Original Article

Analysis of Chromosomal Aberrations in Patients with Mental Retardation Using the Array-CGH Technique: a Single Czech Centre Experience

(array-CGH / DNA microarray / mental retardation / chromosomal aberrations)

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Abstract. Submicroscopic structural chromosomal aberrations (microduplications and microdeletions) are believed to be common causes of mental retardation. These so-called copy number variations can now be routinely detected using various platforms for array-based comparative genomic hybridization (array-CGH), which allow genome-wide identification of pathogenic genomic imbalances. In this study, oligonucleotide-based array-CGH was used to investigate a panel of 23 patients with mental retardation and developmental delay, dysmorphic features or congenital anomalies. Array-CGH confirmed or revealed 16 chromosomal aberrations in a total of 12 patients. Analysis of parental samples showed that five aberrations had occurred *de novo*: del(1)(p36.33p36.23), del(4)(p16.3p16.2) joined with dup(8)(p23.3p23.1), del(6)(q14.1q15), del(11)(q13.1q13.4). Three aberrations appeared to be inherited from an unaffected parent: dup(3)(q29), del(6)(q12), dup(16)(p13.11). Six aberrations appeared to be inherited from a parental carrier: del(1)(p36.33) joined with dup(12)(q24.32), del(21)(q22.2q22.3) joined with dup(11)(q24.2q25), del(X)(q22.3) and del(1)(q21.1). In two cases, parents were not available for testing: del(17)(q11.2q12) and del(2)(q24.3q31.1). Our results show that the use of oligonucleotide-based array-CGH in a clinical diagnostic laboratory increases the detection rate of pathogenic submicroscopic chromosomal aberrations in patients with mental retardation and congenital abnormalities, but it also presents challenges for clinical interpretation of the results (*i.e.* distinguishing between pathogenic and benign variants). Difficulties with analysis notwithstanding, the array-CGH is shown to be a sensitive, fast and reliable method for genome-wide screening of chromosomal aberrations in patients with mental retardation and congenital abnormalities.

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Abbreviations: array-CGH – comparative genome hybridization using microarray, BAC – bacterial artificial chromosome, bp – base pair, CVN – copy number variants, DD – developmental delay, *GABA* – γ-aminobutyric acid gene, *GABRR1* – GABA receptor, rho 1 gene, HR-CGH – high-resolution comparative genome hybridization, FISH – fluorescent *in situ* hybridization, ISCN – International System for Human Cytogenetic Nomenclature, kb – kilo base, Mb – mega base, MLPA – multiplex ligation-dependent probe amplification, MR – mental retardation, SLOS – Smith-Lemli-Opitz syndrome, SNP – single-nucleotide polymorphism, TAR – thrombocytopoenia-absent radius.

Introduction

Mental retardation (MR) with developmental delay (DD), dysmorphic features and congenital malformations occur in the general population with an estimated prevalence of 2–3 % (Curry et al., 1997; Leonard and Wen, 2002). MR can be defined by cognitive abilities that are markedly below average (IQ < 70) and by a decreased ability to adapt to one's environment and to society. Causes of MR are heterogeneous and include genetic and environmental factors. In at least 30–50 % of cases, the aetiology of MR remains unexplained (Daily et al., 2000).

Microdeletions and microduplications are believed to be among the most frequent genetic causes of MR. For more than 50 years, karyotyping using G-banding has been a successful method for identifying chromosomal rearrangements. Based on a literature review, the mean

detection rate of chromosomal aberrations in patients with MR and/or congenital malformations using conventional karyotyping is ~9.5 % (van Karnebeek et al., 2005). However, despite its indisputable success, karyotyping using G-banding has limited resolution, and it is usually able to detect large chromosomal changes of at least 5–10 Mb (Kriek et al., 2007; Hochstenbach et al., 2009). This limitation can be overcome by newer techniques like fluorescent *in situ* hybridization (FISH) or multiplex ligation-dependent probe amplification (MLPA). However, these techniques detect only a restricted number of target sequences in the genome. The routine application of FISH and MLPA techniques in clinical practice is primarily focused on identifying well-known critical regions for the common microdeletion and microduplication syndromes (Kirchhoff et al., 2007) and on the subtelomeric regions (Rooms et al., 2004, 2005).

New technologies, especially oligonucleotide-based comparative genome hybridization (array-CGH), have dramatically changed human genomic analysis by combining the targeted high-resolution aspects of the FISH technique and the genome-wide scale of the karyotyping technique (Vissers et al., 2010). Array-CGH is able to scan the whole genome and detect copy number variants (CNV) including microdeletions and microduplications, and the information it provides is directly linked to the physical and genetic maps of the human genome. For these reasons, genome-wide array-CGH is now used to study the role of submicroscopic aberrations in the aetiology of MR and congenital malformations (Thuresson et al., 2007). Currently, there are several commercially available oligo-array DNA platforms, which are used in most clinical diagnostic laboratories under the labels “chromosomal microarray” or “molecular karyotyping” (Miller et al., 2010). According to a comprehensive study, the application of array-CGH in unselected MR/DD patients has increased the detection rate for genomic rearrangements by approximately twofold, to ~19 % (de Vries et al., 2005; Hochstenbach et al., 2009).

The aims of this study were to start up and validate the genome-wide oligonucleotide array-CGH 44K platform with an average resolution of 43 kb for routine clinical diagnostic applications. We report clinical findings and investigation results for 23 DNA samples from a cohort of selected patients with varying degrees of unexplained MR and dysmorphic features. We show that array-GCH is a reliable technique for detecting submicroscopic chromosomal CNV, rendering it suitable for routine diagnostic screening in mentally retarded patients.

Material and Methods

Patients

Samples from 23 unrelated patients (13 male and 10 female) were analysed by using array-CGH to detect chromosomal aberrations. Informed written consent was obtained from patients’ families or guardians. All patients were referred by a clinical geneticist. The level of MR for each patient was known: mild MR occurred in 26 % of all cases (6/23), moderate MR in 26 % (6/23), severe MR in 39 % (9/23) and profound MR in 9 % (2/23). All patients had dysmorphic features, and most of them had additional congenital malformations. Except for one incident (*Case 2*), all patients had a normal G-banded karyotype obtained using standard procedures.

Chromosomal G-banding

Metaphase slides from cultivated peripheral blood lymphocytes were prepared using standard methanol : acetic acid fixation (3 : 1), treated by trypsin (Sigma-Aldrich, Prague, Czech Republic) and stained by Giemsa solution (Sigma-Aldrich) to obtain chromosomal G-banding. The karyotypes were assembled according to ISCN 2009 at 550 band resolution.

Chromosomal-based CGH/HR-CGH

Reference metaphase chromosome spreads were prepared according to standard protocols using phytohaemagglutinin-stimulated peripheral blood lymphocytes from a karyotypically normal male. Test DNA extracted from peripheral blood lymphocytes was co-hybridized with reference DNA (Promega Corporation, Madison, WI) to the metaphase spreads. CGH/HR-CGH experiments were performed according to the manufacturer’s protocol (Abbott Vysis, Inc., Downers Grove, IL), and chromosomal abnormalities were analysed by the software package LUCIA G 4.82 Advanced Statistic (Laboratory Imaging, Prague, Czech Republic) as described previously (Vranová et al., 2007, 2008).

Array-CGH

DNA from all patients was extracted from peripheral blood lymphocytes using standard chloroform extraction. Whole-genome analysis of chromosomal changes was performed using the Human Genome CGH Microarray 44K (Agilent Technologies, Santa Clara, CA) according to the manufacturer’s protocol (Protocol Version 4.0, 2006). Briefly, 1 μg of reference DNA (Promega Corporation) and test DNA were enzymatically digested (*Alu*I, *Rsa*I) and labelled (Cy3-dUTP and Cy5-dUTP) by using a random-priming reaction. Purified (Microcon YM-30 filters, Millipore, Billerica, MA), differentially labelled test and reference DNA were co-hybridized to the array platform. After one day of hybridization and after washing to remove the unhybridized probes, microarrays were scanned with the Agilent Microarray Scanner (Agilent Technologies). Data were obtained using Feature Extraction software (v. 6.1.1) and visualized by CGH Analytics software (v. 3.5.14) (Agilent Technologies). Significant CNV were detected by using the ADM-2 algorithm with a ≥ 3 neighbouring oligos with aberrant intensity ratios patient/reference.

MLPA (multiplex ligation-dependent probe amplification)

For this study, two distinct commercially available MLPA human telomere kits (the SALSA P036 and the SALSA P070) were used for screening (MRC-Holland, Amsterdam, Netherlands). These MLPA kits contain probes for each subtelomeric region of all chromosomes. MLPA was performed by following the manufacturer's protocol (Schouten et al., 2002). MLPA data were processed by the Coffalyser software v. 9.4 (MRC-Holland) and were interpreted as aberrant if copy number values were bellow 0.7 (in the case of deletion) or above 1.3 (in the case of duplication).

FISH verification and parental confirmation

All aberrations detected by both MLPA kits or by array-CGH were confirmed by FISH analysis of cultured peripheral blood lymphocytes (chromosomal metaphase spreads) using commercially available subtelomeric FISH DNA probes (Vysis ToTelVision Abbott Molecular, Downers Grove, IL; BlueGnome, Cambridge, United Kingdom). An Olympus BX 61 fluorescence microscope (Olympus Corporation, Tokyo, Japan) and a Vosskühler 1300D CCD camera (VDS Vosskühler GmbH, Osnabrück, Germany) were used for image acquisition. Image analysis was performed using LUCIA-KARYO/FISH software (Laboratory Imaging, Prague, Czech Republic). CNV were further investigated for *de novo*/inherited occurrence by MLPA, FISH or array-CGH in both parents, if they were available for testing. In the case of small duplications, when FISH may give apparently normal signal, parental DNA was tested by MLPA or array-CGH for confirmation purpose.

Results

To investigate the performance of our array-CGH 44K platform, we selected 23 unrelated patients with unexplained MR and dysmorphic features. All patients (except *Case 2*) had an apparently normal karyotype assigned by standard G-banding at 550 band resolution. The sensitivity and efficiency of array-CGH were first determined by testing DNA samples from patients (*Cases 1-7* and *11*) with known constitutional chromosomal aberrations. These known changes were previously detected by MLPA or HR-CGH techniques.

Array-CGH confirmed or revealed 16 chromosomal aberrations in a total of 12 patients. Table 1 shows overviews of the respective submicroscopic deletions and duplications identified by array-CGH (ISCN 2009) and clinical features. All significant chromosomal aberrations were verified by at least two independent techniques. Two CNV were interpreted as polymorphic according to the online, publicly accessible Database of Genomic Variants (http://projects.tcag.ca/variation), and

Table 1. Clinical features and overview of the detected aberrations

Case	**Aberrations (ISCN)**	**Origin**	**Clinical features**
De novo aberrations			
1	arr 1p36.33p36.23(777,154-7,392,591)x1 dn	*De novo*	profound MR, autism, hypertrichosis, self-destructive behaviour
2	arr 4p16.3p16.2(62,247-3,362,822)x1 dn arr 8p23.3p23.1(181,330-6,901,636)x3 dn	*De novo*	severe MR, speech delay, hypotrophy
3	arr 6q14.1q15(83,665,726-90,600,084)x1 dn	*De novo*	severe MR, congenital malformation, autism, hydronephrosis
4	arr 11q13.1q13.4(66,703,962-71,769,178)x1 dn	*De novo*	severe MR, cleft palate, heart defect, short stature, hypertelorism, prominent forehead, clinodactyly
Inherited aberrations			
5	arr 1p36.33p36.32(749,422-2,532,453)x1 mat arr 12q24.32q24.33(132,217,409-132,289,149)x3 mat	Maternal	moderate MR, eye defect, hypotonic syndrome, delayed motor development
6	arr 11q24.2q25(125,579,307-133,951,511)x3 pat arr 21q22.2q22.3(39808811-46881029)x1 pat	Paternal	moderate MR, speech defect, clinodactyly, congenital heart defect
7	arr 3q.29(197,224,799-198,996,325)x3 pat	Paternal	severe MR, growth retardation
8	arr 6q12(67,931,393-68,743,086)x1 mat	Maternal	severe MR, congenital malformation, quadriparesis, hypotrophy
9	arr Xq22.3(106,888,316-109,892,856)x0 mat	Maternal	severe MR, bowed legs, elliptocytosis, metaphyseal dysplasia
10	arr 1q21.1(144,124,545-144,458,771)x1 mat	Maternal	mild MR, bilateral radial aplasia, annulled ulna, foramen ovale apertum, deficit of megakaryopoesis
2	arr 16p13.11(14,956,052-16,213,378)x3 pat	Paternal	
Parents unavailable			
11	arr 17q11.2q12(24,239,553-31,461,788)x1		severe MR, autism, pulmonary hypertension
12	arr 2q24.3q31.1(166,568,894-169,601,247)x1		moderate MR, neurological seizures, microcephaly, clinodactyly

those variants were excluded from further analyses (dup(14)(q11.2), dup(15)(q11.2)).

De novo submicroscopic aberrations

Case 1 was a 17-year-old boy with profound MR, dysmorphic features (high forehead, trimerous face, low-set ears), autism, self-destructive behaviour and hypertrichosis. The deletion 1p36 was first detected by MLPA during subtelomeric screening. This result was confirmed and clarified using array-CGH, and the deletion size was estimated as 6.6 Mb. This deletion on 1p36 is known as microdeletion syndrome 1p36, and it is the most common subtelomeric microdeletion syndrome previously described (prevalence 1 : 5000) (Battaglia, 2005; Gajecka et al., 2007).

Case 2 was a severely mentally retarded 9-year-old boy with dysmorphic features, speech delay and hypotrophy. The standard G-banding showed derivate chromosome 4 (46,XY,der(4)). Using MLPA subtelomeric screening, two aberrations were found (a deletion on 4pter and a duplication on 8pter). These changes were specified by further investigation using array-CGH. A deletion of approximately 3.3 Mb located in chromosomal band 4p16 and a duplication approximately 6.7 Mb located in band 8p23 were identified by array-CGH. An additional novel duplication on 16p13 with size 1.25 Mb occurred in this patient. The same duplication on 16p13 was later detected in the phenotypically normal father, and it is in the region with a high frequency of common polymorphic CNV according to the Database of Genomic Variants. This duplication was considered to be most likely only DNA polymorphism with no influence on the patient's phenotype. The deletion on 4p16.3p16.2 spans three genes in the "critical region" associated with Wolf-Hirschhorn syndrome: *WHSC1*, *WHSC2* and *LETM1* (Zollino et al., 2000, 2003).

Case 3, an 11-year-old boy with severe MR, dysmorphic features, congenital malformation, autism and hydronephrosis, displayed an interstitial deletion on 6q14.1q15 using HR-CGH. The result was verified by array-CGH, and by means of this technique, the size of the deletion was estimated to be approximately 6.9 Mb. The deletion on 6q14.1q15 overlaps a region with the gene *SNAP91* (the synaptosomal-associated protein), which is associated with cleft lip/palate (Letra et al., 2010). Other genes in the region 6q14.1q15: *HTR1E* encodes the 5-hydroxytryptamine (serotonin) receptor and its abnormal function has been implicated in a variety of neurologic and psychiatric diseases (Levy et al., 1994), *GABRR1* (γ-aminobutyric acid (GABA) receptor, rho 1) codes for the major inhibitory neurotransmitter in the mammalian brain (Mejía et al., 2007).

Case 4 was an 8-year-old girl with severe MR, dysmorphic features (deep-set eyes, hypertelorism, prominent forehead, micrognathia), cleft palate, clinodactyly, heart defect (patent ductus arteriosus) and short stature. A deletion in band 11q13 was detected by HR-CGH. Array-CGH confirmed the size of the deletion to be approximately 5.14 Mb. There are two interesting genes in the deleted region. The first gene, *SHANK2*, encodes a scaffolding protein present in excitatory synapses. The mutation or deletion of this gene may lead to autism or MR (Berkel et al., 2010). The second gene, *DHCR7* (7-dehydrocholesterol reductase), is connected with the Smith-Lemli-Opitz syndrome (SLOS). Deficiency of this enzyme is characterized by growth retardation, moderate to severe MR, dysmorphic facial features, cleft

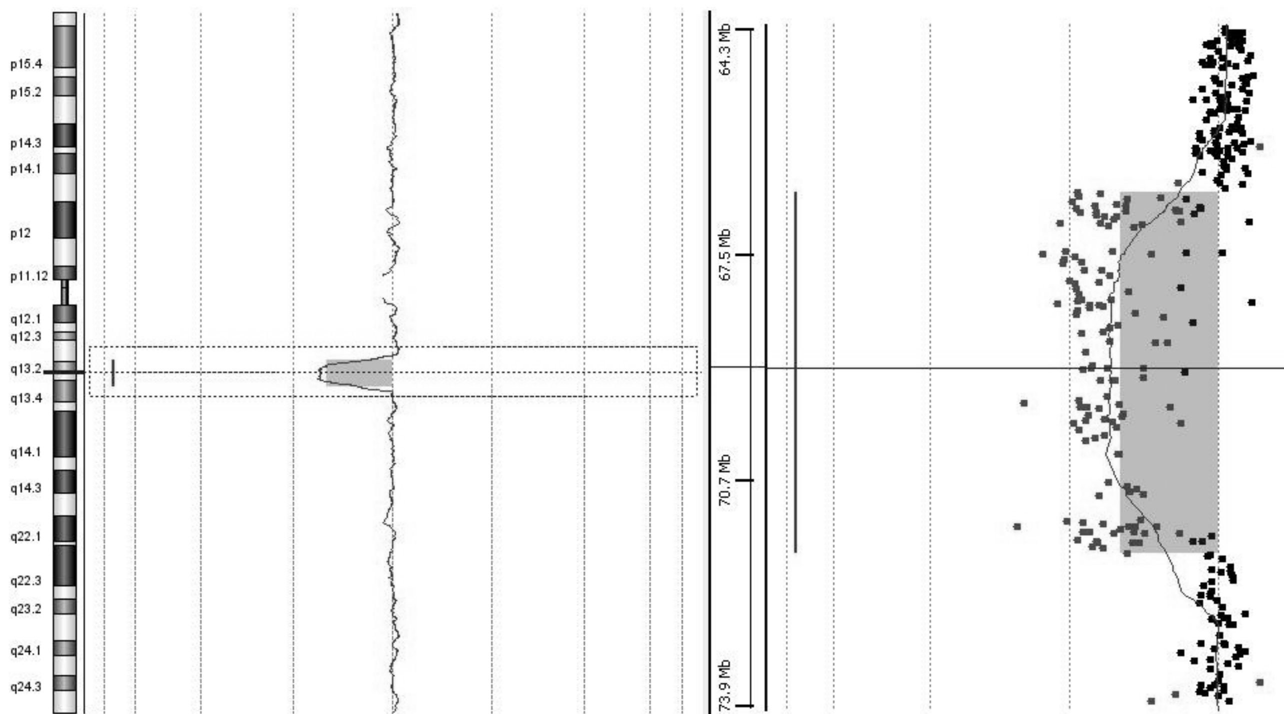

Fig. 2. Array-CGH profile of patients with *de novo* microdeletion del(11)(q13.1q13.4), such as *Case 4.*

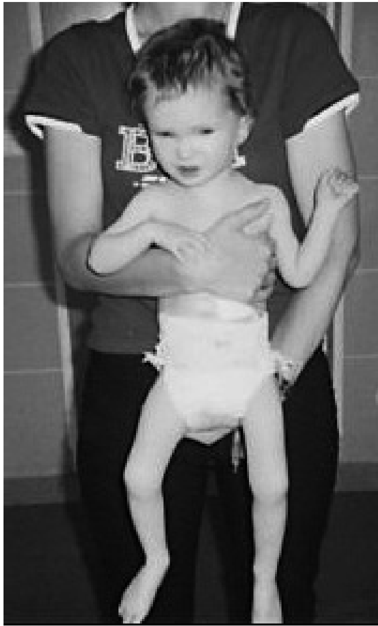

Fig. 1. Facial view of *Case 4* with *de novo* microdeletion of 5.14 Mb in chromosomal band 11q13. Patient presents with severe MR and dysmorphic features (deep-set eyes, hypertelorism, prominent forehead, micrognathia).

palate, cardiovascular defects, postaxial polydactyly, and 2-3 syndactyly of the toes (Yu and Patel, 2005).

Familial submicroscopic aberrations

Case 5, with a deletion on 1pter and a duplication on 12qter as detected by subtelomeric screening using MLPA, was a 6-year-old girl suffering from moderate MR, discrete facial dysmorphism (low-set ears, slight nose inbreak, antimongoloid eye slits), hypotonic syndrome, eye defects (strabism, hyperopia) and delayed motor development. Analysis was performed by 44K and 244K array-CGH. With the higher resolution of the 244K chip it was shown that the duplication on 12q24.32 is very small, approximately 95 kb, containing one gene that likely does not influence the patient's phenotype. A 1.88 Mb deletion on 1p36.33 seems to be the causative chromosomal aberration. This deletion is situated in the same region as in *Case 1* and it is implicated as the cause of microdeletion syndrome 1p36 (Battaglia 2005; Gajecka et al., 2007). Chromosomal rearrangement in this patient developed from segregation of a balanced translocation t(1;12)(p36.33;q24.33), which was confirmed by FISH analysis in the phenotypically normal mother.

Case 6 was an 8-year-old boy with moderate MR, dysmorphic features (low-set ears, epicanthi), congenital heart malformation, speech defect and clinodactyly. MLPA subtelomeric screening revealed two aberrations (a deletion on 21qter and a duplication on 11qter). Array-CGH analysis confirmed the presence of a 7 Mb deletion on chromosome 21q22.2q22.3 and the presence of an 8.37 Mb duplication on 11q24.2q25. In this case, chromosomal changes were inherited from the phenotypically normal father via a balanced translocation t(11;21)(q24.2;q22.2). Partial monosomy 21q has been reported with normal to severe phenotypic consequences depending on the size of the monosomic region (Chettouh et al., 1995; Huret et al., 1995). Study of monosomy 21q22.2q22.3 showed that this aberration may also lead to mild phenotypes comprising thin marfanoid build, facial anomalies and mild MR (Ehling et al., 2004). One case of a balanced t(11;21)(q23;q22) translocation that segregates into the next generation as unbalanced has been described previously (Jacobsen et al., 1973).

Case 7 was a 3-year-old girl presenting with severe MR, dysmorphic features and growth retardation. MLPA subtelomeric examination using a P036 kit revealed a duplication of 3q29, in a region containing the *BDH* gene. Array-CGH analysis confirmed a 1.77 Mb duplication on 3q29. The same duplication was also detected in the father by using the MLPA P036 kit. Duplication of 3q syndrome has been described in at least three recent reports as a clinically relevant abnormality, presenting with varied dysmorphic features (Ballif et al., 2008; Goobie et al., 2008; Lisi et al., 2008).

Case 8 was an 8-year-old girl with severe MR, congenital malformation, quadriparesis and hypotrophy. A deletion in band 6q12 with an estimated size of 811 kb was identified by array-CGH. The same deletion was also detected in the mother by array-CGH. Blood samples for confirmation of this 6q12 deletion were not available. The deletion is situated in a gene gap and is most likely only a polymorphism with no influence on the phenotype.

Case 9, a non-verbal 5-year-old boy with severe MR, dysmorphic features (bradymacrocephaly, low-set ears, antimongoloid slants, hypoplastic midface), bowed legs, elliptocytosis, metaphyseal dysplasia, and aortopulmonary collaterals. We detected an interstitial 3 Mb deletion on Xq22.3 using array-CGH. The deletion Xq22.3 was confirmed by FISH in the patient and also in the unaffected mother. We examined additional members of the family and found the same symptoms and the same deletion in the maternal uncle. Mother and grandmother are unaffected carriers of microdeletion Xq22.3 (data not shown). Alport syndrome with MR, midface hypoplasia, and elliptocytosis (AMME or ATS-MR) is caused by a deletion spanning several genes in Xq22.3. The symptoms of this X-linked disease result from defects in the genes *COL4A5*, *NXT2*, *GUCY2F* and *AMMECR1*. This family was the first known familial incidence of ATS-MS syndrome in the Czech Republic. Internationally, it was the fourth documented family suffering from this X-linked disease (Jonsson et al., 1998; Meloni et al., 2002; Rodriguez et al., 2010).

Case 10 was a 2-year-old boy with mild MR, bilateral radial aplasia, annulled ulna, foramen ovale apertum and deficit of megakaryopoiesis. We identified a 340 kb microdeletion on 1q21 by array-CGH. FISH analysis confirmed the deletion using the RP11-315I20 (BlueGnome) probe. Both parents of the proband were also investigated by FISH. The deletion was also found in

the unaffected mother. The deletion 1q21 is known as a cause of TAR syndrome (thrombocytopoenia-absent radius syndrome). It is a well-defined malformation syndrome comprising limb deformities, thrombocytopoenia and cardiological abnormalities (Hall et al., 1969; Hedberg and Lipton, 1988; Klopocki et al., 2007).

Aberrations with undetermined origin

Case 11 was a 15-year-old boy suffering from severe MR, dysmorphic features, autism and pulmonary hypertension. HR-CGH analysis revealed a deletion on 17q11.2q12. Independent confirmation was performed using array-CGH, and the deletion size was estimated to be 7.2 Mb. The parents were not available for testing. The candidate causal gene in the region 17q11.2q12 is *CDK5R1*, which encodes the protein p35, a neuron-specific activator of CDK5 (serine threonine kinase) (Brunetti-Pierri et al., 2007).

Case 12 was a 10-year-old girl with moderate MR, dysmorphic features, neurological seizures, microcephaly and clinodactyly. Array-CGH showed a 3 Mb deletion on 2q24.3. The deletion has not been confirmed by another independent method because of the unavailability of blood material. The child is currently placed in an infant institution. For the same reason, the parents have not been tested. About 30 children with a 2q interstitial deletion have been reported with similar symptoms (Pescucci et al., 2007).

Discussion

Genome-wide copy-number detection using array-CGH is becoming particularly effective for the investigation of patients with MR/DD, dysmorphic features and congenital anomalies. However, the implementation of array-CGH into routine clinical laboratory practice still presents problems associated with obtaining sufficient funding and with the difficult clinical interpretation of the results. If array-CGH became more affordable for routine clinical diagnostics, patients would benefit from targeted treatments derived from improved diagnosis and prognosis.

The aims of this study were to start up and validate the genome-wide oligonucleotide array-CGH 44K platform (Agilent Technologies 4x44K) for routine application in clinical laboratory practice. The array 44K platform contains 43000 *in situ* synthesised oligonucleotides in the range of 60 bp and covers coding as well as non-coding sequences of the whole genome with an average resolution of 43 kb. Thanks to the high resolution of this platform, novel genomic markers were identified in some solid tumours and haemato-oncological malignancies (Largo et al., 2007; Scaruffi et al., 2007). We report clinical findings from array-CGH 44K analysis in a cohort of 23 DNA samples from selected patients with various degrees of unexplained MR/DD and dysmorphic features.

In a group of 23 patients, array-CGH confirmed or revealed chromosomal rearrangement in 12 cases. In eight patients (*Cases 1-6, 9-10*), we found chromosomal aberrations which are already considered pathological with relevance to the observed phenotype. In one incident (*Case 8*), the detected deletion was assumed to be a benign polymorphism. In three patients (*Case 7, 11* and *12*), the observed chromosomal changes could not be declared causative with certainty due to the lack of parental samples or due to the unavailability of additional tissue samples.

The detected genomic rearrangements in our patients can be divided into two groups. The first group of four patients had relatively large *de novo* chromosomal deletions and duplications (*Case 1-4*). Such large rearrangements are generally assumed to be disease-causing. The second group of patients (*Case 2, 5-10*) had rearrangements that were also observed in an apparently normal parent. The presence of the same aberration in an unaffected parent decreases the likelihood that it is pathogenic. In these cases, only a careful clinical examination of the patient and the parents can decide whether the aberration is related to the clinical manifestations in the patient or not. Some inherited CNV can be dismissed as unimportant polymorphisms as in *Case 8* and *Case 2*.

Pure subtelomeric duplication as a cause of MR was described in a previous study, but was found only infrequently (Ruiter et al., 2007). The repeated sequence of a chromosomal region (containing one or more genes) could be gene-dosage sensitive and responsible for the aberrant phenotype (Lupski, 1998). Duplication on 3q29 (*Case 7*) was previously described as a possible cause of dysmorphic features (Ballif et al., 2008; Goobie et al., 2008). Even if the same aberration is present in the unaffected father, this duplication cannot be immediately dismissed as the causative aberration. There could be incomplete penetrance for the allele containing the deletion, allowing the parent to present a normal phenotype. A three-generation family featuring five individuals with reciprocal 3q29 microduplications has been described previously (Lisi et al., 2008).

Inherited chromosomal aberrations from parents in whom the variant was insufficient to cause disease might still be causative for an MR/DD phenotype in the next generation. This hypothesis has been proposed for TAR syndrome patients such as our *Case 10*. A recent study, in which 30 individuals with TAR syndrome have a microdeletion of 1q21 (in 75 % of cases inherited from normal parent), suggests that TAR syndrome is associated with a deletion on chromosome 1q21.1 but that the phenotype develops only in the presence of an additional as-yet-unknown modifier (mTAR) (Klopocki et al., 2007).

In two patients (*Cases 5* and *6*) the aberrations were inherited from the phenotypically normal parental carrier. The chromosomal rearrangements in the patients developed from the segregation of parental balanced translocation. The parents in both cases were healthy because there was no loss or gain of genetic material, and the breakpoints were not in an important location affecting phenotype. The risk of a severe phenotype exists for

the next generation because of the segregation of rearranged chromosomes during meiosis. Unbalanced translocations can occur (as in *Cases 7* and *8*), which are likely to be disease-causing.

Recent array-CGH studies have led to the identification of not only new microdeletion syndromes, but also new microduplication syndromes. Duplications may be pathogenic or may exist as benign variants in the human population (Rudd et al., 2009). The question of the role of duplications in pathogenesis has not been definitively answered. Another study suggests that duplications may represent risk factors that by themselves are not disease-causing, but in combination with other factors might result in disease (Sebat et al., 2004). In three patients (*Cases 2, 5* and *6*) we detected duplications occurring together with deletions. We are not able to say with certainty how important the role of duplications may be.

The detection rate for chromosomal abnormalities and/or genomic rearrangements in patients with MR/DD depends on the clinical pre-selection of individuals (de Vries et al., 2001) and on various levels of array-CGH resolution (Hochstenbach et al., 2009). In our study we confirmed the effectiveness and reliability of array-CGH 44K in diagnostic practice by verification of all previously described chromosomal abnormalities that have been detected using techniques like MLPA or HR-CGH (*Cases 1-7* and *11*). In addition, Agilent technology of array-CGH 44K was able to discover even further submicroscopic chromosomal abnormalities (*Case 2*).

In the analysis of additional 15 patients with no findings by the other methods (G-banding, MLPA, HR-CGH), we found presumably causative chromosomal changes in three incidents (*Cases 9, 10* and *12*). Three positive cases from 15 patients represent detection rate of ~20 %. This detection rate should not be considered generalizable because of the small cohort size and patient pre-selection process in place here. If array-CGH 44K is implemented in routine clinical diagnostics for all patients with idiopathic MR, then according to recent literature reviews the same type of the array-CGH platform should be expected to have a detection rate of ~12–15 % (Fan et al., 2007; Jaillard et al., 2010; Xiang et al., 2010). Review studies including different array-CGH platforms (BAC, oligo, SNP) with various resolutions indicate that the overall rate of detection of genomic abnormalities ranges from 11 % (Koolen et al., 2009) to 19 % (Hochstenbach et al., 2009) in unselected patients with MR/DD.

Higher-density oligonucleotide-based arrays (such as 1Mb, 1x244K or 2x105K Agilent) are now available for detection of genomic imbalances, and it is important to determine the optimal resolution of array-CGH for routine diagnostics. Studies in the last few years (Aradhya et al., 2007; Jaillard et al., 2010; Xiang et al., 2010) have shown that the 44K oligonucleotide platform appears suitable for diagnostic purposes. Recent studies using array-CGH 244K showed an even higher detection rate of chromosomal imbalances, but clinical application of such a high resolution approach has been complicated by the frequent occurrence of very small CNV with uncertain clinical relevance (Fan et al., 2007). Therefore, the higher resolution array-CGH 244K is suitable for target detail analysis in selected cases or for confirmation of some uncertain results from array-CGH 44K.

In conclusion, our results show that the application of the genome-wide high-resolution Agilent 44K oligonucleotide array-CGH platform in clinical laboratory diagnostics increases the detection rate of pathogenic submicroscopic chromosomal aberrations in patients with MR/DD and congenital abnormalities, but it also presents new difficulties with clinical interpretation of the results. Our understanding of benign and pathogenic CNV is still very incomplete, and therefore both clinicians and cytogeneticists are encouraged to contribute information to worldwide databases, such as DECIPHER and ECARUCA, to increase the knowledge on phenotype/genotype correlations (Vermeesch et al., 2007). A very useful tool for determining polymorphisms is also a publicly available database containing normal genomic variants (e.g. Database of Genomic Variants) or the Human Genome Browser (http://genome.ucsc.edu/).

The presented data emphasise the strengths of high-resolution array-CGH 44K for the detection of genomic imbalances associated with phenotypes of unknown genetic aetiology. Thanks to the high sensitivity of array-CGH Agilent 44K, we are able to identify candidate genes that could be causal for MR/DD. The identification of genetic abnormalities can lead to specific genetic counselling strategies for afflicted families and possibility to targeted prenatal diagnostics. Thorough phenotype/genotype correlation will be needed to determine the clinical significance of newly detected genomic imbalances. High-resolution screening of patients with idiopathic MR by array-CGH 44K has the potential to become an important tool in the clinical diagnostic setting.

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