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CASE REPORT

DICENTRIC CHROMOSOME 14;18 PLUS TWO ADDITIONAL CNVs IN A GIRL WITH MICROFORM HOLOPROSENCEPHALY AND TURNER STIGMATA

Sireteanu A[1], Voloşciuc M[2], Grămescu M[1], Gorduza EV[1], Vulpoi C[3], Frunză I[4], Rusu C[1,*]

***Corresponding Author:** Cristina Rusu, M.D., Ph.D., "Grigore T. Popa" University of Medicine and Pharmacy, Medical Genetics Department, Stradă Universităţii 16, Iaşi 700115, Romania; Tel./Fax: +40-232-272-754; Mobile: +40-745-432-077; E-mail address: abcrusu@gmail.com

ABSTRACT

We report a 20-year-old female with features evocative of Turner syndrome (short stature, broad trunk, mild webbed neck), dysmorphic face, minor features of holo-prosencephaly (HPE), small hands and feet, excessive hair growth on anterior trunk and intellectual disability. Cytogenetic analysis identified a pseudodicentric 14;18 chromosome. Genome wide single nucleotide polymorphism (SNP) array showed a terminal deletion of approximately 10.24 Mb, from 18p11.32 to 18p11.22, flanked by a duplication of approximately 1.15 Mb, from 18p11.22 to 18p11.21. In addition, the SNP array revealed a duplication of 516 kb in 16p11.2. We correlated the patient's clinical findings with the features mentioned in the literature for these copy number variations. This case study shows the importance of microarray analysis in the detection of cryptic chromosomal rearrangements in patients with intellectual disability and multiple congenital anomalies.

Keywords: 18p - syndrome; Holoprosencephaly (HPE); Single nucleotide polymorphism (SNP) array; 16p11.2 duplication

[1] Department of Medical Genetics, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania

[2] Medical Genetics Center, "Sf. Maria" Children's Hospital, Iaşi, Romania

[3] Department of Endocrinology, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania

[4] Department of Neurology, CF Hospital, Iaşi, Romania

INTRODUCTION

Monosomy 18p was first reported in 1963 by de Grouchy *et al.* [1] and has an incidence of 1:50,000 live-born infants [2]. About two-thirds of cases are *de novo* deletions, one-sixth are due to *de novo* translocations between the long arms of an acrocentric and 18q, and the rest come from familial translocations, inversions, complex translocations or direct transmission [3]. The main clinical features of monosomy 18p are mild to moderate intellectual disability (ID), postnatal growth retardation, and dysmorphic features including ptosis, hypertelorism, strabismus, broad flat nose, micrognathia, and low-set large ears [4]. We report on a girl with ID, mild dysmorphic face and masculine body habitus who had a *de novo* 14;18 translocation. Copy number analysis with single nucleotide polymorphism (SNP) array detected a terminal deletion flanked by a duplication on 18p and a duplication of 16p11.2.

MATERIALS AND METHODS

Case Report. The patient is a 20-year-old female, the first child of non consanguineous, healthy Caucasian parents (mother was 24 years old and father was 27 years old when the proband was born). There was no family history of ID, congenital anomalies or psychiatric disorders. The pregnancy was uneventful; she was born at term by normal delivery with a birth weight of 2,200 g (below the 3rd percentile), length and head circumference were not recorded. All de-

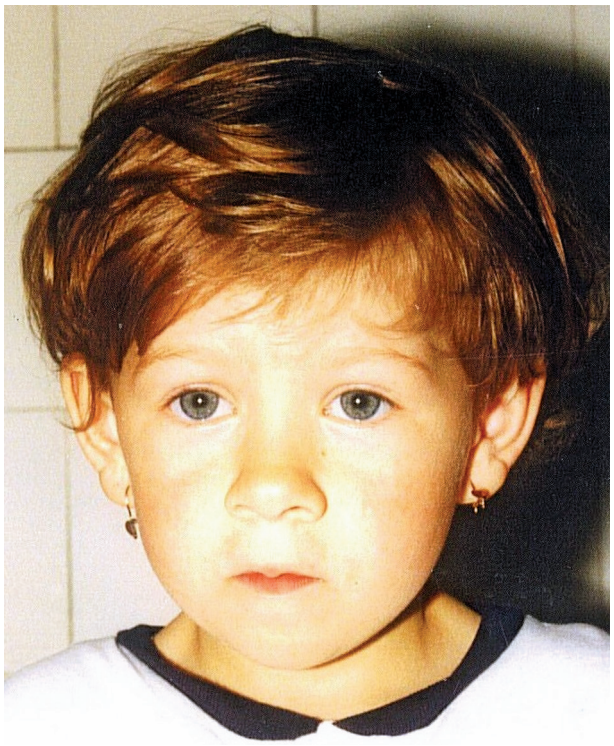

Figure 1. Patient at age 5.5 years.

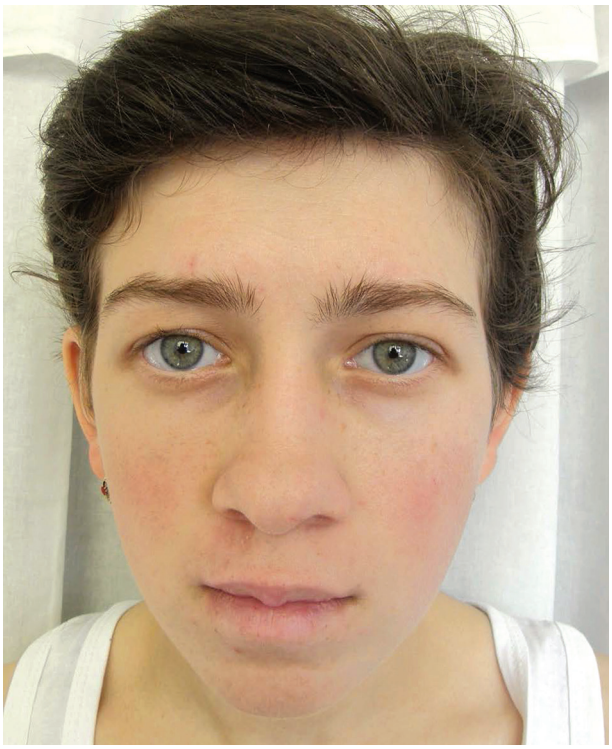

Figure 2. Patient at age 20 years.

velopmental milestones were delayed: she achieved head control at 6 months, walked without support at 2 years, spoke first clear words at 1.5 years. The girl was referred for genetic evaluation at the age of 5.5 year old, due to ID and single central maxillary incisor. Her growth parameters were: height 95.5 cm [-3.68 standard deviation (SD)], weight 13 kg (-2.48 SD), and head circumference 47 cm (-2.76 SD). She had a triangular face, horizontal palpebral fissures, blue sclera, short, slightly protruding philtrum and upper lip, blunted Cupid's bow, slightly everted lower lip, mild microretrognathia, bilateral preauricular sinus (Figure 1). Oral cavity examination showed absent maxillary and mandibular frenulum and single central maxillary incisor. Her language was limited to single words (she could not produce sentences). She had nocturnal and diurnal enuresis, for which she received therapy. No hearing impairment has been identified. Echocardiography showed an atrial septal defect. Abdominal ultrasound, routine biochemical and hematological tests, endocrine investigations [growth hormone (GH), free thyroxine (T4), thyroid-stimulating hormone (TSH)] were normal. No metabolic tests have been performed. Cranio-cerebral computed tomography (CT) scan and magnetic resonance imaging (MRI) of the spine did not show any changes.

When examined at the age of 20, her height was 147 cm (-2.89 SD), weight was 43 kg [-1.76 SD, body mass index (BMI) 19.9 kg/m^2), and head circumference was 53 cm (-1.75 SD). We have noticed her standing and walking position (leaning slightly forward, with widened base of support), as well as slowness in motion and action. The face became elongated, mature for age, with slightly coarse features (Figure 2). She had mild webbed neck, broad chest and narrow hips, normal posterior hairline, kyphoscoliosis, pectus excavatum, short and wide hands and feet (below the 3rd percentile), with mild brachydactyly. Puberty was normal, but she developed asymmetric mammary glands and excessive hair growth in pre-sternal, circumareolar and subumibilical regions (Ferri-man-Gallwey score of 2). Psychological testing established a moderate ID (IQ 45), with impaired speech and language skills, difficulties with interpersonal relationships and oppositional behavior. She presented giggle incontinence. Endocrine investigations were as follows: normal levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, prolactin, elevated plasmatic levels of testosterone, dehydroepiandrosterone (DHEA)-sulfate, and 17-hyroxyprogesterone (OH).

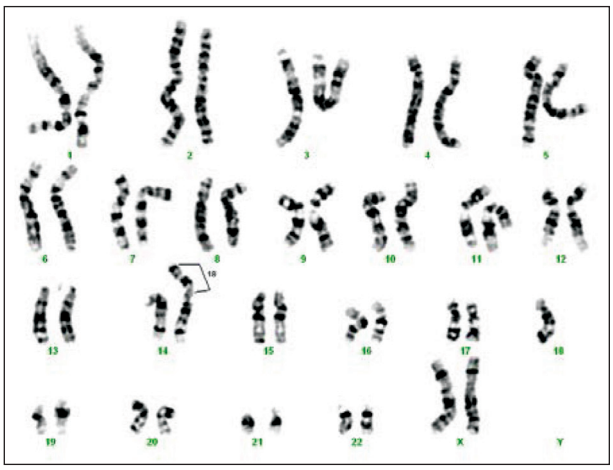

Figure 3. Karyotype of the proband demonstrating the 14;18 translocation.

Figure 4. View of the C-banded derivative chromosome from two different metaphases showing the presence of two centromeres.

Cytogenetic Studies. The chromosome analysis was performed for the patient and her parents using the G-banding technique on metaphase chromosomes from peripheral blood lymphocytes, according to standard protocol. Chromosome C-banding was performed by the standard BSG (barium hydroxide/saline solution/Giemsa) method [5] with slight modifications.

Single Nucleotide Polymorphism Array. Genomic DNA was purified from peripheral blood using Wizard Genomic DNA Purification Kit (Promega Corp., Madison, WI, USA). The SNP array was performed using Human CytoSNP-12 v2.1 BeadChip platform (Illumina Inc., San Diego, CA), containing approximately 300,000 SNPs per sample, according to the manufacturer's instructions. The data were processed using Genome Studio V2010.1 software (Illumina). Genomic positions were defined according to the GRCh37/hg19 Assembly of the Human Genome (February 2009).

RESULTS

The cytogenetic G-banding revealed an unbalanced translocation between chromosomes 14 and 18 (Figure 3). The C-banding showed two centromeres on the derivative chromosome; in all metaphases examined the 14 chromosome centromere was inactivated (Figure 4). Thus, the karyotype was 45,XX,psu dic(14;18)(p11.1;p13.1). The parental karyotypes were normal. The SNP array analysis detected a terminal deletion of approximately 10.24 Mb, from 18p11.32 to 18p11.22 (274-10,242,742), flanked by a duplication of approximately 1.15 Mb, from 18p11.22 to 18p11.21 (10,249,343-11,401,062) (Figure 5). In addition, the SNP array revealed a duplication of 516,590 bp in 16p11.2 (29,568,718-30,085,308). The relatively small size of the duplications did not allow for fluorescent *in situ* hybridization (FISH) to determine the orientation. Blood samples from the parents were not available for SNP array analysis.

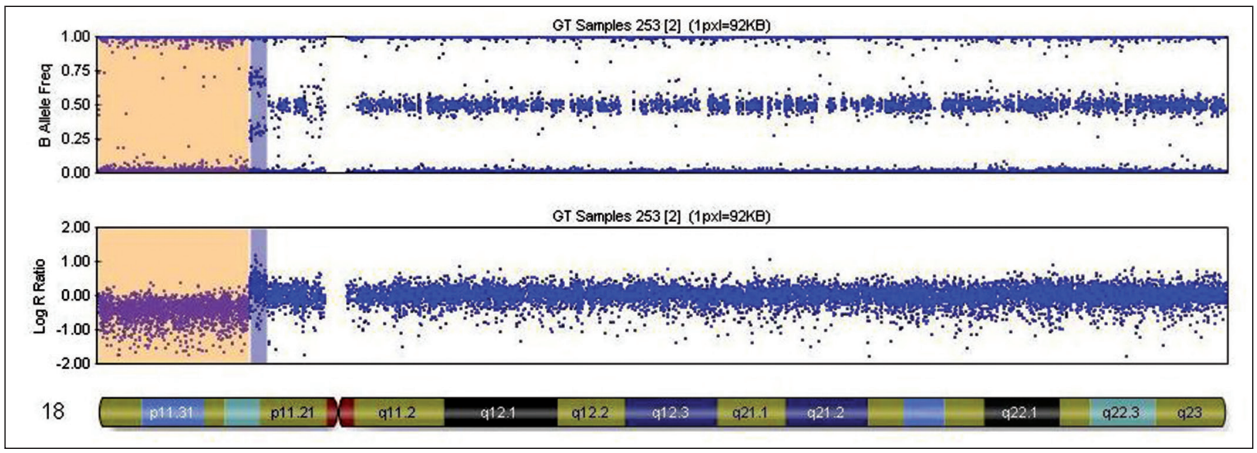

Figure 5. The SNP array results of chromosome 18 in the patient, showing a 10.24 Mb deletion in 18p11.32-p11.22 and a duplication of 1.15 Mb in 18p11.22-p11.21.

DISCUSSION

We describe a female patient with some signs of Turner syndrome, mild dysmorphic face, minor features of holoprosencephaly (HPE), small hands and feet, excessive hair growth on anterior trunk and ID. The karyotype showed an unbalanced translocation between chromosomes 14 and 18 resulting in the formation of a dicentric derivative chromosome. Single nucleotide polymorphism array analysis revealed three abnormalities: an 18p deletion flanked by a duplication, and a 16p11.2 duplication. The translocation is *de novo* as both parents had a normal karyotypes. Non Robertsonian dicentric autosomes are rare findings, reported in only 26 cases in a review by Lemyre *et al.* [6]. The majority of cases involve the acrocentric chromosomes, with a short arm breakpoint, followed in frequency by chromosome 18. Most of the heterodicentric autosomes have only one primary constriction on metaphase chromosomes, and the constriction is noticed mostly at the site of the non-acrocentric centromere [6], as in our case.

Deletions of p arms of acrocentrics containing nucleolus organizer regions (NOR) regions are not known to be associated with phenotypic anomalies, and have therefore probably not contributed to the phenotype. Also, the 1.15 Mb 18p duplication is not likely to have contributed to the phenotype, since most patients with trisomy 18p have normal or mild phenotypes, and may or may not have ID [7]. Our patient displays some of the features of 18p- syndrome, such as ID, features of the HPE spectrum (mild microcephaly, single central maxillary incisor), and features evocative of Turner syndrome (short stature, mild webbed neck, pectus excavatum, broad trunk and narrow hips) (Table 1). Facial dysmorphism (triangular face, blue sclera, bilateral preauricular sinus) is different from that described for 18p deletion, excepting the oromandibular region. Facial appearance has changed over time, becoming elongated (Figure 2), as described also by Tsukahara *et al.* [8]. Congenital cardiac defects, present in our case, have been observed in 10.0% of cases of 18p- [9]. Although the phenotype described above is not characteristic for monosomy 18p, the standing position with widespread legs and leaning slightly forward as well as marked slowness in motion and action are very suggestive for this chromosomal syndrome.

Recurrent 16p11.2 microduplications were initially associated with phenotypes ranging from normal to ID, autistic spectrum disorders and psychiatric problems [10-13]. Other studies showed that these duplications can manifest with dysmorphic features without a recognizable pattern, microcephaly, congenital anomalies (including torticollis, cleft lip and palate, pectus excavatum, pectus carinatum, mild scoliosis, hypospadias, phimosis, tethered cord, pes planus), and seizures [14]. Jacquemont *et al.* [15] showed

Table 1. Comparison of clinical findings in the present case and literature data (modified fromTurleau [2]).

Clinical Features	18p Deletion	Our Patient
ID (variable severity)	[+]	[+]
Speech delay	[+]	[+]
Behavioral disorders	[+]	[+]
Muscular hypotonia	[+]	[+]
Short stature	[+]	[+]
Microcephaly (mild)	[+]	[+]
Variable features of the HPE spectrum	[+]	[+]
Round, flat face	[+]	[–]
Triangular face	[–]	[+]
Ptosis/epicanthal folds/strabismus	[+]	[–]
Blue sclera	[–]	[+]
Flat nasal bridge	[+]	[–]
Short protruding philtrum/upper lip	[+]	[+]
Blunted Cupid's bow	[+]	[+]
Wide mouth	[+]	[–]
Irregularly set teeth, excessive caries	[+]	[–]
Microretrognathia	[+]	[+]
Large protruding ears	[+]	[–]
Preauricular sinus	[–]	[+]
Short, webbed neck	[+]	[–/+]
Broad trunk	[+]	[+]
Pectus excavatum	[+]	[+]
Asymmetric mammary glands	[–]	[+]
Kyphoscoliosis	[+]	[+]
Wide short hands	[+]	[+]
Wide short feet	[–]	[+]
Brachydactyly	[–]	[+]
Cardiac malformations	[+]	[+]
Autoimmune diseases	[+]	[–]
Alopecia	[+]	[–]
Mild hirsutism	[–]	[+]
Dystonia	[+]	[–]
Enuresis	[–]	[+]

that 16p11.2 duplication is associated with a BMI <18.5 kg per m^2 in adults and <-2 SD from the mean in children. Among the features mentioned above, our patient exhibited mild microcephaly, pectus excavatum, mild scoliosis and ID, but these features are also described in 18p deletion. She was underweight during childhood, but recovered later, her BMI being within normal range as an adult. Considering that empiric estimate for penetrance of proximal 16p11.2 duplication established a penetrance of 27.2%, and the likelihood of a normal phenotype is ~73.0% [16], we cannot clearly conclude how this copy number variation (CNV) influences the phenotype. More recently, a patient with thoracolumbar syringomyelia and a 16p11.2 duplication has been described [17]. Although our patient presented kyphoscoliosis and nocturnal enuresis, MRI of the spine showed no changes.

In a study of three patients with 18p deletion, Portnoi *et al.* [18] suggested that there might be a critical region for GH deficiency between 18p11.23 and 18pter. Our patient has a deletion which includes that region, but the level of GH is normal and the craniocerebral CT did not show any pituitary gland anomalies. The critical region for ID has been tentatively mapped between 18p11.1 and 18p11.21 [4]. Our patient has a deletion distal to this point and moderate ID, but this feature may be due to the 16p11.2 microduplication. Brenk *et al.* [19] proposed round face to map to the distal 1.6 Mb of 18p, and post-natal growth retardation and seizures to the distal 8 Mb. Our patient has a terminal deletion larger than 10 Mb, but she had no history of seizures, and the face was triangular in childhood and elongated in adulthood. Considering that a pointed chin can be noticed in five out of 13 patients with 16p11.2 duplication for which the facial features were presented [10,14], we appreciate that the triangular aspect of the face may be due to this rearrangement. Ptosis and short neck, frequently associated with 18p- [2], were attributed by Brenk *et al.* [19] to the proximal half of 18p. These features were absent in our patient, in whom the proximal 5.1 Mb of 18p was not deleted. Thus, haploin-sufficiency of genes located in this region may be responsible for these features.

Our patient has a microform of HPE, although only 10.0% of patients carrying an 18p deletion (including the *TGIF* gene) present HPE [3]. Holoprosencephaly is a complex developmental disorder in which multiple genetic and environmental factors can affect the severity of the phenotype [20]. A recent array CGH study of a large group of HPE patients demonstrated a high frequency of submicroscopic anomalies involving known but also novel HPE loci, including 16p11.2 [21]. Therefore, the 16p11.2 micro duplication present in our patient can be a second genetic event contributing to HPE manifestation.

In conclusion, we report a female patient with a pseudodicentric 14;18 chromosome that carries two additional CNVs. These CNVs confer phenotypic variability to 18p- syndrome, leading to difficulties in establishing the contribution of each abnormality to the phenotype. Although the phenotype of 18p- syndrome is not as typical as for other syndromes, HPE microform and Turner stigmata associated with characteristic posture and marked slowness in motion and action is very suggestive for this syndrome. Microarray analysis of our patient allowed us to define precise molecular characterization of the translocation breakpoints and to uncover two unsuspected cryptic abnormalities, improving genotype-phenotype correlations and management.

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Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES

1. de Grouchy J, Lamy M, Theffry S, Arthuis M, Salmon C. Dysmorphic complexe avec oligophrenie: deletion des bras courts d'un chromosome 18. CR Acad Sci. 1963; 256: 1028-1029.
2. Turleau C. Monosomy 18p. Orphanet J Rare Dis. 2008; 3: 4.
3. Schinzel A. Catalogue of Unbalanced Chromosome Aberrations in Humans, 2nd ed. Berlin: Walter de Gruyter; 2001.
4. Wester U, Bondeson ML, Edeby C, Anneren G. Clinical and molecular characterization of indi-

viduals with 18p deletion: a genotype-phenotype correlation. Am J Med Genet A. 2006; 140(11): 1164-1171.

5. Sumner AT. A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res. 1972; 75(1): 304-306.
6. Lemyre E, der Kaloustian VM, Duncan AM. Stable non-Robertsonian dicentric chromosomes: four new cases and a review. J Med Genet. 2001; 38(1): 76-79.
7. Marical H, Le Bris MJ, Douet-Guilbert N, Parent P, Descourt JP, Morel F, *et al.* 18p Trisomy: a case of direct 18p duplication characterized by molecular cytogenetic analysis. Am J Med Genet A. 2007; 143A(18): 2192-2195.
8. Tsukahara M, Imaizumi K, Fujita K, Tateishi H, Uchida M. Familial Del(18p) syndrome. Am J Med Genet. 2001; 99(1): 67-69.
9. Digilio MC, Marino B, Giannotti A, Di Donato R, Dallapiccola B. Heterotaxy with left atrial isomerism in a patient with deletion 18p. Am J Med Genet. 2000; 94(3): 198-200.
10. Fernandez BA, Roberts W, Chung B, Weksberg R, Meyn S, Szatmari P, *et al.* Phenotypic spectrum associated with de novo and inherited deletions and duplications at 16p11.2 in individuals ascertained for diagnosis of autism spectrum disorder. J Med Genet. 2010; 47(3): 195-203.
11. Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, *et al.*; Autism Consortium. Association between microdeletion and microduplication at 16p11.2 and autism. N Engl J Med. 2008; 358(7): 667-675.
12. Kumar RA, Marshall CR, Badner JA, Babatz TD, Mukamel Z, Aldinger KA, *et al.* Association and mutation analyses of 16p11.2 autism candidate genes. PLoS One. 2009; 4(2): e4582.
13. McCarthy SE, Makarov V, Kirov G, Addington AM, McClellan J, Yoon S, *et al.* Microduplications of 16p11.2 are associated with schizophrenia. Nat Genet. 2009; 41(11): 1223-1227.
14. Shinawi M, Liu P, Kang SH, Shen J, Belmont JW, Scott DA, *et al.* Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioural problems, dysmorphism, epilepsy, and abnormal head size. J Med Genet. 2010; 47(5): 332-341.
15. Jacquemont S, Reymond A, Zufferey F, Harewood L, Walters RG, Kutalik Z, *et al.* Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus. Nature. 2011; 478(7367): 97-102.
16. Rosenfeld JA, Coe BP, Eichler EE, Cuckle H, Shaffer LG. Estimates of penetrance for recurrent pathogenic copy-number variations. Genet Med. 2013; 15(6): 478-481.
17. Schaaf CP, Goin-Kochel RP, Nowell KP, Hunter JV, Aleck KA, Cox S, *et al.* Expanding the clinical spectrum of the 16p11.2 chromosomal rearrangements: three patients with syringomyelia. Eur J Hum Genet. 2011; 19(2): 152-156.
18. Portnoi MF, Gruchy N, Marlin S, Finkel L, Denoyelle F, Dubourg C, *et al.* Midline defects in deletion 18p syndrome: clinical and molecular characterization of three patients. Clin Dysmorphol. 2007; 16(4): 247-252.
19. Brenk CH, Prott EC, Trost D, Hoischen A, Walldorf C, Radlwimmer B, *et al.* Towards mapping phenotypical traits in 18p-syndrome by array-based comparative genomic hybridisation and fluorescent in situ hybridisation. Eur J Hum Genet. 2007; 15(1): 35-44.
20. Ming JE, Muenke M. Multiple hits during early embryonic development: digenic diseases and holopros-encephaly. Am J Hum Genet. 2002; 71(5): 1017-1032.
21. Bendavid C, Rochard L, Dubourg C, Seguin J, Gicquel I, Pasquier L, *et al.* Array-CGH analysis indicates a high prevalence of genomic rearrangements in holopros-encephaly: an updated map of candidate loci. Hum Mutat. 2009; 30(8): 1175-1182.