

ArrayCGH in diagnosing copy number alterations in mental retardation

Björn Menten

About one to three percent of the human population is afflicted by mild to severe mental retardation, often in association with congenital abnormalities (MR/CA). These abnormalities in normal human morphogenesis may express themselves as subtle dysmorphic signs not causing any harm or present as severe disabling and life-threatening malformations such as congenital heart defects. It is well established that constitutional chromosomal aberrations are an important cause for MR/CA. The screening for such chromosomal rearrangements is done by widely used routine analysis of banded metaphase chromosomes (karyotyping). Given the limited resolution of such analyses (5-10 Mb), it was anticipated that a significant number of submicroscopic deletions or duplications (DNA copy number variations, CNV) were overlooked in patients with idiopathic mental retardation with or without congenital anomalies.

We performed one of the first exhaustive studies of this patient group using a new and more sensitive method for detection of CNVs (1). This technique, termed array comparative genomic hybridization (array CGH), allows the genome wide screening for submicroscopic aberrations in one single experiment. Array CGH uses reporter DNA molecules more or less evenly spread throughout the entire genome which are spotted or synthesized in an array on a glass slide. Each reporter is used to interrogate the DNA copy number of a specific genomic region through the competitive hybridization of differentially fluorescent labeled patient and control DNA. Together with the tedious optimization of the technique, also a web based open source (MySQL) database platform was developed for the analysis and visualization of large amount of array CGH data (medgen.ugent.be/arrayCGHbase) (2).

A total of 140 carefully clinically selected patients with mental retardation and/or congenital abnormalities were analyzed for hidden chromosomal aberrations in a collaborative effort with the Center for Medical Genetics Leuven (KUL). This initial study together with a review of other published investigations, allowed for the first time to establish a reliable figure of the number of submicroscopic CNVs in this patient population. When excluding patients with subtelomeric imbalances which could be identified through FISH or MLPA analyses, array CGH still allowed to detect CNVs in an additional ~8% of patients (1).

A major challenge resulting from this new flow of information is the search and description of new microdeletion/microduplication syndromes. Although most CNVs seemed to be scattered across the entire genome we were able to describe a new microdeletion syndrome characterized by osteopoikilosis, mental retardation and short

stature. This observation was facilitated through the identification of *LEMD3* as the causal gene for osteopoikilosis, Buschke-Ollendorff syndrome (BOS) and melorheostosis in the 12q14.3 deleted interval and subsequent, the finding of two additional patients with a 12q14.3 microdeletion (3).

ArrayCGH can also play an important role in the delineation of the critical region for recurrent deletion syndromes (4). In this study we identified a small interstitial deletion on chromosome 18q12.3 in a patient with clinical features of the del(18)(q12.1q21.1) syndrome. We were able to delineate the critical region for this syndrome to an interval of 1.8 Mb, enabling hereby the determination of the crucial genes for this microdeletion syndrome (4).

In conclusion, arrayCGH has several important applications in the field of clinical cytogenetics. The use of this new performant methodology will greatly improve the diagnostic yield in patients with unexplained mental retardation, provide more insights into genotype-phenotype correlations and ultimately lead to the identification of the causal genes. Functional studies of these gene products will enhance our understanding of the genetic regulation in normal human morphogenesis, embryogenesis and brain functioning. Finally, it is my believe that implementation of array CGH will represent a major and perhaps last wave of innovation in cytogenetics, as the latter may become largely redundant. Ultimately and perhaps earlier than we can anticipate, sequencing of the whole genome of a patient may eventually emerge as the method of choice.

References:

1. Menten B, Maas N, Thienpont B, Buysse K, Vandesompele J, Melotte C, de Ravel T, Van Vooren S, Balikova I, Backx L, Janssens S, De Paepe A, De Moor B, Moreau Y, Marynen P, Fryns JP, Mortier G, Devriendt K, Speleman F, Vermeesch JR. 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.* 2006 Aug;43(8):625-33.
2. Menten B, Pattyn F, De Preter K, Robbrecht P, Michels E, Buysse K, Mortier G, De Paepe A, van Vooren S, Vermeesch J, Moreau Y, De Moor B, Vermeulen S, Speleman F, Vandesompele J. arrayCGHbase: an analysis platform for comparative genomic hybridization microarrays. *BMC Bioinformatics.* 2005 May 23;6:124.
3. Menten B, Buysse K, Zahir F, Hellemans J, Hamilton SJ, Costa T, Fagerstrom C, Anadiotis G, Kingsbury D, McGillivray BC, Marra MA, Friedman JM, Speleman F,

Mortier G. Osteopoikilosis, short stature and mental retardation as key features of a new microdeletion syndrome on 12q14. *J Med Genet.* 2007 Apr;44(4):264-8.