INVERTED DUPLICATION DELETION OF 8P: CHARACTERIZATION BY STANDARD CYTOGENETIC AND SNP ARRAY ANALYSES

Adriana Sireteanu, Elena Braha, Roxana Popescu, Mihaela Gramescu, E.V. Gorduza, Cristina Rusu
University of Medicine and Pharmacy "Grigore T. Popa" - Iasi
Faculty of Medicine
Discipline of Medical Genetics

INVERTED DUPLICATION DELETION OF 8P: CHARACTERIZATION BY STANDARD CYTOGENETIC AND SNP ARRAY ANALYSES (Abstract): Inverted 8p duplication deletions are recurrent chromosomal rearrangements that most often arise through non-allelic homologous recombination (NAHR) during maternal meiosis between segmental duplications made up of the olfactory receptor (OR) gene clusters. The presence of a paracentric inversion polymorphism in 8p23.1, found in ~26% of European population, may trigger meiotic misalignment and NAHR between the OR gene repeats. We report clinical, cytogenetic, and molecular findings in a 4 year 8 month-old female with concomitant inverted duplication and terminal deletion of chromosome 8p. The girl, the first child of unrelated parents, was born at term, by normal delivery, after an uneventful pregnancy. Clinical examination revealed dysmorphic features, pectus excavatum, hypertonia, severe developmental delay. Brain ultrasound and MRI showed agenesis of the corpus callosum without other abnormalities. Conventional cytogenetic analysis identified additional material on chromosome 8 at band p21. SNP array analysis further characterized the abnormality as a duplication of about 31.3 Mb, from 8p23.1 to 8p11.1, and additionally revealed a terminal deletion of about 6.8 Mb, from 8p23.3 to 8p23.1. Genomic microarray also identified a region of disomy between deletion and duplication. Chromosome analysis of both parents revealed normal results. Based on clinical examination, conventional cytogenetics and SNP array, we established the diagnosis of inverted duplication deletion of 8p. SNP array analysis precisely defined the breakpoints of rearrangement and, by identifying a region of disomy between the duplication and deletion, indicated that NAHR between segmental duplications was the most likely mechanism for this type of abnormality. **Keywords**: INVERTED DUPLICATION DELETION OF 8P, SNP ARRAY, AGENESIS OF CORPUS CALLOSUM.

Inverted duplication deletion of 8p [inv dup del(8p)] is a recurrent chromosomal rearrangement first described in 1976 by Weleber et al. (1). It has an estimated prevalence of about 1:20,000 newborns (2), around 50 cases being reported worldwide.

Clinical manifestations include: intellectual disability (ID), agenesis of the corpus callosum, minor facial abnormalities, congenital heart defects, hypotonia with a tendency to develop progressive hypertonia and severe orthopedic problems (3, 4).

Inverted duplications with terminal deletions on 8p most often arise through NAHR during maternal meiosis between segmental duplications made up of OR

gene clusters (OR-REPD and OR-REPP). The presence of a paracentric inversion polymorphism in 8p23.1, found in ~26% of the European population, may trigger meiotic misalignment and NAHR between the inverted OR gene repeats (5).

We report the clinical, cytogenetic, and molecular findings in a girl with concomitant inverted duplication and terminal deletion of chromosome 8p and compare the phenotype of the patient with the cases reported in the literature, highlighting the importance of microarray technique in the identification of genomic disorders and their underlying mechanisms.

CASE PRESENTATION

The patient, a 4 year 8 month-old female, was the first child of non-consanguineous, healthy parents (mother - 31 years old, father - 37 years old at birth of the proband). There was no family history of ID, congenital anomalies or psychiatric disorders. Pregnancy was uneventful. She was born at term, by normal delivery, with a birth weight of 3,150 g, length of 50 cm and head circumference of 33 cm; Apgar score was 9 both at 1 and 5 minutes; physical examination revealed left parietal cephalohematoma and hypotonia. She presented neonatal seizures and cyanotic crises. Echocardiography showed a patent foramen ovale. Abdominal ultrasound, routine biochemical and hematological tests, as well as ophthalmologic examination and TORCH test were normal. No hearing impairment was identified. Brain ultrasound and MRI revealed agenesis of the corpus callosum without other abnormalities.

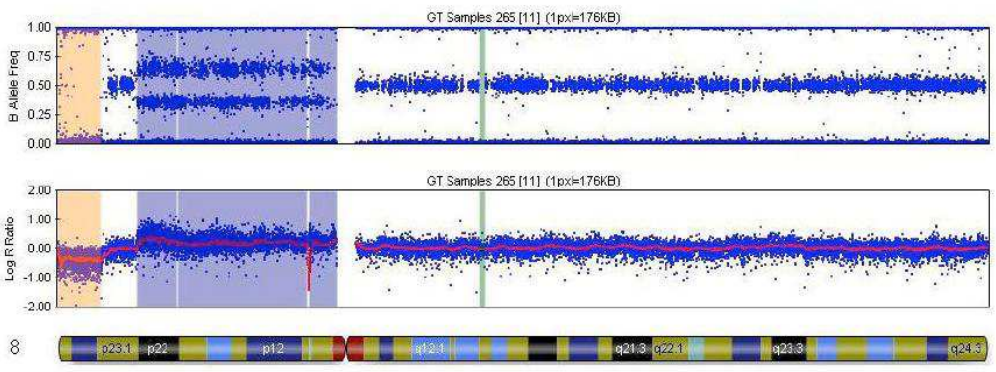

Fig. 1. SNP array results of chromosome 8 in the patient, showing a 6.8 Mb deletion in 8p23.3-p23.1 and a 31.3 Mb duplication in 8p23.1-p11.1

She achieved head control at 9 months and spoke her first word at the age of 12 months. Clinical examination at the age of 4 years and 8 months showed height of 106 cm (+0.43 SD), weight of 13.5 kg (-2.64 SD), and head circumference of 47 cm (-2.58 SD). She had facial dysmorphic features including square face, high forehead, hypoplastic zygomatic bones, large mouth, thin upper lip and everted lower lip, high palate and abnormal dental development, large ears, and dry, curly hair that receded from the temples. Other features were a short neck, pectus excavatum, slender arms and legs with long, tapering fingers, joint contractures in the legs and hips. She showed severe developmental and speech delay: she could not sit alone and she only said few single words.

Standard G banding chromosomal analysis identified an additional material on chromosome 8 at band p21: 46,XX,add(8)(p23.1). Parental karyotypes were normal. A SNP array was performed in University Medical Center Groningen using HumanCytoSNP-12 v 2.1 BeadChip platform (Illumina Inc., San Diego, CA) containing approximately 300.000 SNPs per sample. The data were processed using Genome Studio V 2010.1 software (Illumina). Genomic positions were defined using GRCh37/hg19. SNP array analysis detected a terminal deletion of approximately 6.8 Mb, from 8p23.3 to 8p23.1 (52,041-6,928,066), and a duplication of approximately 31.3 Mb, from 8p23.1 to 8p11.1 (12,599,114-43,951,038) (fig. 1). The deleted and duplicated regions contained 15 and 144 OMIM genes, respectively.

Based on clinical examination, conventional cytogenetics and SNP array, we established the diagnosis of inverted duplication deletion of 8p.

DISCUSSION

Our patient is severely affected compared with patients reported in the literature. The concomitant presence of deletion and duplication makes genotype/phenotype correlations difficult. Considering the size of the duplicated region and the large number of OMIM genes involved, the phenotypic findings in our patient are mainly due to duplication.

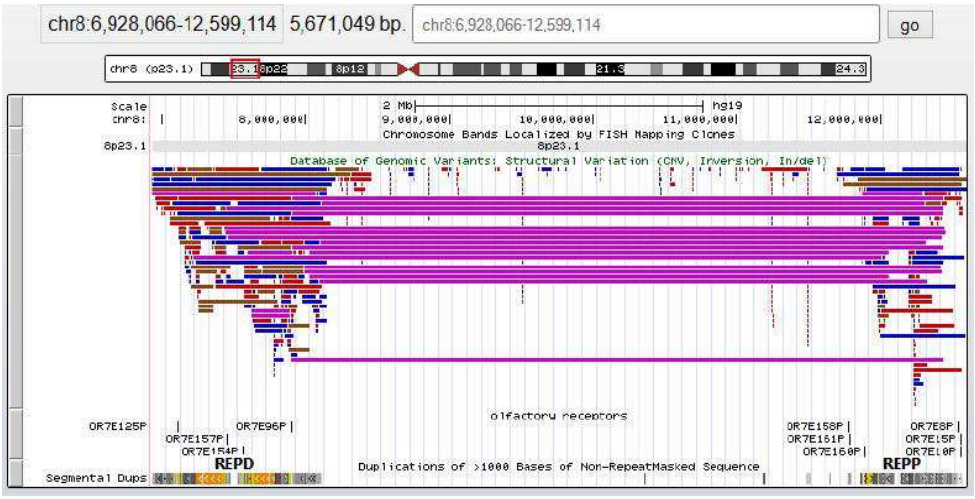

Fig. 2. Annotated screenshot of 5.6 Mb of band 8p23.1 (UCSC Genome Browser on Human Feb. 2009 Assembly (hg19), http://genome.ucsc.edu/). From bottom to top:
- segmental duplications that contain the OR repeats (labelled REPD and REPP);
- olfactory receptor genes based on The Human Olfactory Data Explorer (HORDE);
- multiple copy number variations of REPD and REPP in the Database of Genome Variants (sideways) and common polymorphic inversion between REPD and REPP (middle lines)

Three mechanisms may explain the formation of this abnormality: recombination within a paracentric inversion, recombination between inverted low copy repeats, or U-type exchange following a double strand break. All three mechanisms involve the formation of a dicentric chromosome 8 which subsequently breaks ei-

ther during a meiotic division or during early stages of embryonic development to produce an inv dup del(8p). Observation of a region of disomy between duplication and deletion can distinguish the U-type exchange from the other two mechanisms. The inv dup del(8p) in our patient can be explained by the second mentioned mechanism because the duplication was separated from the 8pter deletion by a ~5.6 Mb single copy region flanked by OR-REPD and OR-REPP regions (fig. 2). Stabilization of the broken chromosome ends can be achieved by: direct addition of telomeric repeats (''telomere healing'') (6); ''telomere capture'', in which broken chromosomes obtain the telomeric end of another chromosome (7); or by formation of a ring chromosome (8). Using high-resolution microarray no additional duplications could be identified, therefore in our patient the broken chromosome end of 8p has been stabilized by telomere healing.

The rearrangement is de novo as both parents had normal karyotypes. Dual colored FISH can be performed in the parents to investigate whether the meiotic recombination event among the OR gene clusters could have been stimulated by the presence of an inversion heterozygosity at 8p23.1. Even if inv dup del(8p) is a recombinant product of a parental 8p23.1 heterozygous inversion, there is no recurrence risk for this rearrangement and no invasive prenatal investigations are indicated for the next pregnancy.

CONCLUSIONS

In conclusion, microarray analysis permited precise molecular characterization of the rearrangement and indicated that NAHR between segmental duplications is the most likely mechanism for this abnormality. Also, it indicated that the terminal deletion was stabilized by telomere healing.

REFERENCES

1. Weleber RG, Verma RS, Kimberling WJ, Fieger HG, Lubs HA. Duplication-deficiency of the short arm of chromosome 8 following artificial insemination. Ann Genet 1976; 19(4): 241-247.
2. Floridia G, Piantanida M, Minelli A et al. The same molecular mechanism at the maternal meiosis I produces mono- and dicentric 8p duplications. Am J Hum Genet 1996; 58(4): 785-796.
3. de Die-Smulders CE, Engelen JJ, Schrander-Stumpel CT et al. Inversion duplication of the short arm of chromosome 8: clinical data on seven patients and review of the literature. Am J Med Genet 1995; 20; 59(3): 369-374.
4. Fryns JP. 8p inverted duplication/deletion syndrome. [updated 2013 Jan; cited 2013 May]. Available from: http://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=en&Expert=96092.
5. Giglio S, Broman KW, Matsumoto N et al. Olfactory receptor-gene clusters, genomic-inversion polymorphisms, and common chromosome rearrangements. Am J Hum Genet 2001; 68(4): 874-883.
6. Wilkie AO, Lamb J, Harris PC, Finney RD, Higgs DR. A truncated human chromosome 16 associated with alpha thalassaemia is stabilized by addition of telomeric repeat (TTAGGG)n. Nature 1990; 346(6287):868-871.
7. Ballif BC, Gajecka M, Shaffer LG. Monosomy 1p36 breakpoints indicate repetitive DNA sequence elements may be involved in generating and/or stabilizing some terminal deletions. Chromosome Res 2004; 12(2): 133-141.
8. Knijnenburg J, van Haeringen A, Hansson KB et al. Ring chromosome formation as a novel escape mechanism in patients with inverted duplication and terminal deletion. Eur J Hum Genet 2007; 15(5): 548-555.