ORIGINAL ARTICLES

Cryptic Chromosomal Rearrangements in Children with Idiopathic Mental Retardation in the Czech Population

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Aims: The aim of our study was to scan for cryptic rearrangements using the multiplex ligation probe amplification method in a cohort of 64 probands with mental retardation or developmental delays in combination with at least one of the following symptoms: hypotonia after birth, congenital anomalies, or face dysmorphisms; but without a positive cytogenetic finding. The study contributes to the knowledge of microdeletion syndromes and helps disclose their natural phenotypic variability. *Results:* In total, 10 positives (16%) were detected, particularly 3 duplications (Xpter-p22.32; 17p11.2; 22q11) and 6 different deletions (1p36; 7q11.23; 10p15; 15q11-q13; 17p11.2; 17p13.3), 1 of these in 2 probands. Besides the well-characterized syndromes, less-often described rearrangements with ambiguous phenotype associations were also detected. *Conclusions:* Some rearrangements, particularly duplications, are associated with vague phenotypes; and their frequency could be underestimated.

Introduction

MENTAL RETARDATION MAY occur as either isolated or associated with several malformations or dysmorphias. The underlying cause of mental retardation remains unknown in up to 80% of all patients (Rauch *et al.*, 2006). Chromosomal aberrations are the most commonly known cause of mental retardation. Standard cytogenetic banding techniques hold resolution limits of 5–8 Mb; and are, thus, not powerful enough to detect chromosomal rearrangements below 5 Mb in size (Salman *et al.*, 2004). It is for this reason these types are referred to as "cryptic" rearrangements.

Cryptic chromosomal rearrangements have been detected on all human chromosomes (Ballif *et al.*, 2007) and may arise all over the genome; however, some chromosomal areas are more prone to them than are others. Microdeletions/duplications mostly arise as the result of frequent unequal crossover events, between sites sharing more than a 97% homology (the so-called, low copy segments, LCRs) (Shaffer *et al.*, 2007). Such high-homology sites evolved during genome evolution by chromosomal segment duplication (Conrad and Antonarakis, 2007). Subtelomeric regions are more frequently the subject of microdeletion than are the other parts of the chromosomes (Mefford and Trask, 2002). There are DNA sequences adjacent and centromeric to the $(T_2AG_3)_n$ sequence; these are the so-called telomere associated repeats, made up of blocks of repetitive DNA (Flint *et al.*, 1997). These sequences are shared among many different subsets of telomeres and evolved as a result of nonhomologous chromosome recombination (Shaffer and Lupski, 2000).

Over the last several years, the employment of modern cytogenetic and molecular genetic techniques has enabled progressive diagnosis of distinctive cryptic rearrangements as the underlying causes of idiopathic mental retardation (Rooms *et al.*, 2006). However, due to the extensive phenotypic variation, as well as mutually overlapping phenotypes, the correct syndrome diagnosis is often difficult. Thus, our study contributes to the level of knowledge of these microdeletion syndromes and helps further disclose their natural phenotypic variability.

Materials and Methods

Subjects

Overall, 64 probands were included in the study. Study subjects were recruited from the Department of Medical Genetics (1st Faculty of Medicine and General Teaching Hospital, Charles University in Prague) in close cooperation with both the Department of Neurology and the Department of Pediatrics and Adolescent Medicine, which are the specialist consultancy locations for these matters within the entire Czech Republic.

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Patients' selection criteria were the presence of either mild-to-severe mental retardation or developmental delay in combination with at least one of the following symptoms: hypotonia after birth, congenital anomalies, and face dysmorphisms; but without a positive cytogenetic finding during standard cytogenetic karyotyping. The introduction of a patient into the project was the sole decision of the clinical geneticist. All patients who were introduced (or their official legal representatives) signed an informed consent form for the taking of blood, DNA analysis, and agreement to submitting to the study. If required, an informed consent form for blood taking and DNA analysis was signed with the proband's relative, as well.

DNA analysis

The EDTA blood was stored at 4°C and then processed within 48 h after venisection. Genomic DNA was isolated from the peripheral blood using QIAGEN spin columns on a QIAcube (QIAGEN, GmbH). Both DNA purity and concentration was measured on a NanoPhotometer (IMPLEN GmbH). DNA concentration was adjusted to 30–80 ng/μL before further analysis. The detection of microdeletion rearrangements was carried out by using the multiplex ligation probe amplification (MLPA) method (MRC-Holland), which enables simultaneous analysis of tens of genomic regions (Schouten et al., 2002).

Each proband was analyzed by use of MLPA kit P245 to detect the 21 most common microdeletion syndromes. Additional microdeletion syndromes were analyzed using MLPA kit P297. The subtelomeric regions were analyzed with the use of the MLPA kit P036. Additional MLPA kits (P018, P064, P147, P250, and ME028) were used for further deletion/duplication verification and specification; and if used are mentioned in the results section of a particular case. The MLPA reaction was run with 50–150 ng of DNA, according to the manufacturer's instructions. The MLPA analysis was carried out by fragmentation analysis conducted on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Visual examination of the peak patterns and peak areas normalization was done for each sample according to the instructions of the manufacturer for each particular MLPA kit.

Results

A total of 64 probands were screened for cryptic deletion/duplication using the MLPA method. The patients' ages ranged from 2 months to 15 years, and the male-to-female ratio was 1.4:1 (37:27). The most frequent age at diagnosis was between 1 and 4 years (34 probands). Mental retardation could only be assessed in those patients who were at least 3 years old ($n = 38$).

Overall, 10 positives (16%) were detected; specifically 3 duplications and 7 deletions using the MLPA kits P245, P036, and P297 (Table 1).

In the case of the 1p36 deletion syndrome, only the TNFRSF4 probe of MLPA kit P245 was involved. The GNB1 probe and the GABRD probe were both outside of the deleted region. Additional deletion specification (P147) detected two separate deletions. One deletion, <1 Mb, spanned from the TNFRS18 probe to the DVL1 probe; the second and largest (about 4 Mb) deletion covered the chromosomal area from the AJAP1 probe to the SLC45A1 probe. The nondeleted region between the two deletions is about 3 Mb. The 3-year-old boy involved was 95 cm tall and 17 kg in weight. Considerable characteristic craniofacial dysmorphisms were detected. This boy suffered from mild mental retardation, hypotonia, and epilepsy. No auditory findings were detected. Both phenotypically healthy parents were analyzed using the MLPA kit P147 to exclude the presence of a 3 Mb duplication, which can mask the proband's nondeleted area. No such duplication was detected in either parent.

In the case of Williams syndrome (7q11.23), Smith-Magenis syndrome (17p11.2), and Miller-Dieker syndrome (17p13.3), all probes from the MLPA kit P245 were deleted. Both probands with Williams syndrome display the deletion of all appropriate probes from the P064 MLPA kit. The deletion extends >1 Mb and encompasses the ELN and LIMK1 genes, among others. In a 3-year-old boy, the Williams syndrome was combined with the typical osteogenesis imperfecta (OI). This proband possessed the characteristic facial features, developmental delay, short stature, as well as pulmonary artery and aortic stenosis. The second proband with Williams syndrome was a 7-month-old girl. She was found to be having intrauterine growth retardation, characteristic facial features,

TABLE 1. SUMMARY OF ALL REARRANGEMENTS FOUND AND THE MULTIPLEX LIGATION PROBE AMPLIFICATION KITS USED FOR THEIR DETECTION AND SPECIFICATION

Proband no.	MLPA kit			Aberration	Additional MLPA kits
	P245	P297	P036		
MD4	+	−	+	1p36 deletion	P064; P147
MD6	−	−	+	Xpter-p22.32 (SHOX) duplication	P018
MD20	+	−	−	7q11.23 deletion	P064
MD21	+	−	−	22q11 duplication	P250
MD22	+	−	−	17p13.3 deletion	P064
MD27	+	−	−	17p11.2 duplication	P064
MD39	+	−	−	15q11.2-q1.2 deletion	ME028
MD48	+	−	−	17p11.2 deletion	P064
MD53	+	−	−	10p15 (GATA3) deletion	P250
MD63	+	−	−	7q11.23 deletion	P064

+ indicates a positive analysis; − indicates a negative analysis.
MLPA, multiplex ligation probe amplification.

and developmental delay; but no cardiac developmental defects were detected. A proband with Smith-Magenis syndrome displayed the deletion of all P064-relevant probes. The deletion extends > 2.5 Mb. The 6-year-old boy displayed hypotonia after birth, craniofacial dysmorphism, microcephaly, and mild mental retardation. Additionally, sleep disturbances and auto-aggressive behaviors were detected. In the case with Miller-Dieker syndrome, three of seven MLPA P064 probes were deleted. The deletion encompasses the METT10D and LIS1 genes, and its range is below 1.5 Mb. The girl proband was hospitalized at 2 months because of repeated apnoeic pauses and seizures. She was further found to be having developmental delay, microcephaly, extension hypertonia, and severe congenital brain defects (lyssencephaly type I; agyria).

In the case with Prader-Willi syndrome (PWS), all of the MLPA kit P245 probes were deleted. Further analysis (ME028) displayed the paternal origin of the deleted fragment. The 2-month-old boy suffered from severe hypotonia, cryptorchidism, characteristic facial dysmorphism, and developmental delay.

In one case, the 10p deletion of one MLPA P245 probe was detected. The deleted probe targeted the GATA3 gene exon 1 and corresponds to the DiGeorge 2 region (10p12-15). The MLPA kit P250 revealed the GATA3 probe deletion targeting exon 4. The 9-year-old girl displays intrauterine growth retardation, developmental delay, microcephaly, short stature, auditory defects, nephropathy, and syndactyly (incomplete simple syndactyly between the third and fourth finger of the upper limbs and complete simple syndactyly between the second and third toes).

Three various duplications were detected. In one case, the P245 analysis showed a duplication of all three 17p11.2 probes, corresponding to the Smith-Magenis region. The duplication was verified using the P064 MLPA kit; and all relevant 17p11 probes were duplicated. The 18-year-old girl suffered from moderate mental retardation, language impairment, short stature, a gait defect, facial dysmorphism (narrow face, prominent nose, short philtrum, pointed chin), and a cataract. A 22q11 duplication was detected in a 10-year-old girl with mild mental retardation, learning disability, disharmonic emotional development, mild craniofacial dysmorphism, and slight stature. All MLPA P425 probes were duplicated. The MLPA kit P250 was used for additional specification; showing that the duplication covers the region 22q11 A-D, and spans > 2.5 Mb. The same duplication was found in the proband's mother.

Surprisingly, in one case with mild mental retardation, the interstitial duplication in the PAR1 region (including the SHOX gene) was detected using the P036 MLPA kit, and then the P018 kit for additional specification. The 3-year-old girl displayed hypotonia, macrocephaly, facial dysmorphism, and short stature. The same duplication was also found in the proband's father.

Discussion

We used the MLPA method to search for cryptic deletions/duplications in a cohort of 64 probands. The high methodological effectiveness (16%) in our study sample was established by stringent introduction of the probands by clinical geneticists with abundant experience in the fields of mental retardation and dysmorphias in children. The detection rate of molecular karyotyping could be around 20% in such a cohort of patients (Gijsbers et al., 2009). Besides the well-characterized syndromes, less-often described rearrangements with ambiguous phenotype associations were also detected.

Monosomy 1p36 (MIM 607872) is the most common terminal deletion syndrome, with an estimated prevalence of 1 in 5000 (Shapira et al., 1997; Shaffer and Lupski, 2000). In our case, two distinct interstitial deletions were detected: a small deletion, < 1–1.5 Mb distant from the p-telomere; as well as a large deletion, 4 to 8(9) Mb distant from the p-telomere. Such complex rearrangements have also been described in other studies (Heilstedt et al., 2003). In our proband, a number of characteristic craniofacial symptoms are present; however, the common auditory findings were not detected (Shapira et al., 1997; Heilstedt et al., 2003). It seems that the critical auditory region is outside of the deleted area. Windpassinger et al. (2002) suggested that the GABRD gene (gene ID: 2563) may be a candidate for the neurodevelopmental and neuropsychiatric anomalies seen in this syndrome. Surprisingly, the GABRD gene was not affected in our proband.

In two cases, the deletion of 7q11.23 was detected as associated with Williams syndrome (WBS; MIM 194050). The estimated WBS frequency is between 1 in 7500 and 1 in 20,000 (Greenberg, 1989; Strømme et al., 2002). Three large region-specific segmental duplications are responsible for recurrent chromosomal rearrangements in 7q11.23 locus (Valero et al., 2000). In both our cases, the deletion is > 1 Mb long, and it encompasses both the ELN (gene ID: 2006) and LIMK1 genes (gene ID: 3984). Consequently, we assume the most common 1.55 Mb deletion exhibited in most patients (95%) (Bayés et al., 2003). Mutations/deletions of the ELN gene are responsible for vascular and connective tissue abnormalities (Ewart et al., 1993). In one proband, the ELN deletion is not associated with congenital cardiac defects; however, considering her age at diagnosis, some less-severe heart abnormalities such as heart murmurs could later emerge. In the proband with OI, the condition was inherited from the mother (OI type III; MIM 259420), and the 7q11.23 deletion seems to be a coincidental event.

Two probands with a 17p11.2 rearrangement were detected. The overall phenotype, including sleep defects and self-injury in our proband with the 17p11.2 deletion, is in accordance with the diagnosis of Smith-Magenis syndrome (MIM 182290). The phenotype of the proband with the 17p11.2 duplication fulfills the characteristics for Potocki-Lupski syndrome (MIM 610883). The cataract condition was inherited from the mother. Both syndromes are the result of a nonallelic homologous recombination between region-specific LCRs (Chen et al., 1997; Potocki et al., 2000). In both our cases with Smith-Magenis syndrome and site duplication, the rearrangement area is > 2.5 Mb long. The most frequent recurrent deletion/duplication is ∼3.7 Mb long (Juyal et al., 1996; Chen et al., 1997).

The 17p13.3 deletion is associated with the Miller-Dieker syndrome (MIM 247200). A deletion or mutation in the LIS1 gene only (gene ID: 601545) appears to cause the isolated lissencephaly (ILS; MIM 607432) (Cardoso et al., 2003). The common prevalence of Miller-Dieker syndrome and ILS is 1 in 40,000 live births (Fleck et al., 2000). In our proband, the deletion is < 1.5 Mb in range (and no facial dysmorphisms were detected); so, ILS seems to be the correct diagnosis.

PWS (MIM 176270) is a neurogenetic disorder usually caused by chromosomal deletion on chromosome 15q11-q13, by uniparental disomy or by imprinting defects. The chromosomal alterations result in an aberrant expression profile of gene loci that are subject to imprinting. The clinical features, as well as methylation status of CpG islands in the *SNRPN* gene (gene ID: 6638), in our proband are in compliance with a diagnosis of PWS. The estimated frequency of PWS is ∼1 in 10,000 (Steffenburg *et al.*, 1996; Cassidy, 1997). Carrozzo *et al.* (1997) suggested recombination or an intrachromosomal loop as the mechanisms that underlie the interstitial *de novo* deletions at 15q11-q13 locus.

The 22q11 duplication is a reciprocal rearrangement to the deletion mainly associated with DiGeorge syndrome (MIM 188400) with birth prevalence of at least 1 in 4000 (Devriendt *et al.*, 1998). The high frequency of 22q11 copy number changes is due to the presence of several copies of a repeat sequence (LCR22). The extent of the 22q11 rearrangement is variable, although 87% extends from the first (LCR22-A) until the fourth (LCR22-D) repeat (Shaikh *et al.*, 2000). Unlike deletion, the site duplication is associated with many variable and ambiguous phenotypes (Courtens *et al.*, 2008). In our case, the duplication was detected to the same extent in the proband's mother (who displays neither pathological features nor behaviors). Similarly, most individuals in whom the 22q11.2 duplication was diagnosed and with obvious clinical features have inherited the duplication from a parent with a normal or near-normal phenotype (Courtens *et al.*, 2008). It could be a susceptible genotype for mental retardation without full penetrance; or simply an ascertainment bias, where the duplication represents population variability with no direct effect on the phenotype.

The DiGeorge 2 critical region (MIM 601362) is located on 10p13-14. It seems that the more telomeric (10p14-10pter) deletion is responsible for the HDR syndrome phenotype (MIM 146255) (Van Esch *et al.*, 2000). Haploinsufficiency of the *GATA3* gene (gene ID: 2625) is only associated with the HDR syndrome (MIM 146255). *GATA3* is essential in the embryonic development of the parathyroids, auditory system, and kidneys (Van Esch *et al.*, 2000). Our proband displays hearing defects and nephropathy; however, no hypoparathyroidism. However, symptoms of the HDR syndrome are variably expressed between and within families (Muroya *et al.*, 2001). It seems that the proband is simultaneously a carrier of the syndactyly type 1 (MIM 185900).

In one of our probands, the interstitial duplication of the *SHOX* gene (gene ID: 6473) (PAR1 region; Xp) was detected. Deletions of the *SHOX* gene are well documented (Bertorelli *et al.*, 2007). In contrast, *SHOX* duplication is rare; only a few cases have thus far been described, and the associated phenotype is highly variable (Thomas *et al.*, 2009). Recently, *SHOX* duplication was associated with type I Mayer-Rokitansky-Kuster-Hauser syndrome (MIM 277000) (Miozzo *et al.*, 2010). In our proband, we found a short stature without complying with the increased *SHOX* gene copy number; additionally, the overall phenotype does not satisfy the criteria for the Mayer-Rokitansky-Kuster-Hauser syndrome. *SHOX* duplications are likely to be under-ascertained, and more cases need to be well characterized, to accurately determine their phenotypic consequences.

The MLPA method is sufficient to reasonably analyze a number of susceptible chromosomal regions at the same time. It is particularly beneficial for probands with vague phenotypes. However, the MLPA method is not able to detect reciprocal rearrangements. Our data support the assumption that the majority of the symptoms may be the result of a small deletion within critical regions. Consequently, it is essential to precisely evaluate the extent of the rearrangement, to enable the determination of such syndrome critical regions and to assess symptom penetrance and variable expressivity. Besides the role of genes in the involved interval, there are multiple factors such as regulatory sequences, epigenetic mechanisms, parental origin, and nucleotide variations in the nonaffected allele that may also be important.

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Disclosure Statement

No competing financial interests exist.

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