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Interstitial duplication of 22q13.2 in a girl with short stature, impaired speech and language, and dysmorphism

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Abstract. The 22q13.3 deletion syndrome has been widely reported, with a known phenotype including global developmental delay, normal to accelerated growth and a characteristic facial appearance. A duplication syndrome involving this region has also been reported, with a somewhat more variable phenotype including psychomotor retardation, growth restriction, characteristic facial appearance differing from that seen in the deletion syndrome, and multiple malformations. The majority of reported patients have terminal duplications, with only three previous reports of interstitial duplication of the region. Herein we report a young woman with a *de novo* 569 kb interstitial duplication of 22q13.2 and short stature, speech and language impairment, refractive amblyopia, menorrhagia and facial dysmorphism. Comparison of her phenotype to previously reported patients with interstitial duplications reveals common traits including growth restriction, craniofacial anomalies and developmental delays. Included in the duplicated region is the gene *EP300*, mutations and deletions of which are implicated in Rubinstein-Taybi syndrome and thyrotroph embryonic factor, which has been proposed to be related to the pituitary hypoplasia seen in one patient with a large duplication, and several other genes without clear relation to disease.

Keywords: Copy number variation, microduplication, speech and language impairment, array comparative genomic hybridization

1. Introduction

Duplications of 22q13 to 22qter have been described, with common clinical features including psychomotor retardation, prenatal and postnatal growth retardation, cleft palate +/– cleft lip, micrognathia, microcephaly, hypertelorism, low set ears, congenital heart defects, renal and genital anomalies, skeletal abnormalities and hypotonia, with a highly reduced life expectancy [1–3]. However, different sized duplications have shown variable phenotypic severity, as well as inconsistency in phenotype even in affected members of the same family. For example, in a small sample of patients with different sized terminal duplications in this region, including subjects with terminal duplications starting at or proximal to the 22q13.2 region, some exhibited upward obliquity of the palpebral fissures, epicanthic folds, and a short neck, whereas these features were absent in patients with more distal duplications [4]. An additional patient was reported with a large terminal duplication and a much broader phenotype including craniosynostosis, gliosis and a hypoplastic, ectopic pituitary gland [5]. This variability in phenotype implies

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that more patients with duplications of this region need to be evaluated to make correlations between size and position of the duplication and the presence of specific clinical findings.

The reciprocal 22q13.3 deletion syndrome (22q13DS), also known as the Phelan-McDermid syndrome, has been very well-reported, and is associated with global developmental delay, normal to accelerated growth, absent or delayed speech, autistic-like behavior and generalized hypotonia. Physical features of the disorder include dolichocephaly, ptosis, abnormal ears, relatively large hands and dysplastic toenails [6–8]. The most terminal sub-band, 22q13.33, contains 40 known genes, including the genes implicated in causing phenotypes associated with the syndrome. One of the genes, named *ProSAP2 (SHANK3)* is thought to be responsible for the neurologic phenotype [9–11].

One consistent feature of interest with relation to the 22q13 deletion is that patients with the deletion often have normal to accelerated growth, in contrast to the short stature seen in patients with duplications [7]. This suggests that similar genes may be responsible for some anomalies in both disorders, with opposing phenotypes related to gene dosage.

There have been significantly fewer reports of interstitial duplications involving this region. Herein we report a young woman with an interstitial microduplication involving 22q13.2, with an associated phenotype that partially overlaps with previously reported patients.

2. Case report

The patient was born at her home in Ecuador via full term normal spontaneous vaginal delivery with a birth weight of 3.4 kg (on the 50th percentile), to a 25-year-old G1P0 mother and a 30-year-old father. Prenatally, her mother took an unknown medication for nausea, but denied toxic habits. Mother felt quickening at 6 months' gestation and reported poor fetal movement. At the patient's initial genetics visit at 16 years of age, she was taking iron supplements for anemia. She wore corrective lenses for refractive amblyopia. An echocardiogram performed previously for a heart murmur was normal. She had menarche at 10 years of age, with heavy menstrual flow. Developmentally, the patient walked at 1 year and began talking at 3 years. At age 16, she was attending a special education program with a diagnosis of speech and language impairment. Family history was notable for healthy non-consanguineous parents of Ecuadorian descent, both of short stature measuring 152.4 cm tall. Mother had no pregnancy losses. The patient had one female paternal cousin with "comprehension problems" and two male paternal cousins with speech delay.

On physical examination, the patient's height was 145 cm (below 5th percentile), weight 51.1 kg (between 25–50th percentile) and head circumference 54 cm (50th percentile) with brachycephaly. Facial dysmorphism (Fig. 1A) included hypertelorism with outer canthal distance 11 cm (above 97th percentile) and inner canthal distance 4 cm (above 97th percentile) and bilateral epicanthal folds. She had thickened and over folded upper helices of both auricles. She had tortuous and caudally deviated external auditory canals. Her palate was high arched. Her neck was short and broad, but with full range of motion, and she had a low posterior hairline. (Figs. 1A–C) The patient had an anterior open bite. She also had brachydactyly with bilateral Sydney lines and small feet. Cervical spine X-ray was normal.

Initial clinical evaluations showed a normal karyotype 46, XX (band resolution 550–650, Quest Diagnostics) and normal Fragile X DNA analysis (29 CGG repeats x2). Targeted bacterial artificial chromosome (BAC) array comparative genomic hybridization (CGH) was performed at Quest Diagnostics, and revealed a duplication of DNA on 22q13.2. Fluorescence in-situ hybridization (FISH) assay with BAC CTD-2549E21 (Hg18; chr22: 39841605-40030292, 189 kb) exhibited enhanced hybridization signal for one of the chromosome 22 homologs, suggesting a duplication. Thyroid function tests were normal.

After obtaining informed consent (IRB#1999-201), the array was repeated in our research laboratory, and the duplication size and breakpoints were clarified to a duplication of approximately 569 kb extending from chr22:39,791,457-40,360,012 (Fig. 2A). Interphase FISH clearly validated the presence of amplified genomic DNA as shown in Fig. 2B. Parental FISH, aCGH and karyotypes (band resolution 550) were all normal.

Genomic DNA from the patient and her parents was isolated from lymphocytes transformed with Epstein Barr virus using the Gentra Pure Gene kit according to manufacturer's protocol in the DNA Isolation and Cell Expansion Core of the Human Genetics Program at the Albert Einstein College of Medicine (Qiagen Inc, Valencia, CA). Sample preparation with 1 μg of DNA was performed according to manufacturer's protocol (protocol 2008 version 6.0, Agilent Technologies, Santa Clara, CA). The patient's DNA sample was compared to a female

Fig. 1. A) Frontal view of patient. B) Profile. C) Posterior neck. Note patient's hypertelorism, short and broad neck, and low posterior hairline.

control DNA (Promega G1521; Promega Corporation, Madison, WI).

An Agilent 44k aCGH (2514950) test was performed on the isolated DNA. Samples were labeled and hybridized, washed, scanned and analyzed (Feature Extraction 10.1.1.1 and CGH Analytic 3.5.14 software) according to manufacturers' protocol (Agilent Technologies, Santa Clara, CA).

Sample and DNA probes as well as the FISH analysis were prepared and analyzed as described for the patient and her parents [12]. FISH analysis was performed to validate the array result on metaphase chromosomes obtained from Epstein Barr virus transformed lymphoblastoid cell lines with probes from BACs RP11-395B24 (duplicated region-red; chr22: 40,119,849–40, 294,546; labeled with Spectrum Orange- Abbot Molecular, Des Plains, IL) and RP11-81N15 (reference-green; chr22:40,935,055-41,103,649; labeled in Biotin and detected streptavidin Alexa 488 - Roche Indianapolis, IN and Invitrogen, Carlsbad, CA) (Fig. 2B). The BAC clones were obtained from the BACPAC Resources Center at Oakland, CA (http://bacpac.chori.org/).

3. Discussion

Though there have been many case reports of terminal duplications involving the 22q13 region, there are very few with an interstitial duplication. To our knowledge, only three such patients have been described (Table 1).

The patient described here has a significantly smaller duplication size than those previously reported (Table 1, Fig. 3). However, features common to nearly all four patients include developmental delay, short stature, hypertelorism, epicanthal folds and ear abnormalities. The patient described by Shimojima et al. [13] had C6–C7 fusion; the patient here has a short, broad neck but a normal X-ray of the cervical spine. Our patient did not have prenatal growth deficiency, as seen in the other two patients, suggesting the critical region for this phenotype may be proximal or distal to the duplicated area in our patient. A more proximal interstitial duplication, extending into the 22q13 region was reported in 1995, prior to the use of microarray technology to delineate breakpoints [14]. This subject had some features common to the subjects with more distal duplications described above, including developmental delay and postnatal growth restriction. However, he also had many features not described for the others including a low posterior hairline, prognathism, tongue protrusion,

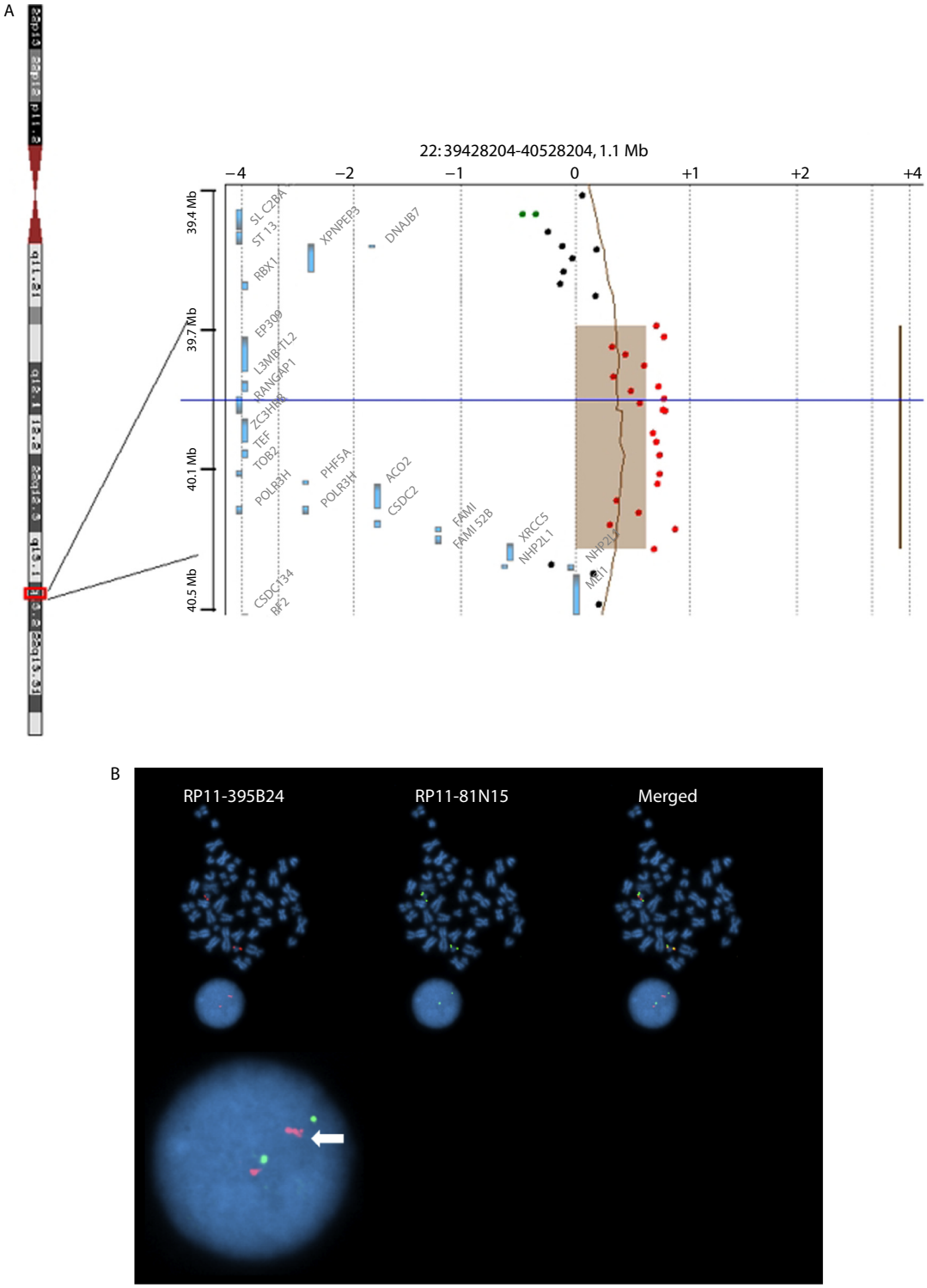

Fig. 2. A) On the left is a cartoon of chromosome 22, oriented from p-arm on top to q-arm on the bottom. The patient's duplicated region is indicated by the box (http://genome.ucsc.edu). The image on the right shows results of the 44Kb Agilent microarray demonstrating a 569 Kb duplication of chromosome 22, with breakpoints 39,791,457-40,360,012. B) Fluorescence in situ hybridization confirming our patient's duplication. Control bacterial artificial chromosome probe RP11-81N15 is labeled in green; bacterial artificial chromosome probe to duplicated region RP11-395B24 is labeled in red. Arrow is pointing to the duplicated region.

Table 1
Characteristics of patients with interstitial 22q13 duplications

Features	Pramparo et al. 2008 (1)	Shimojima et al. 2009 (13)	Prasher et al. 1995 (14)	Our patient
Cytogenetics				
Duplication size	6.9 Mb	6 Mb	No array; only cytogenetic	569 Kb
Breakpoints	22q13.1-13.2	22q13.1-q13.31	22q11.2-q13; inversion	22q13.2
Array comparative genomic hybridization	Bet 36.197-36.297Mb-bet 43.268-43.436Mb	From figure - ~38.750Mb-44.500Mb	Not done	39.791Mb-40.360Mb
Maternally derived	+	+		
Constitutional				
Intrauterine growth restriction	+	+	–	–
Postnatal growth restriction	+	+	+	+
Craniofacial				
Prominent forehead	+		+	–
Hypertelorism	+	+		+
Small palpebral fissures	+			–
Epicanthal folds	+	+		+
Ptosis		+		–
Exotropia	+	–		–
Myopia	+	–		–
Arched eyebrows		+		–
Small low-set/deformed ears	+	+		+
Short/shallow philtrum	+			–
High arched palate				+
C6–C7 fusion		+		–
Other physical				
Brachydactyly			+	+
Abnormal palmar crease			+	+
Toe syndactyly	+	–		–
Neurologic				
Hypotonia	+			–
Mental retardation/Developmental disability	+	+	+	+
Seizures	+	–		–
Abnormal Electroencephalography		–		
Behavior/psych.	+		–	–
Structural brain anomalies	+	–		
Hearing loss	–	+		–

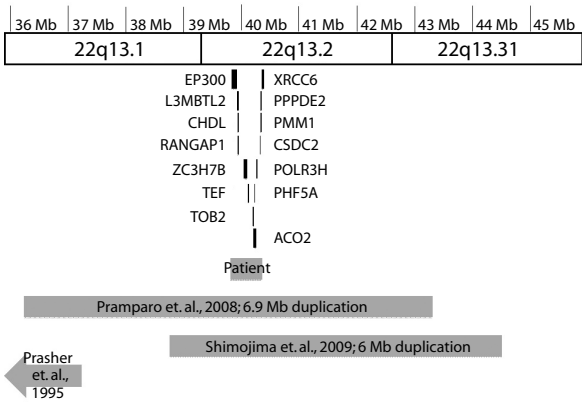

Fig. 3. Cartoon of chromosome 22 is oriented from centromere (left) to telomere (right). Duplication breakpoints of our patient and those of prior reports of interstitial duplications are indicated by grey boxes. Genes included in our patient's duplication are shown.

thick fleshy lips, coloboma and poor vision, kyphosis, and small phallus and testes [14].

While copy number gains and losses have been reported within this region, ranging from 765 bp to over 333 kb (http://projects.tcag.ca/variation/?source=hg18; Fig. 4), we believe our patient's duplication to be pathogenic based on its *de novo* occurrence, and overlap with the phenotypes of the patients above.

One of the genes within our patient's duplicated region is *EP300*, a histone acetyltransferase, which has been associated with Rubinstein-Taybi syndrome (RTS). RTS is a disorder characterized by mental and growth retardation, dysmorphic facies, and broad thumbs and great toes [15]. RTS is most commonly attributable to mutations in, or microdeletions involving, *CREBBP* on chromosome 16p13.3. This disorder has also rarely been caused by mutations in *EP300*, which

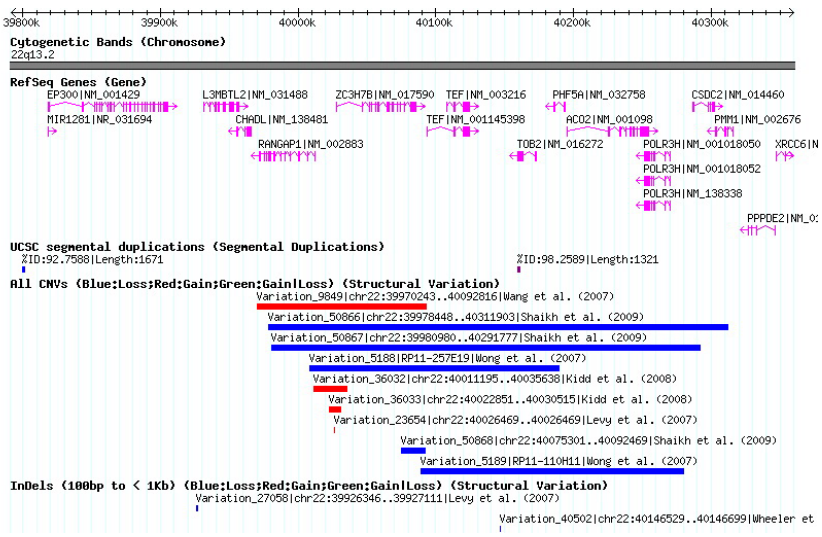

Fig. 4. Copy number polymorphisms within region of our patient's duplication. Line at the top represents genomic material duplicated in our patient. Red (light grey) lines represent copy number gain polymorphisms, and blue (dark grey) lines represent copy number loss polymorphisms. Image produced using http://projects.tcag.ca/variation/?source=hg18.

shares homology at its binding site for transcription factors with *CREBBP*. In a study of 92 patients with RTS, 36 had a mutation or copy number change in *CREBBP,* and three had mutations or deletions in *EP300* [15]. Our patient has some of the clinical characteristics of RTS, including mental retardation and growth retardation, but lacks many of the more salient features. This would be expected since our patient has a duplication and previous patients with RTS due to *EP300* aberrations have had deletions.

EP300 has also been implicated as a tumor suppressor gene. Truncating mutations with loss of the second allele have been identified in both primary epithelial cancers and cancer cell lines [16]. It has been suggested that haploinsufficiency for this gene may promote tumorigenesis, or that promoter hypermethylation may inactivate the gene and thereby lead to cancer [17]. However, our patient has a duplication of this gene, rather than a deletion or truncating mutation, and therefore would not be predicted to be at increased risk of developing cancer related to this genetic aberration.

None of the other 13 genes in our subject's duplicated region have known clear relation to our patient's disease. The thyrotroph embryonic factor, which is contained within our subject's duplicated region, has been proposed to be related to the pituitary hypoplasia seen in one child with a large terminal duplication of the long arm of chromosome 22 [5]. However, our patient does not have known pituitary hypoplasia, though neither an magnetic resonance imaging of the brain nor growth hormone studies have been performed. Since our patient's duplication is so small, and she does have some overlapping features with other described patients, it is clear that at least one of the genes in this gene-rich region is critical for the post-natal growth retardation, craniofacial malformations and intellectual impairment seen in this disorder.

While many microduplication and microdeletion syndromes are caused by non-allelic homologous recombination [18], there are no low copy repeats flanking the breakpoints of our patient's duplication. Parental karyotypes were normal, making a balanced translocation in one of the parents an unlikely mechanism for her duplication. Because of the small size of her duplication, it is possible that one of the parents has an inversion of the region, too small to be detected by karyotype. This may have predisposed to the duplication, in a similar mechanism to that described for the deletion causing Williams syndrome and for other microdeletion and microduplication syndromes [19,20].

This patient underscores the importance of using array CGH in the diagnosis of children with unexplained intellectual impairment and dysmorphism. Despite normal karyotype, our subject does have a chromosomal microduplication that is clinically relevant, providing a much-desired answer to the family for their child's symptoms. The parents' normal karyotypes and FISH rule out a balanced translocation as the etiology of the

duplication, which may be reassuring to the subject's brother for his recurrence risk. However, due to the small size of the duplicated region, we were unable to definitively rule out an inversion in one of the parents as the predisposing event, which could have been passed to the brother. Additionally, if our patient or her brother were to pursue having children of their own, the patient has a clear identified risk of having affected children, and either would be able to undergo prenatal testing or preimplantation genetic diagnosis for this condition if of interest.

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References

[1] Pramparo T, de Gregori M, Gimelli S, Ciccone R, Frondizi D, Liehr T, et al. A 7 Mb duplication at 22q13 in a girl with bipolar disorder and hippocampal malformation. Am J Med Genet A 2008; 146A: 1754–60.

[2] Peeters H, Vermeesch J, Fryns JP. A cryptic duplication 22q13.31 to qter leads to a distinct phenotype with mental retardation, microcephaly and mild facial dysmorphism. Genet Couns 2008; 19: 365–71.

[3] Okamoto N, Kubota T, Nakamura Y, Murakami R, Nishikubo T, Tanaka I, et al. 22q13 Microduplication in two patients with common clinical manifestations: a recognizable syndrome? Am J Med Genet A 2007; 143A: 2804–9.

[4] Feenstra I, Koolen DA, Van der Pas J, Hamel BC, Mieloo H, Smeets DF, et al. Cryptic duplication of the distal segment of 22q due to a translocation (21;22): three case reports and a review of the literature. Eur J Med Genet 2006; 49: 384–95.

[5] Brunetti-Pierri N, Patel A, Brown CW, Rauch RA, Heptulla RA. De novo terminal 22q12.3q13.3 duplication with pituitary hypoplasia. Am J Med Genet A 2009; 149A: 2554–6.

[6] Nesslinger NJ, Gorski JL, Kurczynski TW, Shapira SK, Siegal-Bartelt J, Dumanski JP, et al. Clinical, cytogenetic, and molecular characterization of seven patients with deletions of chromosome 22q13.3. Am J Hum Genet 1994; 54: 464–72.

[7] Phelan MC, Rogers RC, Saul RA, Stapleton GA, Sweet K, McDermid H, et al. 22q13 deletion syndrome. Am J Med Genet 2001; 101: 91–9.

[8] Koolen DA, Reardon W, Rosser EM, Lacombe D, Hurst JA, Law CJ, et al. Molecular characterisation of patients with subtelomeric 22q abnormalities using chromosome specific array-based comparative genomic hybridisation. Eur J Hum Genet 2005; 13: 1019–24.

[9] Wilson HL, Wong AC, Shaw SR, Tse WY, Stapleton GA, Phelan MC, et al. Molecular characterisation of the 22q13 deletion syndrome supports the role of haploinsufficiency of SHANK3/PROSAP2 in the major neurological symptoms. J Med Genet 2003; 40: 575–84.

[10] Bonaglia MC, Giorda R, Borgatti R, Felisari G, Gagliardi C, Selicornia A, et al. Disruption of the ProSAP2 gene in a t(12;22)(q24.1;q13.3) is associated with the 22q13.3 deletion syndrome. Am J Hum Genet 2001; 69: 261–8.

[11] Durand CM, Betancur C, Boeckers TM, Bockman J, Chaste P, Fauchereau F, et al. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. Nat Genet 2007; 39: 25–7.

[12] Downing TE, Oktay MH, Fazzari MJ, Montagna C. Prognostic and predictive value of 16p12.1 and 16q22.1 copy number changes in human breast cancer. Cancer Genet Cytogenet 2010; 198: 52–61.

[13] Shimojima K, Tanaka K, Yamamoto T. A de novo intrachromosomal tandem duplication at 22q13.1q13.31 including the Rubinstein-Taybi region but with no bipolar disorder. Am J Med Genet A 2009; 149A: 1359–63.

[14] Prasher VP, Roberts E, Norman A, Butler AC, Krishnan VH, McMullan DJ. Partial trisomy 22 (q11.2-q13.1) as a result of duplication and pericentric inversion. J Med Genet 1995; 32: 306–8.

[15] Roelfsema JH, White SJ, Ariyurek Y, Bartholdi D, Niedrist D, Papadia F, et al. Genetic heterogeneity in Rubinstein-Taybi syndrome: mutations in both the CBP and EP300 genes cause disease. Am J Hum Genet 2005; 76: 572–80.

[16] Gayther SA, Batley SJ, Linger L, Bannister A, Thorpe K, Chin SF, et al. Mutations truncating the EP300 acetylase in human cancers. Nat Genet 2000; 24: 300–3.

[17] Bryan EJ, Jokubaitis VJ, Chamberlain NL, Baxter SW, Dawson E, Choong DY, et al. Mutation analysis of EP300 in colon, breast and ovarian carcinomas. Int J Cancer 2002; 102: 137–41.

[18] Lupski JR. Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. Trends Genet 1998; 14: 417–22.

[19] Hobart HH, Morris CA, Mervis CB, Pani AM, Kistler DJ, Rios CM, et al. Inversion of the Williams syndrome region is a common polymorphism found more frequently in parents of children with Williams syndrome. Am J Med Genet C Semin Med Genet 2010; 154C: 220–8.

[20] Sharp AJ. Emerging themes and new challenges in defining the role of structural variation in human disease. Hum Mutat 2009; 30: 135–44.