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NEWS AND COMMENTARY

Diagnostics

Genomic copy number analysis in mental retardation: finding the needles in the haystack

Joris A Veltman

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Technological advancements are rapidly changing the way the human genome is being analysed for the presence of structural or numerical abnormalities associated with health and disease. Until recently, the major diagnostic tools available in a cytogenetics laboratory were chromosome banding and fluorescence *in situ* hybridization. The diagnostic yield of these approaches in the general population of patients with unexplained mental retardation is approximately 10%.[1] Thanks to the human genome sequencing project and the development of microarray-based genome profiling methods such as array-based comparative genomic hybridization (array CGH[2]), we are now able to screen the human genome for the presence of genomic dosage variations at an unprecedented resolution. These methods will rapidly find their way in novel diagnostic applications if validation and clinical interpretation become straightforward. In the September issue of *EJHG* Saugier-Veber *et al*[3] described the application of a novel method, quantitative multiplex PCR of short fluorescent fragments (QMPSF), for high-throughput analysis of targeted genomic alterations in patients with mental retardation. This study nicely demonstrates the capacity of this relatively simple and affordable approach to detect genomic dosage variation and its diagnostic potential in mental retardation.

Although diagnostic genome profiling by microarrays is rapidly revealing hundreds of novel submicroscopic dosage variations in mental retardation,[4,5] interpretation of genomic microarray results is not trivial. Dosage variations may constitute false positive findings or represent genomic polymorphisms without clinical consequences. Therefore, it is essential in a diagnostic setting to validate these findings by an independent technology, test unaffected parents if available, and/or a large cohort of ethnically matched controls. In addition, after establishing the link of a novel genomic dosage variation with disease, one would like to establish the frequency of such a variation in the diseased population by rapidly testing a large cohort of patients with a similar condition. Approaches are needed for these various downstream analyses that are semiquantitative, high-throughput, relatively cheap and easy to implement, and QMPSF is such an approach. Saugier-Veber *et al*[3] selected 12 candidate loci known to be involved in mental retardation, developed locus-specific primers and validated the assay by using positive controls. In the second part of their study, they screened these loci by QMPSF in almost 300 patients with unexplained mental retardation. This resulted in the identification of three novel genomic rearrangements, which were validated by locus-specific QMPSF assays and are likely to be disease causing as the affected loci were known to be associated with mental retardation and for one rearrangement *de novo* occurrence was proven.

The QMPSF method was developed in 2000 by Charbonnier *et al*[6] for the detection of deletions and duplications of mismatch repair genes in HNPCC, and has been used to screen for genomic dosage variations in genes like TP53, CFTR and APP. It is important to note that the technology has been used predominantly by a single research group, and therefore the ease of implementation is at present unknown (although the method seems straightforward). In addition, in most studies in which QMPSF has been used a maximum of 12 loci were tested in a single assay. It is unclear whether this number can easily be increased without affecting the sensitivity of the assay. Next to QMPSF, there are several other semi-quantitative multiplex PCR assays for the detection of genomic copy number variation. These assays include multiplex amplifiable probe hybridization (MAPH[7]), multiplex ligation-dependent probe amplification (MLPA[8]), and real-time qPCR.[9] Both MAPH and real-time qPCR are quite laborious and difficult to implement in a routine setting, this in contrast to MLPA which has become the most widely used method for targeted genomic dosage measurements. MLPA has the advantage over QMPSF that it allows the analysis of up to 40 loci in a single multiplex reaction, and, because of the required ligation step, is very specific, allowing copy number analysis of regions with high sequence homology. Primer design, on the other hand, appears less critical for the QMPSF method and this approach may be more cost-effective and suitable for rapid validation experiments of loci with unique sequences. The performance of both methods has not been compared, and therefore it remains speculative which technology is most suited for targeted high-throughput genomic copy number analysis.

In conclusion, the combination of microarray-based genome-wide copy number analysis with high-throughput targeted approaches such as QMPSF will rapidly result in the identification and characterization of the majority of genomic loci with dosage variation associated with mental retardation. Most submicroscopic alterations identified by array CGH until now are unique, identification of patients with overlapping alterations by technologies like QMPSF is essential for clinical interpretation and counselling

purposes. In addition, the genes located within these recurrent loci will be prime candidates for mental retardation and their analysis will improve our insight into the biological pathways disturbed in this frequent disorder ■

Joris A Veltman is at the Department of Human Genetics, Radboud University Nijmegen Medical Centre, PO Box 9101, Nijmegen 6500 HB, The Netherlands. Tel: 31 24 3614941; Fax: 31 24 3668752; E-mail: j.veltman@antrg.umcn.nl

References

1 van Karnebeek CD, Jansweijer MC, Leenders AG, Offringa M, Hennekam RC: *Eur J Hum Genet* 2005; **13**: 6–25.
2 Albertson DG, Pinkel D: *Hum Mol Genet* 2003; **12**: R145–R152.
3 Saugier-Veber P, Goldenberg A, Drouin-Garraud V *et al*: *Eur J Hum Genet* 2006; **14**: 1009–1017.
4 de Vries BB, Pfundt R, Leisink M *et al*: *Am J Hum Genet* 2005; **77**: 606–616.
5 Menten B, Maas N, Thienpont B *et al*: *J Med Genet* 2006; **43**: 625–633.
6 Charbonnier F, Raux G, Wang Q *et al*: *Cancer Res* 2000; **60**: 2760–2763.
7 Armour JA, Sismani C, Patsalis PC, Cross G: *Nucleic Acids Res* 2000; **28**: 605–609.
8 Schouten JP, McElgunn CJ, Waaijer R *et al*: *Nucleic Acids Res* 2002; **30**: e57.
9 Weksberg R, Hughes S, Moldovan L *et al*: *BMC Genom* 2005; **6**: 180.