syndrome. Hum Mol Genet 16:567–572

Sharp AJ, Mefford HC, Li K et al (2008) A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures. Nat Genet 40:322–328

Shaw CJ, Lupski JR (2005) Non-recurrent 17p11.2 deletions are generated by homologous and non-homologous mechanisms. Hum Genet 116:1–7

Shaw-Smith C, Redon R, Rickman L et al (2004) Microarray based comparative genomic hybridisation (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features. J Med Genet 41:241–248

Shaw-Smith C, Pittman AM, Willatt L et al (2006) Microdeletion encompassing MAPT at chromosome 17q21.3 is associated with developmental delay and learning disability. Nat Genet 38:1032–1037

Shendure J, Ji H (2008) Next-generation DNA sequencing. Nat Biotechnol 26:1135–1145

Shendure J, Porreca GJ, Reppas NB et al (2005) Accurate multiplex polony sequencing of an evolved bacterial genome. Science 309:1728–1732

Solinas-Toldo S, Lampel S, Stilgenbauer S et al (1997) Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. Genes Chromosom Cancer 20:399–407

Somerville MJ, Mervis CB, Young EJ et al (2005) Severe expressive-language delay related to duplication of the Williams-Beuren locus. N Engl J Med 353:1694–1701

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

Stankiewicz P, Lupski JR (2002) Genome architecture, rearrangements and genomic disorders. Trends Genet 18:74–82

Stankiewicz P, Shaw CJ, Dapper JD et al (2003) Genome architecture catalyzes nonrecurrent chromosomal rearrangements. Am J Hum Genet 72:1101–1116

Stefansson H, Rujescu D, Cichon S et al (2008) Large recurrent microdeletions associated with schizophrenia. Nature 455:232–236

Thienpont B, Mertens L, de Ravel T et al (2007) Submicroscopic chromosomal imbalances detected by array-CGH are a frequent cause of congenital heart defects in selected patients. Eur Heart J 28: 2778–2784

Tjio H, Levan A (1956) The chromosome numbers of man. Hereditas 42:1–6

Toffolatti L, Cardazzo B, Nobile C et al (2002) Investigating the mechanism of chromosomal deletion: characterization of 39 deletion breakpoints in introns 47 and 48 of the human dystrophin gene. Genomics 80:523–530

Uhrig S, Schlembach D, Waldispuehl-Geigl J et al (2007) Impact of array comparative genomic hybridization-derived information on genetic counseling demonstrated by prenatal diagnosis of the TAR (thrombocytopenia-absent-radius) syndrome-associated microdeletion 1q21.1. Am J Hum Genet 81:866–868

Ullmann R, Turner G, Kirchhoff M et al (2007) Array CGH identifies reciprocal 16p13.1 duplications and deletions that predispose to autism and/or mental retardation. Hum Mutat 28:674–682

Van Buggenhout GJ, van Ravenswaaij-Arts C, Mieloo H et al (2001) Dysmorphology and mental retardation: molecular cytogenetic studies in dysmorphic mentally retarded patients. Ann Génét 44:89–92

Van Esch H, Bauters M, Ignatius J et al (2005) Duplication of the MECP2 region is a frequent cause of severe mental retardation and progressive neurological symptoms in males. Am J Hum Genet 77:442–453

van Karnebeek CD, Jansweijer MC, Leenders AG et al (2005) Diagnostic investigations in individuals with mental retardation: a systematic literature review of their usefulness. Eur J Hum Genet 13:6–25

van Ommen GJ (2005) Frequency of new copy number variation in humans. Nat Genet 37:333–334

Van Prooijen-Knegt AC, Van Hoek JFM, Bauman JGJ et al (1982) In situ hybridization of DNA sequences in human metaphase chromosomes visualized by an indirect fluorescent immunocytochemical procedure. Exp Cell Res 141:397–407

Vanneste E, Voet T, Le Caignec C et al (2009) Chromosome instability is common in human cleavage-stage embryos. Nat Med 15:577–583

Velagaleti GV, Bien-Willner GA, Northup JK et al (2005) Position effects due to chromosome breakpoints that map approximately 900 Kb upstream and approximately 1.3 Mb downstream of SOX9 in two patients with campomelic dysplasia. Am J Hum Genet 76: 652–662

Vermeesch JR, Rauch A (2006) Reply to Hochstenbach et al. "Molecular karyotyping". Eur J Hum Genet 14:1063–1064

Vermeesch JR, Fiegler H, de Leeuw N et al (2007) Guidelines for molecular karyotyping in constitutional genetic diagnosis. Eur J Hum Genet 15:1105–1114

Vissers LE, de Vries BB, Osoegawa K et al (2003) Array-based comparative genomic hybridization for the genomewide detection of submicroscopic chromosomal abnormalities. Am J Hum Genet 73:1261–1270

Vissers LE, Stankiewicz P, Yatsenko SA et al (2007) Complex chromosome 17p rearrangements associated with low-copy repeats in two patients with congenital anomalies. Hum Genet 121:697–709

Voelkerding KV, Dames SA, Durtschi JD (2009) Next-generation sequencing: from basic research to diagnostics. Clin Chem 55:641–658

Walsh T, McClellan JM, McCarthy SE et al (2008) Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. Science 320:539–543

Warburton D (1991) De novo balanced chromosome rearrangements and extra marker chromosomes identified at prenatal diagnosis: clinical significance and distribution of breakpoints. Am J Hum Genet 49:995–1013

Weterings E, Van Gent DC (2004) The mechanism of non-homologous end-joining: a synopsis of synapsis. DNA Repair (Amst) 3:1425–1435

Willatt L, Cox J, Barber J et al (2005) 3q29 microdeletion syndrome: clinical and molecular characterization of a new syndrome. Am J Hum Genet 77:154–160

Wong KK, deLeeuw RJ, Dosanjh NS et al (2007) A comprehensive analysis of common copy-number variations in the human genome. Am J Hum Genet 80:91–104

Xu B, Roos JL, Levy S et al (2008) Strong association of de novo copy number mutations with sporadic schizophrenia. Nat Genet 40: 880–885

Yobb TM, Somerville MJ, Willatt L et al (2005) Microduplication and triplication of 22q11.2: a highly variable syndrome. Am J Hum Genet 76:865–876