7q Deletion/12q Duplication Is the Possible Cause of an Alobar Holoprosencephaly Case

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Established Facts

- Holoprosencephaly (HPE) is a multifactorial developmental disorder of the forebrain affecting 1 in 250 gestations.
- The disorder can be divided into genetic and nongenetic factors, with brain and face malformations ranging from very mild to severe.

Novel Insights

- A pregnancy affected by alobar HPE is described, probably due to a 7q deletion and a 12q duplication.
- Five genes (*REPIN1* or *AP4*, *CDK5*, *DPP6*, *EN2*, and *ANKLE2*) are linked to alobar HPE.
- We hypothesize that disruption of key elements of the developing brain, taking environmental factors into account, contributes to the HPE phenotypic spectrum.

Keywords

Array CGH · Deletion 7q · Duplication 12q · Holoprosencephaly

Abstract

Holoprosencephaly (HPE) spectrum disorder is the most common congenital malformation of the human brain with absence of or incomplete midline cleavage. Its cause is heterogeneous, making genetic counseling a challenge. In this case report, a pregnancy affected by alobar HPE is described. Using aCGH, an 8.9-Mb deletion at 7q36.1q36.3 together

with a 4.9-Mb duplication at 12q24.32q24.33 is assumed to be the possible reason for this alobar HPE case. It is discussed that disruption of key elements of the developing brain, taking environmental factors into account, contributes to the HPE spectrum. The use of aCGH for invasive prenatal testing is starting to become the standard technique, providing accurate information about the cause of congenital diseases for couples receiving genetic counseling.

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Holoprosencephaly (HPE) is the most common congenital malformation of the brain in humans, affecting 1 out of 250 gestations and only 1 out of 8,000 live births [Lacbawan et al., 2009]. The midline cleavage of the developing brain during the third and fourth weeks of gestation is either absent or incomplete, and it is accompanied by abnormal craniofacial features such as midline facial clefting or hypotelorism. The spectrum of severity, defined by the extent of brain malformation, is described as alobar, semilobar, and lobar forms [Corsello et al., 1990]. The complete spectrum of HPE includes middle interhemispheric variants, septopreoptic HPE, and microforms characterized by midline defects such as single maxillary median incisor or hypotelorism without brain malformations typical of HPE [Lazaro et al., 2004].

The high phenotypic variability of HPE is characterized by heterogenic etiology. The causes of HPE can be divided into genetic and nongenetic. The nongenetic causes are smoking, alcohol consumption, gestational diabetes, or transplacental infections [Croen et al., 2000]. The genetic causes are chromosome abnormalities such as trisomy 13, 18, and triploidy. Further, 4 genes responsible for HPE have been characterized (*SHH*, *ZIC2*, *SIX3*, and *TGIF*), while another 5 genes (*PTCH1*, *GLI2*, *DISP1*, *NODAL*, *FOXH*) and 4 chromosomal loci (21q22.3, 2q37.1q37.3, 14q13, 1q41q42) seem to play a minor role in the occurrence of HPE [reviewed in Roessler and Muenke, 2010]. Pathogenic mutations in an HPE-associated gene or locus can also be consistent with a normal face and brain, which can be attributed to incomplete penetrance [Pineda-Alvarez et al., 2010]. Combinations of mutations in major and/or minor HPE genes lead to the occurrence of HPE and may account for the variability of this complex disease [Ming and Muenke, 2002]; thus, genetic counseling remains a challenging issue for families affected by HPE.

In this case report, a pregnancy affected by alobar HPE is described. Using aCGH, an 8.9-Mb deletion at 7q36.1q36.3 together with a 4.9-Mb duplication at 12q24.32q24.33, due to paternal translocation, is assumed

to be the possible cause of alobar HPE. The couple also have a daughter with severe intellectual disability, while most of the daughter's cousins from the father's side have abnormal phenotypes. aCGH technique has an incremental yield over conventional cytogenetics of 0.9–1.6%, which has been mostly highlighted in cases of fetuses with structural defects [Papoulidis et al., 2015]. The use of aCGH was crucial for this case to delineate the exact genotype of the fetus and to coordinate genetic counseling.

Clinical Report

The 23-year-old female patient with a clear family medical history was referred to our unit for a programmed noninvasive first-trimester combined screening test. The proposita reported a previous obstetrical history of a daughter with severe intellectual disability without anatomical defects and a first-trimester termination pregnancy for HPE. The patient, originating from Albania, had a BMI of 22. She was a nonsmoker, and she was not taking any systemic medication. Our sonographic findings of absent cerebral hemispheres and fused thalami were compatible with the diagnosis of alobar HPE (data not shown). Thus, chorionic villus sampling was performed, and the sample was analyzed with conventional karyotyping and aCGH.

Materials and Methods

Karyotyping was carried out on chorionic villus sample according to standard procedures (Giemsa staining, G-banding), while the conventional karyotyping of parental peripheral blood samples were carried out after PHA stimulation and thymidine treatment.

High-resolution molecular karyotyping was performed with an aCGH platform of 60,000 oligonucleotides (Agilent technologies). Briefly, DNA was extracted from the chorionic villus sample with Promega Maxwell 16, and it was hybridized with the human reference DNA of the same gender (Promega Biotech). The statistical test used as a parameter to estimate the number of copies was ADM-2 (provided by the DNA analytics software, Agilent Technologies) with a window of 0.5 Mb and a threshold of 6. Only those copy number changes that affected at least 5 consecutive probes with identically oriented change were considered as copy number variations. Consequently, for most of the genome, the average resolution of this analysis was 200 kb.

Mutational analysis of the *SHH*, *ZIC2*, *SIX3*, and *TGIF* genes was done after PCR amplification of the coding regions and the immediate flanking intron-exon boundaries of these genes. The amplicons were sequenced using an ABI 3100 genetic analyzer.

Results

Although there were no clinically significant mutations found in the *SHH*, *ZIC2*, *SIX3*, and *TGIF* genes after mutational analysis, aCGH analysis revealed a fetus with an 8.9-Mb deficit at the chromosomal region

Fig. 1. aCGH results showing the duplicated region (A) and the deleted region (B).

7q36.1q36.3 (chr7:150,027,807–158,909,738; hg19) including 51 genes of the OMIM database, and a 4.9-Mb duplication at the chromosomal region 12q24.32q24.33 (chr12:128,854,408–133,767,986; hg19) including 25 genes (Fig. 1). After genetic counseling, pregnancy was terminated and parental karyotyping was performed in order to interpret these chromosomal defects. The results showed that the father is a carrier of a chromosome 7/12 translocation (Fig. 2). A more detailed medical history revealed that both father's sisters had children with intellectual disability (Fig. 3). The parents decided to perform assisted reproduction with preimplantation genetic diagnosis (PGD) and intracytoplasmic sperm injection (ICSI) after considering the higher family risk.

Discussion

The development of the human forebrain is controlled by a delicate balance of multiple signaling pathways, such as hedgehog signals, fibroblast growth factors, bone mor-

phogenetic proteins, retinoic acid, and canonical and noncanonical Wnt signals [reviewed in Roessler and Muenke, 2010]. Focusing on the function of only one gene in order to understand the development of the human forebrain and the HPE spectrum disorder would be much too simple [Roessler and Muenke, 2010]. Therefore, we suggest disruption of key elements of the above-mentioned pathways as responsible, reflecting the HPE spectrum.

SHH haploinsufficiency may be one of the key elements that points to the “alobar” aspect of the HPE spectrum, taking environmental factors into account. The *SHH* gene, being involved in the current case, is a regulator that activates the *SHH*-*PTCH*-*GLI* pathway and plays an important role in brain development [reviewed in Vilavicencio et al., 2000]. In the most recent study, which describes the mutations of the *SHH* gene, it has been stated that these mutations are associated with the microform of HPE, though a proportion of probands were diagnosed with semilobar or alobar HPE [Mercier et al., 2011]. Sporadic environmental factors such as alcohol

Fig. 2. Paternal karyotype. The black arrows show the translocation between chromosomes 7 and 12.

Fig. 3. Pedigree of the family. I-5 and I-7 were aborted family members. II-1, II-5, and II-6 were all examined by a genetic counselor. III-1 is intellectually disabled. III-2 terminated pregnancy at 12.5 weeks. III-3 is 10 years old. She does not walk or talk and has no visual contact. She also has major hearing loss. III-4 was a stillbirth. III-5 is 5 years old. He walks, but he does not talk, and he has unilateral microphthalmia. III-6 had holoprosencephaly.

consumption during the third and fourth weeks of gestation may contribute to the “shift” from the microform to semilobar and alobar form.

In the present case, there is an 8.9-Mb deletion at 7q36.1q36.3 and a 4.9-Mb duplication at 12q24.32q24.33. It is assumed that when a 7q deletion is accompanied by partial trisomy of another chromosome, the result is a more severe cerebral phenotype, due to extra gene dosage [Ayub et al., 2016]. Furthermore, there are 4 genes (*REPIN1* or *AP4*, *CDK5*, *DPP6*, and *EN2*) at the deleted and 1 gene (*ANKLE2*) at the duplicated region, which may

play a role in brain development. The *AP4* protein is active in nonneural cells and has a decreased expression in Down syndrome fetal cortex compared to a normal one [Kim et al., 2006]. Furthermore, it is a transcriptional repressor of *PAHX-API*, a neuronal gene that is developmentally upregulated in the adult mouse brain [Kim et al., 2006]. Therefore, *AP4* may contribute to brain development by determining the cell fate of nonneural tissues next to the brain. A *CDK5* mutation is cosegregated with autosomal recessive lissencephaly accompanied by cerebellar hypoplasia in a highly consanguineous Israeli Mos-

lem family [Magen et al., 2015]. The loss-of-function variations in *DPP6* are associated with autosomal dominant microcephaly and intellectual disability. It is found that mice with a knockdown of transcript 1 of *Dpp6* had smaller brains than their wildtype littermates. *DPP6* appears to play a major role in the regulation of proliferation and migration of neurons in neurogenesis, most likely by participating in neuronal electrical excitability, synaptic integration, and plasticity [Liao et al., 2013]. Furthermore, human genetic and mouse behavioral analyses suggest that engrailed-2 (*EN2*) contributes to neurodevelopmental disorders; it is shown that the forebrain regions of *En2*-knockout mice exhibited reduced growth [Genestine et al., 2015]. Yamamoto et al. [2014], combining *Drosophila* and human mutant screen datasets, found that variants in *ANKLE2* may be the cause of an apparent recessive microcephaly. The human data identified variants of the *ANKLE2* in a family with this disease. Their child has an extreme small head circumference, and brain MRI in the newborn period demonstrated a low forehead, several scalp rugae, and mildly enlarged extra-axial space with communication between the posterior lateral ventricles and the mesial extra-axial space. Other brain abnormalities included a simplified gyral pattern, mildly thickened cortex, small frontal horns of the lateral ventricles with mildly enlarged posterior horns of the lateral ventricles, and agenesis of the corpus callosum. Analysis of the brain from *Drosophila Ankle2* mutants showed a decreased number of neuroblasts, decreased mitosis and cell proliferation, and increased apoptosis [Yamamoto et al., 2014]. Altogether these data lead to the assumption that not only sporadic environmental factors, but also haploinsufficiency of *SHH* and genes controlling brain development may contribute to more severe phenotypes of HPE.

It has been suggested that duplication of the 12q terminal region is a clinically recognizable syndrome [McCorquodale et al., 1986]. Although it is difficult to delineate the phenotype of 12q duplication syndrome, in most cases, craniofacial anomalies, occasional brain malformations and heart defects, cryptorchidism, psychomotor delay, and intellectual disability are evident symptoms [Bouman et al., 2013]. The difficulty in diagnosis lies in the fact that usually molecular techniques are not employed; only a few cases with pure 12q duplication have been described [reviewed in Cappellacci et al., 2006]. Moreover, other chromosomal abnormalities have been detected with 12q duplication [reviewed in Ireland et al., 2004; Sathanoori et al., 2007], and some of these patients exhibit features seen in Noonan syndrome because

PTPN11 is included in the duplicated region [Shchelochkov et al., 2008; Chen et al., 2014; Geckinli et al., 2015].

This is the first reported case with alobar HPE and 7q deletion/12q duplication. This is in agreement with the observation that severe forms of HPE are more frequently found in terminal 7q deletion patients associated with another partial trisomy than in those with pure 7q deletion [Lukusa et al., 2005]. Although the most severe forms of HPE cases involve 7q deletion and 3p duplication [Chen et al., 1999], others are accompanied by 2q, 2p, 8p, 18q, 1q, and 22q duplication [reviewed in Ayub et al., 2016]. There are at least 3 exceptional cases of patients without HPE, despite being carriers of a 7q deletion and other chromosome duplications [Kuller et al., 1992; Ginocchio et al., 2008; Ayub et al., 2016]. This could be explained by the fact that none of the genes involved in brain development are present in the duplicated region. The present case describes a fetus with alobar HPE probably due to the *ANKLE2* duplication in 12q, which is implicated to be involved in brain development [Yamamoto et al., 2014].

Nowadays, aCGH plays an important role in routine prenatal genetic testing, allowing the detection of submicroscopic deletions and/or duplications correlating to the patient's phenotype manifestation. This provides more information about congenital diseases for affected couples receiving genetic counseling. HPE is a congenital disease with incomplete penetrance and variable expressivity; therefore, couples with a medical history of HPE should be advised by specialized physicians before and after having an aCGH analysis.

Acknowledgment

The authors would like to thank the family for their collaboration.

Statement of Ethics

The study was approved by the Ethics Committee of "P&A Kyriakou" Children's Hospital, Athens, Greece, and performed with respect to the ethical standards of the Declaration of Helsinki, as revised in 2008.

Disclosure Statement

The authors declare no conflicts of interest.

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