Advanced genome-wide screening in human genomic disorders

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Summary

In the past decades it gradually became clear that genetics play a significant role in idiopathic mental retardation. The search for genetic alterations responsible for idiopathic mental retardation has been dominated by cytogenetic screening methods as chromosome banding. Many genetic alterations, such as trisomies, partial trisomies or monosomies and balanced or unbalanced translocations were detected. However, as the detection limit of classical cytogenetic screening is about 5-10 megabases, this limitation has precluded the finding of a positive genetic cause in a large portion of cases.

In order to detect smaller genetic alterations genome-wide a new technique was developed, called array comparative genomic hybridization (array-CGH). This molecular genetic technique is based on the comparison of two differentially fluorescently labeled genomic DNA samples (typically a test or patient sample and a reference sample of a normal individual), hybridized to an array of immobilized DNA fragments representing specific locations on the genome. The resolution of this array-CGH technique depends on the spacing of spotted DNA fragments and the length of each spotted probe.

In chapter 1 array-CGH is introduced and placed into the spectrum of other cytogenetic and molecular screening techniques currently used. Chapter 2 to 8 of this thesis describe the development and use of an array-CGH platform in the field of molecular and cytogenetics, built up using large insert clones with a spatial resolution of about 1 Megabase throughout the whole euchromatic genome. Added functionality of array-CGH compared to conventional banding techniques is shown in chapter 2. Chromosomal imbalances could be detected more accurately and even be revised in primary cell lines from patients with known abberations.

In a consecutive study array-CGH was compared to current techniques with respect to detection of potential pathogenic genetic alterations. Array-CGH detected 16% more confirmed potential pathogenic genetic alterations in idiopathic mental retardation as shown in chapter 3. This percentage is in concordance with other published studies.

The advantages described above may change the approach that is currently used for postnatal genetic testing. While conventional chromosome banding has been the method of choice for screening for years, in chapter 4 a fundamental new approach is proposed that used a panel of molecular techniques as MLPA and array-CGH as the first step in screening for genetic alterations. This robust, cost-effective and high-throughput procedure may lead to a faster and more precise elucidation of chromosomal imbalances.

The following chapters then explain in a detailed way that array-CGH including high resolution oligo array-CGH, are useful tools to elucidate chromosome imbalances in detail and a convenient step towards characterize alterations down to a single base. This type of studies not only detects genetic alterations, but

also contributes to the common knowledge of the understanding of how chromosome rearrangements take place. They may result in better genotype to phenotype correlation of patients and can pinpoint the influence of a given gene or genetic region to a specific disease. These chapters also show that it is highly important to establish and maintain international collaborations within the field of genetics to share data on copy number variation, in order to distinguish disease related changes and normal variation.

Chapter 5 and chapter 6 describe examples of complex chromosomal imbalances that are elucidated in detail using array-CGH. In chapter 5 a seemingly balanced complex translocation involving three chromosomes and eight breakpoints was additionally found to contain a cryptic deletion. Chapter 6 shows a complex ring chromosome with an additional duplication and triplication, which initially was concluded to only have a simple subtelomeric deletion with standard microscopical evaluation. This chapter proves that in order to obtain a correct genotype to phenotype correlation detailed screening using G-banding analysis in combination with high resolution molecular techniques is necessary.

In chapter 7 a microduplication of chromosome 3q29 found in four different families is described. It is shown that this specific chromosomal rearrangement has a reduced penetrance and a variable phenotype. To conclude causality of a copy number variation like this duplication it is important to document case reports and families with uncommon variation in broadly accessible databases. The phenotypic influence of a copy number variation can then be recognized earlier. On the other hand it remains a challenge to correctly interpret the data on copy number variation that is recorded in these databases, as is shown in chapter 8. Here, a deletion of chromosome 15q15.3 which is described as a normal copy number variant is proven to be causing hearing loss in a syndromic patient in a homozygous state.

Now or in the near future array-CGH techniques will generally be implemented in diagnostic laboratories to support or partially replace the classical cytogenetics. They have proven to be a very valuable addition to the diagnostics of clinical genetics, but in order to correctly interpret structural alterations metaphase chromosome analysis remains an important technique. Nevertheless, the field of genetics proves to be highly innovative as next generation sequencing techniques are already on the doorstep to take over array comparative genomics. These next generation sequence techniques will be able to sequence the whole genome of a patient in search of causal alterations and will result in superior resolution over the previous techniques. It proves that the development within the field of diagnostics has certainly not come to an end.