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Advanced genome-wide screening in human genomic disorders

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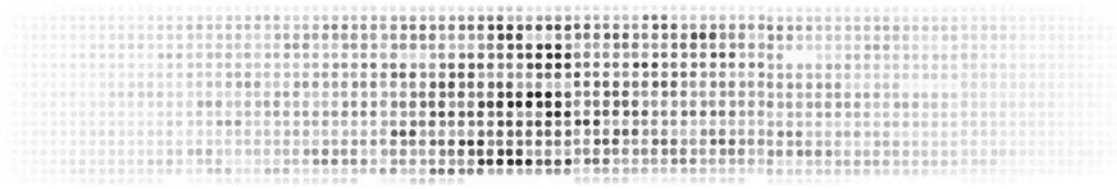
Chapter 9

Discussion



Before the introduction of genome wide molecular screening techniques, structural alterations in chromosomes have been found mainly by G-banding, allowing the detection of aneusomies and structural alterations of at least 5-10 Mb in size. They have been commonly identified based on the principle of "phenotype first", in which patients with the same phenotype have been found to have similar structural or numerical chromosomal alterations. Cryptic alterations were occasionally revealed by the detection of a patient with a balanced translocation in a patient group with similar phenotypic characteristics and no apparent visible structural alterations. The translocation breakpoints in the index case could then pinpoint the region causative for the phenotype and the patient group could subsequently be screened for involvement of this genetic region with FISH or mutation detection by sequencing.
With the introduction of molecular techniques for whole genome analysis a new era has started in clinical cytogenetics. Array-CGH, SNP arrays and massive parallel sequencing provide information on unbalanced structural chromosomal alterations in much higher resolution and with more precision than conventional banding methods. Massive parallel sequencing techniques as paired-end mapping even detect balanced translocations and inversions and SNP arrays can detect copy number neutral alterations as uniparental disomies.
These new tools are needed to unravel the causal defects in patients with idiopathic developmental delay with or without congenital abnormalities. It was already known that the causes for developmental delay could be very diverse, from large chromosomal or numerical alterations to single gene deletions, disruptions or mutations and even to environmental factors or metabolic disease. Nevertheless, the development of array-CGH proved to be of significant value in the diagnostics of mental retardation in terms of resolution and accuracy of the detection of the abnormality. An example for this is shown in chapter 2, where several cell lines of patients with chromosomal alterations were investigated. In one case, G-banding revealed an aberrant chromosome 8 that was judged to be an isochromosome 8q. After array-CGH analysis the marker chromosome was found to have a partial 8p deletion together with a partial 8q duplication, instead of an isochromosome 8q. Subsequently, the mother was found to be carrier of a pericentric inversion of chromosome 8. A second case was identified with a ring chromosome X, while G-banding was unable to resolve the origin of the ring chromosome. In a third case array-CGH showed the capacity to detect different sub-lineages in a ring chromosome 13 cell line, showing the instability of this particular ring chromosome. This instability was proven with FISH.
A separate example of the power and additional value of array-CGH is given in chapter 5. Here, a patient with a complex rearrangement as detected with G-banding, was thought to originate from a balanced three way translocation and a separate insertion. Detailed investigation using array-CGH and FISH revealed eight breakpoints with an additional deletion in one breakpoint region. This and other publications [1-4] proved that a significant portion of complex

rearrangements that appear balanced with G-banding have additional rearrangements or deletions not detected using classical cytogenetics.
Several cases of ring chromosomes have also shown to be more complex when detailed array analysis was performed after conventional G-banding. Chapter 6 describes a ring chromosome 14 which next to a terminal deletion also has an inverted duplication and a triplication. These additional alterations were not seen initially nor retrospectively using cytogenetic G-banding investigation. Based on this chapter and the publication of Rossi et al. [5], one can conclude that screening with high resolution molecular techniques is needed for correct interpretation of acrocentric ring chromosomes.
When patients with idiopathic mental retardation are studied using array-CGH, providing a 10-fold better resolution than G-banding, 10-15% more causal genetic alterations are detected, as shown in this thesis in chapter 3 and other published studies [6]. These figures match the percentages that were estimated at the start of the project to establish array-CGH within our laboratory. The results also emphasized that the alterations found by array-CGH provide superior resolution and are mostly heterogeneous, which limits the possibilities for targeted screening for unknown genetic factors [7].
Nevertheless, many recurrent deletions and duplications are detected by array-CGH. They are often found to be flanked by repetitive sequences, by which the formation of these alterations is mediated. Non-allelic homologous recombination is described as the main responsible mechanism. During meiosis misalignment takes place between the two repeats and subsequent recombination causes deletion or duplication of the unique sequence between the repeats. The finding of such recurrent deletions and duplications resulted in the identification of the underlying genetic cause of several different syndromes. This is sometimes called “genotype-first” type of research, whereby contrary to the phenotype-first studies the genetic alteration of a patient with idiopathic mental retardation is elucidated first, after which the patient can be categorized in relation to other patients with similar genetic alterations. Often the phenotypic characteristics of these patients are retrospectively comparable, but for example because of heterogeneity in the phenotype, it proves to be a difficult task for clinical geneticists to diagnose the right syndrome, without *a priori* whole genome screening results [8].
In chapter 7 an example of a recurrent duplication is described, which is mediated by the recombination of flanking duplicons. This report describes a genetic alteration with a rather variable phenotype. The fact that this recurrent duplication is not found in unrelated normal individuals supports the conclusion that it represents the causal alteration. The variation seen in the phenotype may possibly be explained by other unknown genetic or environmental factors.
In the latter case, as well as in many other individuals with mental retardation, epigenetic factors may also play a role in the origin of the retardation. For instance imprinting is known to play a role in the causality of Prader-Willi

syndrome, Angelman syndrome and Beckwith-Wiedemann syndrome, in which in some cases the expression of a gene is influenced by uniparental disomy of the involved chromosome. In Angelman syndrome a fraction of patients was found with a paternal imprinting pattern on the maternal allele affecting the expression of the involved gene [9]. Probably more syndromes of mental retardation are caused by differences or defects in hypo- or hypermethylation of promoter regions or genes involved in development or of aberrant methylation or acetylation of histones, thereby influencing underlying gene expression.
High resolution whole genome molecular screening such as array-CGH results in the detection of many sub-microscopic copy number variations. Chapter 8 shows clearly that in a homozygous state normal copy number variations may have phenotypic impact and it is rather likely that more regions currently reported as normal variation may have a similar influence. Also one can expect that some regions of variation can be tolerated in a person without consequence, while in another individual the same region causes a phenotype because of a different genetic background. With more detailed investigations and further documentations it is expected that so-called polygenic causes for disease will become a more frequent finding.
To maximally benefit from data on copy number variation it is very important that international arrangements are made for the documentation of the data. The Hospital of Sick Children in Toronto, Canada, started to host a database called the Database of Genomic Variants (DGV) [10,11]. This database contains data of structural variation in normal individuals or cell lines, generated with microarray platforms and mass sequencing techniques. Free accessibility for the whole community is offered allowing everybody to profit from the wealth of information that arises from individual experiments. Nevertheless care must be taken to only implement data of a high quality standard, so that the information collected from the database can be interpreted correctly. The database should also be maintained properly, according to the latest results and insights, since new data could shed different light on the influence of structural variation to disease. Two other databases, a European one named the “European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations” or ECARUCA [12], and an international database called the “DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources” or DECIPHER [13], register chromosomal imbalances that are presumably linked to a phenotype. Registered geneticists and cytogeneticists are able to upload molecular data on reported copy number variations, combined with the phenotypic patient information. These databases are by necessity partly restricted to the registered professionals to protect private patient information. Worldwide submitted data can serve as a reference in explaining found copy number data in a single patient. Also for these databases control and quality assurance of submitted data is a prerequisite. The collaboration that resulted in

the publication of chapter 7 was based on contacts initiated through the DECIPHER platform.
Molecular genetic techniques for whole genome screening have proven to be highly valuable in research settings. In chapter 4 a different strategy for routine screening of developmentally delayed patients is proposed, in which routine screening with MLPA precedes array-CGH and conventional G-banding. This approach may result in a more cost effective screening of patients.
The use of array-CGH in the studies presented in this thesis has been successful. In chapter 3 an increase of 16% of the genetic alterations found in a patient group with mental retardation with or without congenital anomalies was found using array-CGH. This percentage was comparable to percentages found in other studies of array-CGH screening in mental retardation patients. Moreover, the introduction of the technique created a number of possibilities for follow-up research and interesting research questions, resulting in detailed molecular characterization of several genetic alterations as presented in this thesis.
The field of genetics is developing very rapidly, with techniques such as paired-end mapping and mass-sequencing being introduced recently. Therefore, it is far from clear what the gold standard will be for cytogenetic laboratories in the near future. However, as this thesis and many other publications prove, it is certain that the field of cytogenetic screening with mainly G-banding and FISH needs to change dramatically. The latest developments make new diagnostic approaches possible to maximize the yield of genomic information in a cost-efficient way. On the other hand, the era of classical chromosome investigation is not over yet. Correct interpretation and confirmation of the molecular genetic data as well as the detection of Robertsonian and reciprocal translocations and inheritance patterns continue to require chromosome analysis by microscopy.

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