High-resolution karyotyping by oligonucleotide microarrays : the next revolution in cytogenetics

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Chapter 6

Discussion

Conventional karyotyping by G-banding has been in use since the 1970s as the standard technique in many laboratories to detect chromosomal aberrations. With this technique it is possible to identify chromosomal rearrangements of at least 5-10 Mb. Other techniques have been developed to detect smaller aberrations. These methods, such as Fluorescence *in situ* Hybridization (FISH), Multiplex Ligation-dependent Probe Amplification (MLPA) and Quantitative Fluorescence-Polymerase Chain Reaction (QF-PCR), are useful only for identifying microdeletions and subtelomeric regions or for confirming and further characterizing previously identified chromosomal aberrations. In recent years a technique to screen the whole human genome in a single experiment, the array Comparative Genome Hybridization (aCGH), has been developed. It enables the detection of copy number variants (CNVs) (deletions and duplications) that are approximately 100 times smaller than those that can be identified by conventional karyotyping. The first genome-wide CGH arrays were based on large DNA fragments such as bacterial artificial chromosomes (BACs) (Solinas-Toldo et al., 1997; Pinkel et al., 1998). The resolution of these arrays depends on the size of the probes. Other CGH arrays were developed with small oligonucleotide probes (45-85 - mer) (Agilent and Nimblegen) that allowed the detection of smaller CNVs. More recently, the Single Nucleotide Polymorphism (SNP) array, which was originally developed for genome wide association studies, turned out to be ideal also for the detection of CNVs. These oligonucleotide SNP arrays (25-50 - mer) (Affymetrix and Illumina) are based on probes containing a SNP. With this oligonucleotide array technique (both aCGH and SNP) the resolution has increased to approximately 10 - 100 kb and depends on the genomic spacing between the probes.

Mental retardation (MR) occurs in 2-3% of the population and in approximately 5% of these patients a chromosomal aberration can be detected by conventional karyotyping (de Vries et al., 2005). In contrast, the overall yield of CNVs detected by high-resolution array (aCGH and SNP) in MR patients is approximately 17% (Vissers et al., 2003; Shaw-Smith et al., 2004; Schoumans et al., 2005; Tyson et al., 2005; Friedman et al., 2006; Menten et al., 2006; Rosenberg et al., 2006; Fan et al., 2007; Hoyer et al., 2007). We studied 318 MR patients using high-resolution SNP arrays in order to establish the frequency of submicroscopic CNVs in patients with idiopathic MR (**chapter 2**). In this cohort we identified different chromosomal aberrations in 22.6% of the MR patients. It should be noted, however, that most of the above mentioned reports, including our study, represent highly selected patient cohorts. In all these patients conventional karyotyping had been performed already and in a smaller group of patients also locus-specific FISH and subtelomeric MLPA had been done, however no aberration was detected. Testing every MR patient with a SNP array instead of conventional karyotyping would further increase the diagnostic power of this method and we have, therefore, recommended it as a new diagnostic tool to be included in the workflow for MR patients (**chapter 2**). At present, high-resolution array is being successfully implemented in routine clinical diagnostic laboratories. It allows the screening for all known microdeletion and microduplication syndromes as well as novel CNVs in a single experiment. As a consequence, fewer diagnostic tests are needed to facilitate a rapid diagnosis in many patients.

A major advantage of the SNP array is that it provides information on the genotype and enables the detection of regions of loss of heterozygosity (LOH) (**chapter 2**). LOH can be the result of a deletion or of uniparental disomy (UPD), which is known to cause genomic disorders such as Prader-Willi syndrome and Silver-Russel syndrome.

With a SNP array it is possible to distinguish between heterodisomy and isodisomy if both parents are hybridized on an array. Furthermore the genotype information can be used to identify homozygous regions in offspring from consanguineous parents, to resolve questions concerning mix-up of samples and paternity, and to determine the parental origin of a deletion. An additional advantage of the genotype information is that it serves as an extra control step to confirm a deletion or a duplication. In case of a deletion all SNPs appear homozygous because of loss of one allele; duplications show four possible genotypes, including AAA, AAB, ABB and BBB.

Although it was originally suspected that array analysis would not be able to detect mosaicism, it appears that the aCGH and SNP array techniques are actually more sensitive in detecting low-level mosaicism than conventional karyotyping (**chapter 2** and **3.4**). Usually, an insufficient number of cells is counted unless mosaicism is suspected. Also, a single abnormal cell might be interpreted as an artifact of cell culture. So, one can easily fail to detect an aberrant subset of cells with conventional karyotyping. Furthermore, as shown in **chapter 2**, **4.2** and **5.1**, the array technique is more sensitive in detecting unbalanced translocations. Relatively large aberrations (3 – 21 Mb) are missed by conventional karyotyping because telomeric bands of many chromosomes are similar in appearance.

In **chapter 3.2** and **4.1** we have shown that the SNP array is useful in characterizing previously detected microscopically visible chromosomal rearrangements. In **chapter 3.2** we have described apparently balanced translocations and inversions where the SNP array detected additional CNVs. These CNVs were either at the breakpoints of the rearrangement or were on chromosomes unrelated to the previously detected aberration. In **chapter 4.1** we have presented a patient where conventional karyotyping detected extra material of unknown origin on the long arm of chromosome 9. SNP array analysis detected a duplication of 400 kb, a triplication of 2.4 Mb and a duplication of 130 kb of chromosome band 9q34.3. In this case, the SNP array proved to be an accurate method for the identification and delineation of the chromosomal rearrangement. As there is no technique available at present that can detect complex chromosome rearrangements as well as cryptic CNVs in one experiment, conventional karyotyping, FISH and high-resolution array screening remain essential for unravelling complex karyotypes.

The disadvantage of using arrays instead of conventional karyotyping is that arrays cannot detect balanced rearrangements. A large prenatal study has shown that approximately 0.5% and 0.1% of the antenatal cases carry an apparently balanced reciprocal translocation or an inversion respectively (Warburton, 1991). Only 6% of these cases are associated with abnormal phenotypes. The abnormal phenotype can be caused by the disruption of a gene at the breakpoint or by a small duplication or a deletion that is beyond the resolution of the microscope. The SNP array analysis would (depending on the resolution) detect the small abnormalities, though the disruption of genes would remain undetected. A Dutch retrospective study has shown that of all referrals only about 0.78% potentially pathogenic balanced rearrangements would remain undetected by array analysis without conventional karyotyping (Hochstenbach et al., 2009). Conventional karyotyping, on the other hand, will miss a much higher percentage of unbalanced rearrangements if no array analysis is performed.

An ideal technique would combine whole-genome high-resolution screening for CNVs and the detection of inversions, insertions and translocations. This is possible with paired-end mapping, a new sequencing method, whereby aberrations from

approximately 3 kb can be identified (Korbel et al., 2007). It involves fragmentation of genomic DNA to 3 kb fragments; these fragments are circularized and randomly sheared. These products are sequenced and mapped back to the reference sequence. Paired-end mapping has already been used successfully in breakpoint mapping of balanced chromosome rearrangements (Chen et al., 2009). However, experience with this technique in whole-genome screening is limited. More importantly, the costs are high and the process is very laborious. A rapid and routine implementation of this technique is therefore not to be expected soon.

Finally, another example of the power of high-resolution arrays is given in **chapter 5**. Two studies in which the SNP arrays were used to detect CNVs in patients with phenotypes other than MR are presented. In **chapter 5.1** we have reported the detection of CNVs in 33% of patients with a BPES-like phenotype. In **chapter 5.2** we have used high-resolution SNP arrays to detect CNVs in patients with features of the SOTOS syndrome. We found chromosomal aberrations in 15% of these SOTOS-like patients.

The major recurring theme in this thesis is that the clinical consequence of novel CNVs is not always immediately evident in many cases (**chapter 2**, **3.1**, **3.2**, **3.3**, **5.1** and **5.2**). High-resolution array studies have revealed many new microdeletion and microduplication syndromes (reviewed by Slavotinek, 2008). There are other recurrent deletions and duplications for which the clinical significance is not immediately clear. A good example of the difficulty in assigning clinical significance is discussed in **chapter 3.1**. A ~600 kb 16p11.2 deletion was initially detected in patients with autism, but later it was seen also in patients with MR, in healthy individuals, and finally in obese patients (Walters et al., 2010). This suggests that the recurrent 16p11.2 deletion is associated with a variable outcome and it is even uncertain whether it is pathogenic. There are other well known microdeletion syndromes with a wide phenotypic spectrum (e.g. 22q11.2) (Edelmann et al., 1999). Since geneticists often assume that only *de novo* CNVs are pathogenic, one of the first steps in a routine diagnostic workflow is to check the parents for transmission of a CNV. Besides, it has been shown already that patients with known syndromes can inherit this deletion from one of their healthy parents (Edelmann et al., 1999). The cause for this phenotypic variability is unknown. Several hypotheses have been proposed to explain the variable effect of deletions: stochastic variation of gene expression at a lower level (Cook et al., 1998), presence of modifying genes on the other undeleted homologue as was demonstrated recently at the locus for adult polycystic kidney disease, *PKD1* (Rossetti et al., 2009), influence of unlinked genes or epigenetic factors that may play a role. Recently, Girirajan and colleagues suggested a two-hit model for a recurrent 16p12.1 microdeletion (Girirajan et al., 2010). They found that 30% of the affected individuals with a 16p12.1 microdeletion carried a second CNV. These results show for the first time two independent chromosomal changes in a mentally retarded patient, confirming the multifactorial model proposed decades ago. With the increasing resolution of genome analysis similar examples are likely to follow soon. The big challenge, however, is to prove the pathogenicity of each CNV or genomic variant, which is becoming more difficult with the increasing complexity. To gain better insight in the pathogenicity of these CNVs large numbers of patients and their families need to be analyzed in great detail.

Even before the introduction of G-banding it was clear that two chromosomes of a pair were not always alike. As most variants were found in individuals with a clinical abnormality, it was not until large newborn studies that it became evident that

these variants were benign (reviewed by Wyandt, 2004). With the introduction of the whole-genome high-resolution screening technique, a similar problem has appeared; this technique is able to detect smaller abnormalities and thereby also new variants of unknown clinical relevance (**chapters 2** and **3**). It has been estimated that up to 12% of the human genome is involved in CNVs (Redon et al., 2006). Large studies on healthy individuals have revealed many new benign CNVs which are collected in the Database of Genomic Variants (DGV) (Iafrate et al., 2004; Sebat et al., 2004; Sharp et al., 2005; Tuzun et al., 2005; Feuk et al., 2006; Friedman et al., 2006; Hinds et al., 2006; Locke et al., 2006; McCarroll et al., 2006; Redon et al., 2006; Conrad and Hurles, 2007). This database is a valuable tool for geneticists for comparing CNVs that they find in their patients. Although this database is still growing, many rare CNVs identified in MR patients are not reported. As more data becomes available in the next few years, it will become clear whether CNVs that are at present classified as potentially pathogenic are pathogenic or not. Databases like DECIPHER and ECARUCA, which collect CNVs detected in patients can help unravel novel disease-causing CNVs much faster.

In March 2010 the DGV contained 14478 CNV loci and is still regularly updated. The data submitted to this database are not subjected to an editorial screening, the only requirements being that the data is published as a scientific manuscript and that the CNV was identified in a non-disease control sample. The contributors are therefore able to use different methods with a variety of detection rates, error rates, and genomic coverage. In some contributing studies no distinction is made between gains and losses; these CNVs should therefore be excluded for comparison. Caution should be exercised in the interpretation of the submitted CNVs.

Manufacturers producing high-resolution array techniques are developing new arrays with even higher resolution. Recently we have tested the latest available 2.7M (Affymetrix) array. These arrays contain 2.7 million markers, including 400.000 SNP probes and 2.3 million CNV probes. Not only the resolution but also the laboratory procedures are constantly being improved. In diagnostic laboratories, procedures with fewer steps and more automation are highly encouraged, since this means less hands-on time and less possibility of sample mix-up. However, a higher resolution will, per definition, also detect more CNVs of unknown clinical significance.

Next generation sequencing technologies will eventually replace the array technologies in the genetic diagnostic flow of MR. Sequencing will be able to detect mutations as well as deletions, duplications, inversions and translocations. At present, sequencing of whole genomes is feasible and in a few years also affordable; however, analysis of the data is not yet suitable for a large scale. Bioinformaticians will be necessary to solve this problem. Implementation of sequencing will furthermore introduce the same problems on an even larger scale, as the whole-genome high-resolution arrays do at present. Large numbers of new variants will be identified, most of which are probably not disease causing and many others that may be associated with other unexpected diseases. The challenge to distinguish between harmless variants and variants causing or contributing to disease will become even more daunting.

In the series of articles presented in this thesis we have studied patients with MR and/or congenital malformations. The question we have tried to answer by studying the genome of the patient is whether we can find a cause for the signs and symptoms observed in the patient. The SNP array was successfully used for the detection of novel CNVs and has replaced the conventional karyotyping in the routine diagnostic flow in

MR patients in our diagnostic setting. We are therefore able to make a diagnosis in a higher number of MR patients, thus improving medical care and genetic counselling. However, a major complexity is the finding of potentially pathogenic CNVs for which the clinical significance is not immediately clear.

References

Chen W, Ullmann R, Langnick C, Menzel C, Wotschofsky Z, Hu H, Döring A, Hu Y, Kang H, Tzschach A, Hoeltzenbein M, Neitzel H, Markus S, Wiedersberg E, Kistner G, van Ravenswaaij-Arts CM, Kleefstra T, Kalscheuer VM, Ropers HH. 2010. Breakpoint analysis of balanced chromosome rearrangements by next-generation paired-end sequencing. Eur J Hum Genet 18:539-543.

Conrad DF, Hurles ME. 2007. The population genetics of structural variation. Nat Genet 39:S30-36.

Cook DL, Gerber AN, Tapscott SJ. 1998. Modeling stochastic gene expression: implications for haploinsufficiency. Proc Natl Acad Sci USA. 95:15641-15646.

Edelmann L, Pandita RK, Spiteri E, Funke B, Goldberg R, Palanisamy N, Chaganti RS, Magenis E, Shprintzen RJ, Morrow BE. 1999. A common molecular basis for rearrangement disorders on chromosome 22q11. Hum Mol Genet 8:1157-1167.

Fan YS, Jayakar P, Zhu H, Barbouth D, Sacharow S, Morales A, Carver V, Benke P, Mundy P, Elsas LJ. 2007. Detection of pathogenic gene copy number variations in patients with mental retardation by genomewide oligonucleotide array comparative genomic hybridization. Hum Mutat 28:1124-1132.

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

Friedman JM, Baross A, Delaney AD, Ally A, Arbour L, Armstrong L, Asano J, Bailey DK,Barber S, Birch P, Brown-John M, Cao M, Chan S, Charest DL, Farnoud N, Fernandes N, Flibotte S, Go A, Gibson WT, Holt RA, Jones SJ, Kennedy GC, Krzywinski M, Langlois S, Li HI, McGillivray BC, Nayar T, Pugh TJ, Rajcan-Separovic E, Schein JE, Schnerch A, Siddiqui A, Van Allen MI, Wilson G, Yong SL, Zahir F, Eydoux P, Marra MA. 2006. Oligonucleotide microarray analysis of genomic imbalance in children with mental retardation. Am J Hum Genet 79:500-513.

Girirajan S, Rosenfeld JA, Cooper GM, Antonacci F, Siswara P, Itsara A, Vives L, Walsh T, McCarthy SE, Baker C, Mefford HC, Kidd JM, Browning SR, Browning BL, Dickel DE, Levy DL, Ballif BC, Platky K, Farber DM, Gowans GC, Wetherbee JJ, Asamoah A, Weaver DD, Mark PR, Dickerson J, Garg BP, Ellingwood SA, Smith R, Banks VC, Smith W, McDonald MT, Hoo JJ, French BN, Hudson C, Johnson JP, Ozmore JR, Moeschler JB, Surti U, Escobar LF, El-Khechen D, Gorski JL, Kussmann J, Salbert B, Lacassie Y, Biser A, McDonald-McGinn DM, Zackai EH, Deardorff MA, Shaikh TH, Haan E, Friend KL, Fichera M, Romano C, Gécz J, DeLisi LE, Sebat J, King MC, Shaffer LG, Eichler EE. 2010. A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. Nat Genet 42:203-209.

Hinds DA, Kloek AP, Jen M, Chen X, Frazer KA. 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, Nieuwint A, Polstra A, Poddighe P, Ruivenkamp C, Sikkema-Raddatz B, Smeets D, Poot M. 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, Gohring I, Thiel CT, Peippo MM, Rauch R, Hofbeck M, Trautmann U, Zweier C, Zenker M, Huffmeier U, Kraus C, Ekici AB, Ruschendorf F, Nurnberg P, Reis A, Rauch A. 2007. Molecular karyotyping in patients with mental retardation using 100K single-nucleotide polymorphism arrays. J Med Genet 44:629-636.

Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, Scherer SW, Lee C. 2004. Detection of large-scale variation in the human genome. Nat Genet 36:949-951.

Korbel JO, Urban AE, Affourtit JP, Godwin B, Grubert F, Simons JF, Kim PM, Palejev D, Carriero NJ, Du L, Taillon BE, Chen Z, Tanzer A, Saunders AC, Chi J, Yang F, Carter NP, Hurles ME, Weissman SM, Harkins TT, Gerstein MB, Egholm M, Snyder M. 2007. Paired-end mapping reveals extensive structural variation in the human genome. Science 318:420-426.

Le Caignec C, Boceno M, Saugier-Veber P, Jacquemont S, Joubert M, David A, Frebourg T, Rival JM. 2005. Detection of genomic imbalances by array based comparative genomic hybridisation in fetuses with multiple malformations. J Med Genet 42:121-128.

Locke DP, Sharp AJ, McCarroll SA, McGrath SD, Newman TL, Cheng Z, Schwartz S, Albertson DG, Pinkel D, Altshuler DM, Eichler EE. 2006. Linkage disequilibrium and heritability of copy-number

polymorphisms within duplicated regions of the human genome. Am J Hum Genet 79:275-290.

McCarroll SA, Hadnott TN, Perry GH, Sabeti PC, Zody MC, Barrett JC, Dallaire S, Gabriel SB, Lee C, Daly MJ, Altshuler DM; International HapMap Consortium. 2006. Common deletion polymorphisms in the human genome. Nat Genet 38:86-92.

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. 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.

Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, Dairkee SH, Ljung BM, Gray JW, Albertson DG. 1998. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 20:207-211.

Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, González JR, Gratacòs M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME. 2006. Global variation in copy number in the human genome. Nature 444:444-454.

Rosenberg C, Knijnenburg J, Bakker E, Vianna-Morgante AM, Sloos W, Otto PA, Kriek M, Hansson K, Krepischi-Santos AC, Fiegler H, Carter NP, Bijlsma EK, van HA, Szuhai K, Tanke HJ. 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.

Rossetti S, Kubly VJ, Consugar MB, Hopp K, Roy S, Horsley SW, Chauveau D, Rees L, Barratt TM, van't Hoff WG, Niaudet WP, Torres VE, Harris PC. 2009. Incompletely penetrant PKD1 alleles suggest a role for gene dosage in cyst initiation in polycystic kidney disease. Kidney Int 75:848-55.

Schoumans J, Ruivenkamp C, Holmberg E, Kyllerman M, Anderlid BM, Nordenskjold M. 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, Alexander J, Young J, Lundin P, Maner S, Massa H, Walker M, Chi M, Navin N, Lucito R, Healy J, Hicks J, Ye K, Reiner A, Gilliam TC, Trask B, Patterson N, Zetterberg A, Wigler M. 2004. Large-scale copy number polymorphism in the human genome. Science 305:525-528.

Shaffer LG, Coppinger J, Alliman S, Torchia BA, Theisen A, Ballif BC, Bejjani BA. 2008. Comparison of microarray-based detection rates for cytogenetic abnormalities in prenatal and neonatal specimens. Prenat Diagn 28:789-795.

Sharp AJ, Locke DP, McGrath SD, Cheng Z, Bailey JA, Vallente RU, Pertz LM, Clark RA, Schwartz S, Segraves R, Oseroff VV, Albertson DG, Pinkel D, Eichler EE. 2005. Segmental duplications and copy-number variation in the human genome. Am J Hum Genet 77:78-88.

Shaw-Smith C, Redon R, Rickman L, Rio M, Willatt L, Fiegler H, Firth H, Sanlaville D, Winter R, Colleaux L, Bobrow M, Carter NP. 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.

Slavotinek AM. 2008. Novel microdeletion syndromes detected by chromosome microarrays. Hum Genet 124:1-17.

Solinas-Toldo S, Lampel S, Stilgenbauer S, Nickolenko J, Benner A, Döhner H, Cremer T, Lichter P. 1997. Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. Genes Chromosomes Cancer 20:399-407.

Tuzun E, Sharp AJ, Bailey JA, Kaul R, Morrison VA, Pertz LM, Haugen E, Hayden H, Albertson D, Pinkel D, Olson MV, Eichler EE. 2005. Fine-scale structural variation of the human genome. Nat Genet 37:727-732.

Tyreman M, Abbott KM, Willatt LR, Nash R, Lees C, Whittaker J, Simonic I. 2009. High resolution array analysis: diagnosing pregnancies with abnormal ultrasound findings. J Med Genet 46:531-541.

Tyson C, Harvard C, Locker R, Friedman JM, Langlois S, Lewis ME, Van AM, Somerville M, Arbour L, Clarke L, McGilivray B, Yong SL, Siegel-Bartel J, Rajcan-Separovic E. 2005. Submicroscopic deletions and duplications in individuals with intellectual disability detected by array-CGH. Am J Med Genet A 139:173-185.

Vissers LE, de Vries BB, Osoegawa K, Janssen IM, Feuth T, Choy CO, Straatman H, van der Vliet V, Huys EH, van Rijk A, Smeets D, van Ravenswaaij-Arts CM, Knoers NV, van der Burgt I, de Jong PJ, Brunner HG, van Kessel AG, Schoenmakers EF, Veltman JA. 2003. Array-based comparative genomic hybridization

for the genomewide detection of submicroscopic chromosomal abnormalities. Am J Hum Genet 73:1261-1270.

Vries de BB, Pfundt R, Leisink M, Koolen DA, Vissers LE, Janssen IM, Reijmersdal S, Nillesen WM, Huys EH, Leeuw N, Smeets D, Sistermans EA, Feuth T, van Ravenswaaij-Arts CM, van Kessel AG, Schoenmakers EF, Brunner HG, Veltman JA. 2005. Diagnostic genome profiling in mental retardation. Am J Hum Genet 77:606-616.

Walters RG, Jacquemont S, Valsesia A, de Smith AJ, Martinet D, Andersson J, Falchi M, Chen F, Andrieux J, Lobbens S, Delobel B, Stutzmann F, El-Sayed Moustafa JS, Chèvre JC, Lecoeur C, Vatin V, Bouquillon S, Buxton JL, Boute O, Holder-Espinasse M, Cuisset JM, Lemaitre MP, Ambresin AE, Brioschi A, Gaillard M, Giusti V, Fellmann F, Ferrarini A, Hadjikhani N, Campion D, Guilmatre A, Goldenberg A, Calmels N, Mandel JL, Le Caignec C, David A, Isidor B, Cordier MP, Dupuis-Girod S, Labalme A, Sanlaville D, Béri-Dexheimer M, Jonveaux P, Leheup B, Ounap K, Bochukova EG, Henning E, Keogh J, Ellis RJ, Macdermot KD, van Haelst MM, Vincent-Delorme C, Plessis G, Touraine R, Philippe A, Malan V, Mathieu-Dramard M, Chiesa J, Blaumeiser B, Kooy RF, Caiazzo R, Pigeyre M, Balkau B, Sladek R, Bergmann S, Mooser V, Waterworth D, Reymond A, Vollenweider P, Waeber G, Kurg A, Palta P, Esko T, Metspalu A, Nelis M, Elliott P, Hartikainen AL, McCarthy MI, Peltonen L, Carlsson L, Jacobson P, Sjöström L, Huang N, Hurles ME, O'Rahilly S, Farooqi IS, Männik K, Jarvelin MR, Pattou F, Meyre D, Walley AJ, Coin LJ, Blakemore AI, Froguel P, Beckmann JS. 2010. A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. Nature 463:671-675.

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.

Wyandt HE, Tonk VS, editors. 2004. Atlas of human chromosome heteromorphisms. Dordrecht: Kluwer Academic Publishers.