CONGENITAL HYDROCEPHALUS AND HEMIVERTEbrae ASSOCIATED WITH DE NOVO PARTIAL MONOSOMY 6q (6q25.3→qter)

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ABSTRACT

This study was conducted to describe a prenatal case of congenital hydrocephalus and hemivertebrae with a 6q terminal deletion and to investigate the possible correlation between the genotype and phenotype of the proband. We performed an array-based comparative genomic hybridization (aCGH) analysis on a fetus diagnosed with congenital hydrocephalus and hemivertebrae. The deletion, spanning 10.06 Mb from 6q25.3 to 6qter, was detected in this fetus. The results of aCGH, karyotype and fluorescent in situ hybridization (FISH) analyses in the healthy parents were normal, which confirmed that the proband’s copy-number variant (CNV) was de novo. This deleted region encompassed 97 genes, including 28 OMIM genes. We discussed four genes (TBP, PSMB1, QKI and Pacrg) that may be responsible for hydrocephalus while the T gene may have a role in hemivertebra. We speculate that five genes in the 6q terminal deletion region were potentially associated with hemivertebrae and hydrocephalus in the proband.

Keywords: Genotype; Hemivertebrae; Hydrocephalus; Phenotype; 6q Terminal deletion.

INTRODUCTION

Isolated 6q subtelomeric deletions are relatively rare, and few correlative studies have been reported [1-4]. The most common clinical features include mental retardation, developmental delay, dysmorphic features, hypotonia, microcephaly, facial dysmorphism, seizures, cardiac defects and brain anomalies, such as abnormal corpus callosum and hydrocephalus [1,3,5]. Patients with pure 6q terminal deletions usually present multiple anomalies and seldom present less than three malformations. Hydrocephalus associated with a chromosome 6q terminal deletion has been reported in several postnatal and prenatal cases [5]. However, the relationship between the involved region and the genes associated with hydrocephalus is still not well understood.

Studies on hemivertebra due to microdeletions of distal chromosomal regions of 6q are scarce. This malformation often emerges simultaneously with various anomalies of the nervous system, musculoskeletal system, genitourinary tract, cardiac system and gastrointestinal tract, but no report discussing the possible genetic etiology has been published [6-10].
Here we report on a 24-week-old fetus with congenital hydrocephalus and hemivertebrae diagnosed by prenatal ultrasonography with the molecular genetic finding of a 6q terminal deletion. The case described here is rare in that the proband only exhibited two of the deformities that have previously been reported to be associated with terminal 6q deletions [2]. We investigated the genotype-phenotype correlation and sought to identify the relevant region of the 6q terminus and the associated genes that may be responsible for these clinical features.

MATERIALS AND METHODS

A 30-year-old gravida 5 para 0 woman was referred at 24 weeks’ gestation for a routine prenatal examination in the middle trimester of pregnancy. She had experienced four first-trimester spontaneous miscarriages for no known reason. The pregnancy was unremarkable. Both the gravida and her husband were in good health. The ultrasonographic anatomical scan of the fetus identified a single live fetus with hydrocephalus of the bilateral ventricles and lumbar hemivertebrae at L3 (Figure 1A and 1B). Though Figure 1A seems likely to be considered as the holoprosencephaly, we can identify an intact falx cerebri, distinct and separate ventricles, and cannot find the absence of the midline echo and fusion of the thalami that is a feature of holoprosencephaly from the sonogram of this fetus. Thus, this is a typical hydrocephalus. No other abnormalities were detected in the ultrasound screening. Screening for fetal Down syndrome with maternal serum demonstrated a low risk. The couple was counseled about the genetic risk and elected to undergo amniocentesis for molecular cytogenetics diagnosis.

Genomic DNA was extracted from uncultured amniotic fluid (AF) and parental peripheral blood using the DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany). Genomic copy-number variants (CNVs) were detected using Fetal DNA Chip (Agilent Technologies, Inc., Santa Clara, CA, USA), a targeted high-resolution 44K oligonucleotide array specifically constructed for prenatal screening with the intention of targeting common trisomic aneuploidies and most known microdeletion and micro-duplication syndromes. The Fetal DNA Chip included telomeric and pericentromeric regions, covering the genome at a resolution of 100 kb (http://www.fetalmedicine.hk/en/Fetal DNA Chip.asp). This chip is specifically designed to evaluate over 100 known genomic disorders in the fetus (http://www.fetalmedicine.hk/en/Fetal DNA chip/Appendix I.pdf) with most of the known common non pathogenic CNV regions removed. The quality of the array was analyzed using DNA Analytics software version 4.0.81 (Agilent Technologies), and cases where the derivative log ratio spread of the array was >0.25 were excluded from further data analysis. Any CNVs that were detected in our cohort were then checked with the Database of Genomic Variants to exclude known non pathogenic chromosome CNVs [11].

The coordinates of CNVs detected in the specimens are based on the Human February 2009 (hg19) assembly of the International Human Genome Sequencing Consortium. We searched for similar CNVs in the DGV (Database of Genomic Variants: http://projects.tcag.ca/variation/cgi-bin/gbrowse/hg19), DECIPHER (https://decipher.sanger.ac.uk), ISCA (https://www.iscaconsortium.org/isca/ucsc/hg19),
OMIM (http://omim.org) and PubMed (http://www.ncbi.nlm.nih.gov/pmc/) to determine if the CNVs were pathogenic and to investigate the relationship between the CNVs and clinical characteristics.

Genomic copy number variations detected by array-based comparative genomic hybridization (aCGH) were confirmed by conventional karyotyping analysis and fluorescence in situ hybridization (FISH). G-banding chromosome analysis at a 500-band resolution was performed on the cultured amniocytes and parental peripheral blood lymphocytes following the laboratory’s standard protocols. Thirty metaphases were analyzed. The FISH analysis was performed on cultured amniocytes and lymphocyte metaphase preparations using Rp11-196G15 (Empire Genomics, Buffalo, NY, USA) for the 6p22.3 area (spectrum red) and Rp11-37D8 (spectrum green) for the 6q27 deletion area, according to the standard FISH protocol.

RESULTS

The aCGH revealed a deletion in chromosome region 6q25.3→qter with a size of 10.04 Mb (the first and last affected probe at positions 160,857,810 and 170,893,070) in the fetus (Figure 2). The deleted 6q25.3→qter region contains 97 genes, including 28 OMIM genes. The karyotype of the cultured amnio-
The karyotype of the fetus revealed a de novo partial monosomy 6q (Figure 3). Metaphase FISH analysis on cultured amniocytes revealed an absence of a signal for the 6q subtelomeric probe (Figure 4). The G-banding chromosome analysis and FISH confirmed the results of aCGH. There were no abnormal findings by aCGH, karyotyping or FISH testing in the healthy parents, and thus, the aberration was confirmed to be de novo.

**DISCUSSION**

Hemivertebrae is a rare congenital spinal malformation in which only one side of the vertebral body develops, leading to a secondary deformation of the spine, such as scoliosis or kyphosis [7,10]. The condition is found in five to ten per 10,000 births, occurring more commonly in girls [9]. Hemivertebrae may exist alone, but more commonly, hemivertebrae occur with multiple congenital abnormalities, such as skeletal anomalies of the spine, ribs, and limbs; diastematomyelia; cardiac system and genitourinary tract anomalies; and central nervous system (CNS), deformities, either in prenatally or postnatally diagnosed cases [6-10]. Notably, hemivertebrae may be involved in several genetic syndromes, including Jarcho-Levin syndrome, Klippel-Feil syndrome and VATER syndrome (vertebral anomalies, imperforate anus, tracheoesophageal fistula, and renal anomalies) [6,12]. The etiology of hemivertebrae is not clear because it usually occurs sporadically; thus the likelihood of genetic involvement has been considered low. Several chromosome deletions associated with hemivertebrae have previously been reported in earlier studies. These deletions include del(1q4), del(3p2), monosomy 4p, interstitial 5q-deletion, r(15) and interstitial deletion of 17p [13]. However, more recent studies have shown that the correlation between hemivertebrae and chromosomal abnormalities is small. Fetal karyotypes are usually normal whether the hemivertebrae are isolated or non-isolated, although non-isolated hemivertebrae may be associated with an increased risk of aneuploidy [7,12]. Therefore, the complexity and uncertainty of the cause of hemivertebrae contribute to the difficulty of interpreting the genotype-phenotype correlations in our case.

With regard to hemivertebrae associated with 6q terminal deletion, hemivertebrae generally emerges as a deformity accompanying major anomalies associated with 6q subtelomeric deletions. Nevertheless, the 6q terminal region has never been considered as a candidate chromosomal region that could be responsible for hemivertebrae, and the underlying genotype-phenotype correlations require further investigation. The T (also known as Brachyury) gene that maps to 6q27 encodes a transcription factor that is essential for normal mesodermal development and the formation and differentiation of the notochord in all vertebrates. The notochord controls cell migration and differentiation in those tissues that are most often involved in sacral agenesis, the neural tube, and the sclerotomal cells that form the vertebrae. In mice,
mutations of the \( T \) gene lead to the lack of expression of the product and have been recognized as the cause of dominant Brachury and death \textit{in utero}, with an abnormal notochord and absent somites. The \( T \) gene has been proposed as a candidate gene for sacral agenesis in humans. Furthermore, a previous study of congenital vertebral malformations (CVMs) indicated that missense mutations in the \( T \) gene might result in the pathogenesis of human CVMs as one of the genetic components [14]. The CVMs are a class of disorders composed of hemivertebrae, vertebral bars, supernumerary vertebrae, and butterfly- and wedge-shaped vertebrae. Although there is no evidence addressing the direct correlation between the \( T \) gene and hemivertebrae, taken together, it is possible that copy number loss resulting from haploinsufficiency of this gene might have substantially contributed to the hemivertebrae malformation observed in our case.

Congenital hydrocephalus can occur as an isolated abnormality or in combination with many genetic syndromes, referred to as multiple congenital anomalies (MCAs), in various body systems [15,16]. The main clinical sign in most fetuses with congenital hydrocephalus is cerebral ventriculomegaly [16], which is a pathological dilatation of the cerebral ventricular system and might be a consequence of obstruction of the flow of cerebral spinal fluid (CSF), hypersecretion, defective filtration or a developmental anomaly of the intracranial architecture [5].

To date, the specific causes of congenital hydrocephalus in the majority of cases remain under investigation. Garne \textit{et al.} [17] recently demonstrated that 87 fetuses and infants with congenital hydrocephalus exhibited a low rate of karyotype abnormalities (9.0%). However, evidence from previous studies suggests that the genetic etiology probably plays an important role in congenital hydrocephalus. Indeed, approximately 40.0% of cases of congenital hydrocephalus may be attributable to genetic factors, including cytogenetic abnormalities, monogenic or complicated inherited conditions and multifactorial disorders [17], although non syndromic hydrocephalus appears to be less related to these conditions. Based on the literature, except for the most common cytogenetic abnormalities such as aneuploid and multiploid karyotypes, submicroscopic chromosomal aberrations, also known as genomic CNVs, represent an important genetic cause in a growing number of cases. The variant regions in most chromosomes have been previously described. In particular, 6p terminal deletions have been rather frequently reported to be associated with congenital hydrocephalus. However, most previous reports were based on syndromic hydrocephalus (≥1 major and >2 minor anomalies), and non syndromic (no major and ≤2 minor anomalies) cases have rarely been described. In addition, few studies have elucidated the molecular basis of the disease phenotype or the relationship between the CNVs and phenotypic characteristics. In the present study, the proband exhibited features that are known to be associated with the terminal 6q deletion phenotype. This fetus presented bilateral hydrocephalus/ventriculomegaly and a lumbar hemivertebrae but lacked the typical phenotypic features of subtelomeric 6q deletion, such as developmental delay, corpus callosum anomalies, microcephaly, cleft palate and hyperactivity [2,3,5], which was considered unusual for such a large deletion, spanning 10.04 Mb. Until now, very few cases of prenatal ventriculomegaly due to submicroscopic terminal 6q deletions have been reported, and in those cases, the extent of the deletion with respect to non syndromic ventriculomegaly was less than 5 Mb [5,18]. For postnatal cases, Lee \textit{et al.} [3] made a comprehensive summary of 28 patients diagnosed postnatally with subtelomeric 6q deletions of ≤11 Mb and idiopathic intellectual disability, developmental delay and/or dysmorphic features. These authors reported that smaller 6q terminal deletions tended to cause milder anomalies. Accordingly, we speculated that the size of the deletion appears to be correlated with the clinical complexity of the phenotype. Thus, the fetus in our study should have more severe malformations and more types of abnormalities because the deleted region held more functional genes that could contribute to the clinical manifestation. However, it is more likely that several specific genes located within the deleted segment play a role in the genesis of hydrocephalus.

The \( TBP \) gene is most frequently mentioned in relation to the 6q terminal deletion phenotype [3,5,18-20]. This gene encodes a TATA-binding protein, which is a subunit of the RNA polymerase II transcription factor that affects the initiation of transcription. It has been found to be highly expressed in the cerebral cortex, the frontal, parietal and occipital lobes and the caudate nucleus, and it has an important role in CNS morphogenesis [19,20]. Deletion of \( TBP \) may affect the elaboration of cortical neurons, and
cortical maldevelopment could contribute to mental retardation and seizures, which are commonly seen in patients with 6q terminal deletions [20]. Moreover, dynamic mutations that expand the CAG trinucleotide repeat in the TBP gene have been identified to cause spinocerebellar ataxia 17, a neurodegenerative disorder [3]. However, there is no definite proof to verify the correlation between the TBP gene and hydrocephalus. Nonetheless, because a portion of the cases of congenital hydrocephalus result from brain deformity, we hypothesize that haploinsufficiency of TBP, which might cause cortical dysplasia, could be responsible for congenital hydrocephalus.

The PSMB1 gene is a multicatalytic protease complex with a highly ordered ring-shaped 20S core structure that is situated next to the TBP gene both in humans and mice. The PSMB1 gene is tightly associated with TBP as a functional unit in both species. On this basis, PSMB1 and TBP appear to have similar genetic, functional and pathological features [20]. Thus, we suggest that PSMB1 and TBP may be additional candidate genes for the congenital hydrocephalus phenotype associated with the terminal 6q deletion.

Recently, the Quaking (QKI) gene that maps to 6q26 has been proposed to be associated with the clinical phenotype of the 6q terminal deletion [3,21]. In humans, Aberg et al. [22] demonstrated that down-regulation of the QKI gene might cause a decline in the mRNA levels of the myelin-related genes (PLP1, MAG, and TF) that are involved in oligodendrocyte differentiation and maturation in 55 schizophrenic patients when compared with the controls, thus indicating that QKI may play a role in myelin and oligodendrocyte dysfunction in schizophrenia. In addition, Backx et al. [21] reported the disruption of the QKI gene in association with a de novo balanced translocation resulting in a clinical phenotype similar to the common subtelomeric 6q deletion syndrome, though without seizures and brain anomalies. This result suggests that haploinsufficiency of the QKI gene underlies a substantial part of the 6q subtelomeric deletion phenotype.

Deletion of QKI leads to dysmyelination defects, and deletion of PARK2 co-regulated (Pacrg) contributes to mild hydrocephalus [23]. Furthermore, the communicating hydrocephalus phenotype can be rescued by the transgenic expression of Pacrg in the qk− mutant. Considering that the structure and function of ciliary systems is highly conserved between humans and mice, we suggest that haploinsufficiency of the human Pacrg gene located at 6q26 is responsible for the congenital hydrocephalus phenotype.

A recent article reported that the smallest region of overlap spans 1.7 Mb in 6q27 and contained DLL1, THBS2, PHF10, and C6orf70 (ERMARD), which are plausible candidates for the causation of structural brain abnormalities [24]. DLL1 is expressed in the paraxial mesoderm, which is correlated with somito genesis in the nervous system [25]. PHF10 encoding a zing finger domain protein is essential for self-renewal of the multipotent neural stem cells and neuronal differentiation [24]. But the relationship between these candidate genes and CNS abnormalities is not sure, and the underlying pathogenicity is not known. Maybe the molecular basis of these genes for the disorder will be studied in the future. So here we hypothesize the genes to be candidate genes.

A recent article reported seven patients and reviewed 14 patients in previous literature [24]. Here we review 6q terminal deletion patients reported with hydrocephalus and hemivertebrae present in DECIPHER (Figure 5). From this figure, we can find the patients who have the same phenotype as ours. Moreover, their genotype in the 6q terminal deletion is partially or totally similar to ours. So these can nicely illustrate the deletion region that is responsible for our patient’s phenotype.

In summary, we present a prenatal diagnosis of a de novo partial monosomy 6q (6q25.3→qter) by aCGH using uncultured amniocytes from a fetus with congenital hydrocephalus and hemivertebrae. To the best of our knowledge, this report describes the largest detection of submicroscopic 6q terminal deletions because of an atypical prenatal finding of ventriculomegaly and hemivertebrae. We performed a detailed investigation of the genotype-phenotype correlations of the plausible candidate genes TBP, PSMB1, QKI, Pacrg, and T with hydrocephalus and hemivertebrae. Further investigation is required to clarify the genetic mechanisms of the genes responsible for the pheno-type effects.

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