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

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Chapter 4.1

A 400 kb duplication, 2.4 Mb triplication and 130 kb duplication of 9q34.3 in a patient with severe mental retardation

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Eur J Med Genet 2008;51:479-487

Abstract
The presence of a duplication as well as a triplication in one chromosome is a rare rearrangement and not easy to distinguish with routine chromosomal analysis. Recent developments in array technologies, however, not only allow screening of the whole genome at a higher resolution, but also make it possible to characterize complex chromosomal rearrangements in more detail. Here we report a molecular cytogenetic analysis of a 16-year old female with severe mental retardation and an abnormality on the end of the long arm of chromosome 9. Subtelomeric multiplex ligation-dependent probe amplification (MLPA) analysis revealed that the extra material originated from the telomeric end of chromosome 9q. Fine mapping using a high-resolution single nucleotide polymorphism (SNP) array detected a duplication of ~400 kb upstream of a ~2.4 Mb triplication followed by a duplication of ~130 kb of chromosome 9q34.3. This study underscores the value of combining conventional karyotyping with novel array technologies to unravel complex chromosomal alterations in order to study their phenotypic impact.

1. Methods of detection

1.1 Cytogenetic analysis

Conventional cytogenetic analysis on GTG-banded chromosomes from cultured lymphocytes of the patient was performed according to standard techniques. All metaphases studied demonstrated a 46,XX, add(9)(q34) karyotype (Fig. 4.1.1a).

1.2 MLPA

Two specifically designed sets of probes for testing subtelomeric chromosomal imbalances, SALSA P036B and P070 Human Telomere Test Kit (MRC Holland, Amsterdam, The Netherlands), were used for subtelomere screening of the patient and the parents. MLPA experiments were performed as described by MRC Holland (http://www.mlpa.com/pages/indexpag.html) with slight modifications. Amplification products were identified and quantified by capillary electrophoresis on an ABI 3130 genetic analyzer (Applied Biosystems, Nieuwerkerk aan de IJssel, The Netherlands). Peak analysis was performed with the GeneMarker Software V1.51 (SoftGenetics, USA).

1.3 SNP- array

The Affymetrix GeneChip Human Mapping 238K *StyI* array was used. This SNP array contains ~238.000 25-mer oligonucleotides with a ~12 kb resolution. A sample of 250 ng DNA was processed according to the instruction provided in the Affymetrix GeneChip Human Mapping 500K Manual (http://www.affymetrix.com). SNP copy number was assessed in the patient using DNA-Chip Analyzer (dChip) software (version release 02-16-06) [8]. Regions of copy number gain and loss were detected using the hidden Markov model output of dChip.

1.4 Chromosomal anomaly

To further characterize the extent of the aberration found by karyotyping, subtelomeric MLPA was performed. A duplication of both 9q probes, located in the *MRPL41* gene and *EHMT1* gene, was observed (Fig. 4.1.1b).

Fine mapping of the duplication was performed with a 238K SNP array. This array illustrated a terminal duplication of ~2.93 Mb (Fig. 4.1.1c). Due to exceptional high intensity values from both, the MLPA and the SNP array analyses, a 9q34.3

triplication was suggested. Detailed analysis of the SNP array data revealed a ~400 kb duplication followed by a ~2.4 Mb triplication and a ~130 kb duplication (Fig. 4.1.1d). The proximal breakpoint is mapped in 9q34.3 with the last SNP (rs11103754) normal located in 137.24 Mb and the first SNP (rs7043655) duplicated in 137.26 Mb. The second breakpoint is mapped between the duplicated SNP (rs3849220) located in 137.62 Mb and the triplicated SNP (rs7873626) in 137.63Mb. The most distal breakpoint in 9q34.3 is mapped with the last SNP (rs7848769) triplicated in 140.075 Mb and the first SNP (rs7860423) duplicated in 140.076 Mb. Based on this result, the patient's karyotype was refined as:

46,XX,add(9)(q34).arr SNP 9q34.3q34.3(rs7043655->rs3849220)x3,
9q34.3q34.3(rs7873626->rs7848769)x4,9q34.3q34.3(rs7860423->rs1820789)x3 dn

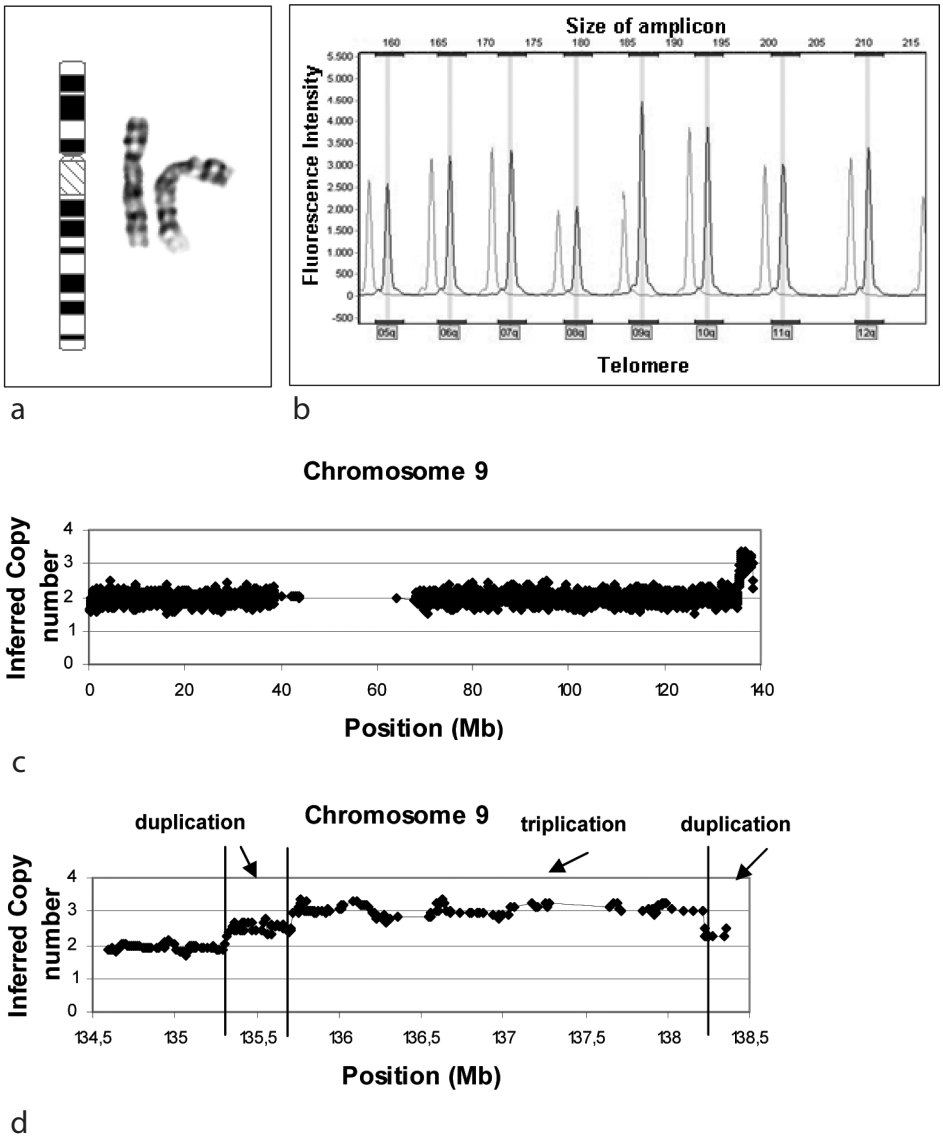

Figure 4.1.1 Cytogenetic and molecular results. (a) Partial karyotype showing both chromosomes 9. Right: abnormal chromosome 9. (b) MLPA analysis revealing an aberration of the 9q probe. Red peaks: control panel; blue peaks: patient. (c) SNP array analysis of the patient revealed an aberration on the long arm of chromosome 9 of approximately 2.93 Mb. The threshold for deletions and duplications is, respectively, 1.6 and 2.4. (d) SNP array copy number plot focused on the duplicated region of chromosome 9, showing a ~400 kb duplication, a ~2.4 Mb triplication and a ~130 kb duplication.

1.5 Methods of confirmation

To verify these results additional MLPA probes were developed within the 2.93 Mb critical interval for 9q. MLPA probes for the duplicated and triplicated region of chromosome 9q34.3 (located in genes *MRPS2*, *CAMSAP1*, *LHX3*, *PTGDS*, *ANAPC2*, *COL5A1*, *OLFM1*, *CACNA1B*) and control probes (genes *GPR64* and *FAM46D* located on chromosome X) were designed at our laboratory and commercially obtained from Biolegio (Malden, The Netherlands). The MLPA experiments were performed as described [15].

Furthermore BAC clones specific for human chromosome 9q were selected based on their physical location within the affected 9q region (http://www.ensembl.org). Fluorescent *in situ* hybridization (FISH) experiments were performed by standard procedures [2]. BAC clones RP11-270D17 and GS-135I17 were used to confirm both duplications (Fig. 4.1.2a). To determine the precise orientation of the fragments involved in the triplication, BAC clones RP11-413M3 and RP11-48E05 were used and labeled with different fluorochromes (Fig. 4.1.2b, c).

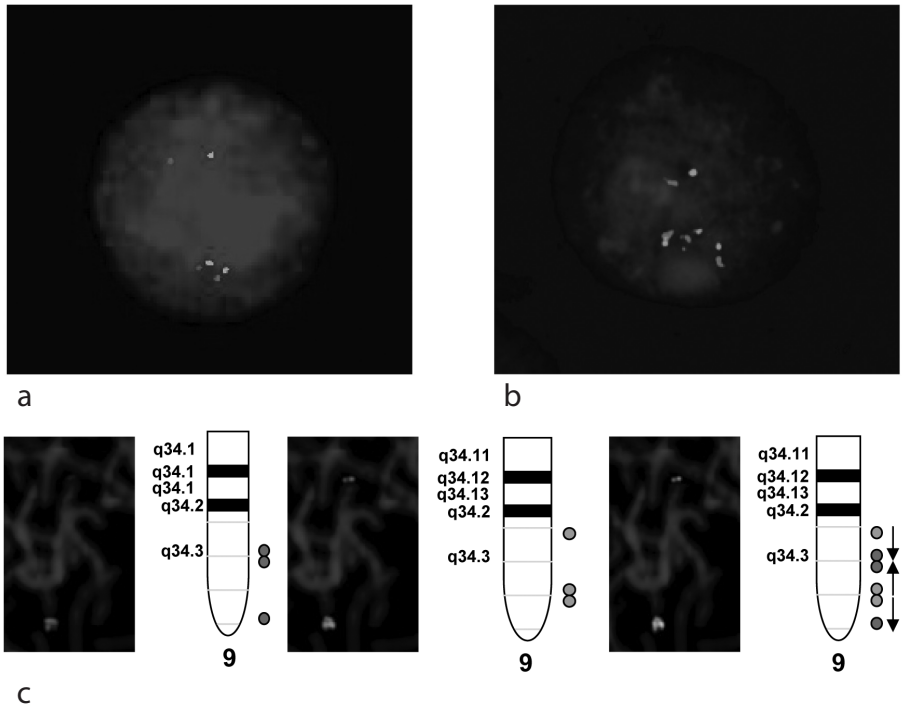

Figure 4.1.2 (a) FISH analysis with RP11-270D17 (green) and GS-135I17 (red) observed a duplication for both the probes confirming the 400 kb and 130 kb duplication, respectively. (b) Hybridization with RP11-413M3 (green, centromeric side of chromosome 9q) and RP11-48E05 (red, telomeric side of chromosome 9q) revealed the presence of four red and four green signals. The signals for the chromosome with the triplicated region showed that the middle repeat is inverted (green-red-red-green-green-red). (c) For probe RP11-48E05 the centromeric signal was of double intensity as compared to the telomeric signal. For probe RP11-413M3 (green) the telomeric signal was stronger than the centromeric signal. This indicates that the repeats of the triplication are normal in the proximal and distal region and inverted in the central region.

1.6 Causative of the phenotype

Conventional karyotyping of the parents presented a normal karyotype. Furthermore the parents showed normal subtelomeric MLPA, FISH and SNP array results. These results indicate that the aberration was *de novo*.

2. Clinical description

The female patient was born after an uncomplicated pregnancy, weighing 2750 g. She is the youngest child in a sibship of three. Parents are healthy and non-consanguineous. A previous pregnancy had resulted in unexplained stillbirth around 38 weeks gestation. She has one healthy older sister.

In early infancy, psychomotor retardation was noted (walking after 2.5 years of age, no speech development). At physical examination at the age of 2.5 years height was 89 cm (10th centile), head circumference 47.5 cm (25th centile). She was reported to have no apparent facial dysmorphism, and apart from tapering fingers and a sacral dimple no phenotypic anomalies were present. Despite extensive investigations at the time, no reason for the developmental delay was found. At the age of 6 years she underwent surgery because of convergent strabismus.

At the age of 16 years she was referred to our department for re-evaluation (Fig. 4.1.3). She was severely mentally retarded. She had limited speech; she only used a few words and was dysarthric. There was constant drooling. She had hearing loss. She was able to communicate using signs and pictures. She had a high threshold for pain. There was no history of sleep disturbance, seizures or constipation. Recently, she developed aggressive behaviour and was found to have more difficulties with walking.

At physical examination her growth parameters were within normal limits. Her facial features showed narrow palpebral fissures and a wide/large mouth. Both earlobes were upturned. Apart from slender fingers with clinodactyly of both fifth fingers and bilateral pes cavus, no dysmorphic features were present.

DNA analysis for Angelman syndrome and Mowat-Wilson syndrome showed a normal methylation pattern and no mutation in the *ZFHX1B* gene, respectively. MRI and metabolic screening showed no abnormalities.

We obtained samples from the patient and family members after acquiring informed consent approved by the Institutional Review Board at the Leiden University Medical Center, Leiden.

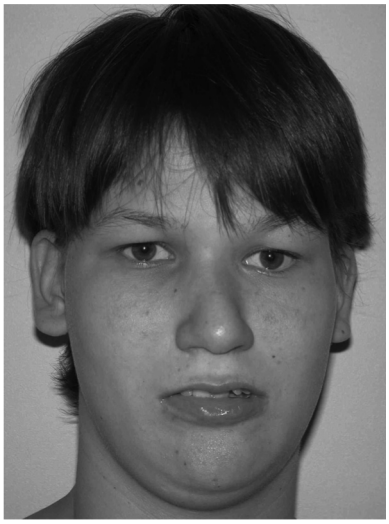

Figure 4.1.3 Picture of the female patient at the age of 16 years. Note mild facial asymmetry and narrow palpebral fissures.

3. Discussion

Cytogenetic karyotyping was performed on a female patient with unexplained severe mental retardation and showed an abnormal karyotype, 46,XX,add(9)(q34). To determine the extent of the aberration on chromosome 9, subtelomeric MLPA analysis was performed showing an abnormality for both probes on chromosome 9q. Subsequent SNP array analysis revealed a ~400 kb duplication followed by a ~2.4 Mb triplication and a duplication of 130 kb of chromosome 9q34.3 demonstrating the added value of this technique to conventional karyotyping.

To the best of our knowledge, this is the first report on a case with a partial duplication and partial triplication of chromosome 9q34.3. In the literature a few cases of duplication 9q are described. In 1975 the first association between clinical features and a partial duplication of chromosome 9q in two patients was reported [12]. Later four families with seven affected children, presumably descending from a common ancestor, in which an inverted insertion (9)(q22.1q34.3q34.1) chromosome was segregating were described [1]. Meiotic recombination led to the duplication of the 9q34 region, and to a phenotype they called the duplication 9q34 syndrome. Recently a new case with a duplication of chromosome 9q34 was described [4]. Furthermore, patients with a duplication of the 9q34 region and an additional aberration elsewhere in the genome were reported [6,9,10]. It may be expected that in those cases the phenotype is also affected by the other observed deletions and duplications in the genome. Nevertheless, the authors recognized a clinical syndrome similar to the duplication 9q34 syndrome [1]. Phenotypic similarities are observed between patients with a duplication of chromosome 9q34 and patients with larger duplications of chromosome 9q [10]. Larger duplications of chromosome 9q appear to be associated with a more severe developmental delay.

Although there are no cases described of partial triplication of this region before, it would be logical to compare the clinical phenotype of our patient with cases with duplication of chromosome 9q34 (Table 4.1.1). Only one study described patients with a pure 9q34 duplication overlapping the 9q34.3 abnormality in our patient [1]. In their patients common clinical features were dolichocephaly, facial asymmetry, deep-set eyes, narrow horizontal palpebral fissures, prominent nasal bridge, beaked nose, small mouth, retrognathia, arachnodactyly, camptodactyly, joint contractures, and scoliosis. The facial phenotype seemed to change with age. Reported medical problems were feeding difficulties and failure to thrive in infancy, strabismus and ptosis. All patients had psychomotor retardation and speech development was frequently impaired. Several patients were reported to have behavioral problems (hyperactivity, tantrums). Our patient seems to have some of the reported facial features. Furthermore, her arachnodactyly, limited speech and behavioral problems may be explained by the chromosome aberration.

It has been described that a microdeletion syndrome can show overlapping features with a microduplication syndrome of the same region, e.g. 22q11 [3]. Patients with a deletion 9q34 however, show a distinct phenotype that does not bear a resemblance to our patient [5]. As yet there are no arguments for overlapping clinical features in microduplication and microdeletion in the 9q34 region.

In the 9q34.3 deletion syndrome, haploinsufficiency of *EHMT1* (MIM 607001) is recognized as the causative gene [7]. The *EHMT1* gene belongs to the histone-lysine methyltransferase family and plays a role in the central nervous system development and function through epigenetic histon modification (http://www.dsi.univ-paris5.fr/

Table 4.1.1 Comparison of clinical features with other reported cases with duplication 9q34

Phenotypic features	Allderdice et al. 9q34.1q34.3	Gawlik-Kuklinska et al. 9q33.3q34.1	Present case 9q34.3
Facial asymmetry	+	+	±
Deep-set eyes	±	+	-
Narrow horizontal palpebral fissures	+	+	+
Abnormally shaped ears	+	-	+
Prominent chin	+	+	-
Beaked nose	+	-	-
Microstomia	+	+	-
Retrognathia	+	+	-
Abnormal position of digits	+	+	-
Arachnodactyly	+	+	+
Camptodactyly	+	+	-
Scoliosis	±	+	-
Overweight/obesity	-	+	-
Tongue/palate abnormalities	-	+	-
Cardiac anomalies	-	-	-
Low birth weight	+	-	-
Hypotonia	+	+	-
Cyanosis	+	+	-
Failure to thrive	+	+	-

genatlas/). It is unknown whether duplication or triplication of this gene results in an equal disturbance of function.

In our case the most distal breakpoint disrupts the *CACNA1B* gene. *CACNA1B* is a calcium channel, voltage dependent, N-type, alpha subunit gene (MIM 601012). It encodes an N-type calcium channel, which controls neurotransmitter release from neurons. It has been suggested that a heterozygous deletion of *CACNA1B* may result in the reduction of N-type channel activity [5]. In this way, in addition to the triplication of *EHMT1*, haploinsufficiency of *CACNA1B* may be a contributing factor for mental retardation and/or epilepsy. However, FISH analysis revealed the orientation of the triplication and supports an inverted orientation of the middle fragment (Fig. 4.1.3b, c). This results in two normal functioning copies of the *CACNA1B* gene and three

interrupted copies.

In short, copy number variations of the *EHMT1* gene may probably explain some features of the phenotype of this patient. However, according to the Ensembl Genome Browser (http://www.ensembl.org/index.html) release 44 based on the NCBI 36 assembly of the human genome (November 2005), the 2.93 Mb duplicated and triplicated regions of our patient contains approximately 100 genes. It is therefore unlikely that the clinical features in our patient are the result of a copy number change in just one gene. We expect that the phenotype associated with this rearrangement will be clarified when other cases with comparable rearrangements are disclosed.

According to the Database of Genomic Variants (http://projects.tcag.ca/variation/) the 9q34.3 region corresponds to a copy number variation region (CNVR), which is an artificial grouping of CNVs overlapping or in close proximity of each other [16]. This CNVR consists of 3 individual CNVs (gains) detected in 1190 controls (0.3%). Since this aberration is repeatedly described as disease causing [1,4,12], it is likely that the complex rearrangement described here causes the phenotype.

The three breakpoint regions of the 9q aberration present no architectural features that might be involved in the mediation of duplications and triplications. We speculate that first a 2.93 Mb duplication occurred and subsequently, an interstitial triplication in the duplicated region. Intrachromosomal triplications are rare and most of them concern the proximal 15q region [13]. Most of the reported triplications seem to result from a common mechanism as indicated by the inverted orientation of the middle repeat [14]. However, it is not clear which mechanism can explain the genesis of these triplications.

In conclusion, using conventional karyotyping characterization of complex rearrangements and identification of the origin of extra chromosomal material is often difficult. The introduction of new molecular cytogenetic methods such as MLPA analysis and SNP arrays provides cytogenetics with new approaches to define these abnormal chromosomes. The resolution of chromosome studies is markedly improved by the availability of SNP arrays that enable high-resolution genome analysis. This technique proves to be a more accurate method for the identification and delineation of chromosomal rearrangements. As a result, precise definition of complex rearrangements can be better established. It is, however, noteworthy that current array technologies are incapable of detecting balanced alterations such as inversions, and only give information regarding genomic gains and losses. In this study we underline the added value of array technologies in defining chromosomal aberrations observed with conventional chromosome analysis.

Acknowledgements

We thank the patient and her family for their collaboration and Marlies Hoogenboom for expert technical assistance with FISH analysis.

References

[1] Allderdice PW, Eales B, Onyett H, Sprague W, Henderson K, Lefeuvre PA, Pal G. 1983. Duplication 9q34 syndrome. Am J Hum Genet 35:1005-1009.

[2] Dauwerse JG, Jumelet EA, Wessels JW, Saris JJ, Hagemeijer A, Beverstock GC, Van Ommen GJB, Breuning MH. 1992. Extensive cross-homology between the long and the short arm of chromosome 16 may explain leukemic inversions and translocations. Blood 79:1299-1304.

[3] Ensenauer RE, Adeyinka A, Flynn HC, Michels VV, Lindor NM, Dawson DB, Thorland EC, Lorentz CP, Goldstein JL, McDonald MT, Smith WE, Simon-Fayard E, Alexander AA, Kulharya AS, Ketterling RP,

Clark RD, Jalal SM. 2003. Microduplication 22q11.2, an emerging syndrome: clinical, cytogenetic, and molecular analysis of thirteen patients. Am J Hum Genet 73:1027-1040.

[4] Gawlik-Kuklinska K, Iliszko M, Wozniak A, Debiec-Rychter M, Kardas I, Wierzba J, Limon J. 2007. A girl with duplication 9q34 syndrome. Am J Med Genet Part A 143A:2019-2023.

[5] Harada N, Visser R, Dawson A, Fukamachi M, Iwakoshi M, Okamoto N, Kishino T, Niikawa N, Matsumoto N. 2004. A 1-Mb critical region in six patients with 9q34.3 terminal deletion syndrome. J Hum Genet 49:440-444.

[6] Houdou S, Yorifugi T, Tsuruta S, Hashida K, Ohta S, Ieshima A. 1987. Distal 9q trisomy syndrome: report of the first oriental case and literature review. Acta Neonatal Jpn 23:347-352.

[7] Kleefstra T, Brunner HG, Amiel J, Oudakker AR, Nillesen WM, Magee A, Geneviève D, Cormier-Daire V, van Esch H, Fryns JP, Hamel BC, Sistermans EA, de Vries BB, van Bokhoven H. 2006. Loss-of-function mutations in euchromatin histone methyl transferase 1 (EHMT1) cause the 9q34 subtelomeric deletion syndrome. Am J Hum Genet 79:370-377.

[8] Li C, Wong WH. 2001. Model-based analysis of oligonucleotide arrays: Expression index computation and outlier detection. Proc Natl Acad Sci 98:31-36.

[9] Mattina T, Pierluigi M, Mazzone D, Scardilli S, Perfumo C, Mollica F. 1997. Double partial trisomy 9q34.1->qter and 21 pter->q22.11: FISH and clinical findings. J Med Genet 34:945-948.

[10] Spinner NB, Lucas JN, Poggensee M, Jacquette M, Schneider A. 1993. Duplication 9q34->qter identified by chromosome painting. Am J Med Genet 45:609-613.

[11] Stewart DR, Huang A, Faravelli F, Anderlid BM, Medne L, Ciprero K, Kaur M, Rossi E, Tenconi R, Nordenskjöld M, Gripp KW, Nicholson L, Meschino WS, Capua E, Quarrell OW, Flint J, Irons M, Giampietro PF, Schowalter DB, Zaleski CA, Malacarne M, Zackai EH, Spinner NB, Krantz ID. 2004. Subtelomeric deletions of chromosome 9q: a novel microdeletion syndrome. Am J Med Genet 128A:340-351.

[12] Turleau C, de Grouchy J, Chavin-Colin F, Roubin M, Brissaud PE, Repesse G, Safar A, Borniche P. 1975. Partial trisomy 9q: a new syndrome. Hum Genet 29:233-241.

[13] Vialard F, Mignon-Ravix C, Parain D, Depetris D, Portnoi MF, Moirot H, Mattei MG. 2003. Mechanism of intrachromosomal triplications 15q11-q13: a new clinical report. Am J Med Genet A 118:229-234.

[14] Wang J, Reddy KS, Wang E, Halderman L, Morgan BL, Lachman RS, Lin HJ, Cornford ME. 1999. Intrachromosomal triplication of 2q11.2-q21 in a severely malformed infant: case report and review of triplications and their possible mechanisms. Am J Med Genet 82:312-317.

[15] White SJ, Vink GR, Kriek M, Wuyts W, Schouten J, Bakker B, Breuning MH, den Dunnen JT. 2004. Two-color multiplex ligation-dependent probe amplification: detecting genomic rearrangements in hereditary multiple exostoses. Hum Mutat 24:86-92.

[16] Zogopoulos G, Ha KCH, Naqib F, Moore S, Kim H, Montpetit A, Robidoux F, Laflamme P, Cotterchio M, Greenwood C, Scherer SW, Zanke B, Hudson TJ, Bader GD, Gallinger S. 2007. Germ-line DNA copy number variation frequencies in a large North American population. Hum Genet 122:345-353.