Split hand/foot malformation with long-bone deficiency and *BHLHA9* duplication: A prenatal diagnosis report

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Distal limb deformities are congenital malformations with phenotypic variability and high genetic heterogeneity. Split hand/foot malformation, also known as ectrodactyly, is a congenital limb malformation characterized by a defect of the central rays of the hands and/or feet. Split hand/foot malformation with long-bone deficiency (SHFLD) is a rare condition related to a 17p13.3 duplication. Recently, genomic duplications encompassing *BHLHA9* have been associated with SHFLD. We report a case of SHFLD presenting with campomelia of the right femur, bilateral agenesis of fibulae, bilateral club feet, and oligosyndactyly of the hands and feet, that was associated with a 17p13.3 duplication, as determined prenatally using array comparative genomic hybridization.

Key words: Split-hand-foot malformation with long bone deficiency, 17p13.3, *BHLHA9*, Comparative genomic hybridization.

Introduction

Split hand/foot malformation (SHFM) is a rare limb deformity with a prevalence of 1 in 8,500–25,000 live births that is caused by abnormal developmental signaling during embryogenesis [1]. SHFM with long-bone deficiency (SHFLD) is characterized by SHFM with the addition of long-bone deficiency usually affecting the tibia [2]. Fibular aplasia with SHFM is a rare disorder that shares several characteristics with the better known tibial aplasia-SHFM syndrome [3]. SHFLD is inherited as an autosomal-dominant trait with variable expression patterns and reduced penetrance [4]. The association of a major locus on 17p13.3 was identified via linkage analysis of a large family with SHFLD [5,6]. The minimal 17p13.3 critical region encompasses a single gene, *BHLHA9*. Genomic duplication of *BHLHA9* is associated with

SHFLD [2]. In this study, we report a case of SHFLD associated with a 17p13.3 duplication within *BHLHA9* that was prenatally diagnosed using array comparative genomic hybridization (array CGH).

Case

1. Case description

A 23-year-old nulliparous woman was referred for fetal level II sonography owing to abnormal ultrasound findings, including skeletal anomalies and oligohydramnios at 20 weeks of gestation. Detailed evaluation of the fetus was difficult due to the absence of amniotic fluid. Thus, we infused normal saline into the amniotic cavity after amniocentesis for fetal karyotyping and further genetic examination. Ultrasonography

Received: 26 November 2015, Revised: 7 December 2015, Accepted: 8 December 2015, Published: 31 December 2015

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Conflict of interest: The authors declare that they do not have any conflicts of interest.

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revealed a normally grown fetus; biometric data of the head and abdomen were compatible with the age of gestation. Visualization of the upper limbs showed malformations with oligosyndactyly of the bilateral hands. Examination of the lower limbs demonstrated abnormal posture of flexion contractures of the knees, agenesis of fibulae, club-foot appearance, and oligosyndactyly (Fig. 1). On the following day, we carried out an ultrasonographic evaluation for fetal well-being, and the scanning view revealed severe oligohydramnios. After extensive parental counseling, the decision was made to terminate the pregnancy. The findings on the postmortem external examination and X-rays were similar to the prenatal findings on ultrasonography (Figs. 2 and 3). Neither she nor her husband showed skeletal abnormalities, nor did any family member. We recommended the patient for parental cytogenetic evaluations; however, she refused further examination.

2. Cytogenetic and CGH analysis

We performed ultrasonography-guided amniocentesis. The amniotic fluid sample was processed and analyzed according to a standardized protocol. Cultivation of the amniotic fluid sample for chromosomal analysis was performed according to the method for long-term cell cultures, in which the 'flask' technique was used. Commercial medium was used for amniotic fluid cultivation (Amniomed, Euroclone, Milan, Italy; Amniomax, GIBCO-Invitrogen, Carlsbad, CA, USA). Conventional GTG-banding analysis was performed on cultured amniocytes. All 20 metaphase chromosomes from the amniotic fluid sample indicated that the fetus had normal chromosomes. We reported the fetal karyotype as 46,XY.

The genomic DNAs for array CGH analysis were extracted from amniotic fluid. The CGH procedure was performed as described by Kallioniemi et al. [7] and analyzed by the Cytoscan 750 K Array (Affymetrix, Santa Clara, CA, USA). The CGH profile

Fig. 1. High-resolution ultrasonography. (A) Right femoral campomelia. (B) Bilateral fibular agenesis. (C) Persistent flexion of the knees; club feet with oligodactyly. (D) Oligosyndactyly of the hands.

Fig. 2. Postmortem external examination. (A) Micrognathia. (B) Oligosyndactyly, right hand. (C) Oligosyndactyly, left hand. (D) External genitalia of the male, contracture of both knees, club feet, oligodactyly of both feet, and rocker-bottom feet.

Fig. 3. Postmortem simple X-ray examination. (A) Whole-body features. (B) Oligosyndactyly, right hand. (C) Oligosyndactyly, left hand. (D) Right femoral campomelia, bilateral fibular agenesis, contracture of both knees, club feet, and oligodactyly of both feet.

Fig. 4. Comparative genomic hybridization analysis. A 211-kb gain of signal on 17p13.3 associated with *ABR*, *BHLHA9*, *TUSC5*, *YWHAE*, and *CRK* genes.

showed prominent amplification of the signal in the region of 17p13.3. We identified a duplication of 211 kb on chromosome 17p13.3 (Fig. 4). The critical genes in this region, *ABR*, *BHLHA9*, *TUSC5*, *YWHAE*, and *CRK*, were reported. In particular, *BHLHA9* is associated with SHFLD, and *YWHAE* is associated with developmental delay. The other genes did not present clear functions.

Discussion

Limb development during embryogenesis is a highly complex process. Deformations of the distal limbs are congenital abnormalities associated with variable phenotypes and high genetic heterogeneity. SHFM, also known as ectrodactyly, is a limb abnormality characterized by a congenital defect involving the central rays of hands and/or feet that is caused by failure to preserve the central region of the apical ectodermal ridge in the

developing autopod [1,8]. SHFM is considered to originate from abnormal developmental signals during limb morphogenesis. The non-syndromic form of SHFM can be isolated (type I) or associated with long bone deficiency (type II, SHFLD), which may also present with other manifestations [1].

The known SHFM loci are associated with chromosomal rearrangements, including small duplications or deletions, of the human genome. These include *SHFM1*, which is associated with mutations to the *DLX5/6* gene on chromosome 7q21 [9], *SHFM2* on Xq26 [1], *SHFM3* associated with heterozygous duplications on 10q24, the *SHFM4* mutation to the TP63 locus on 3q27, *SHFM5* on 2q31, and the *SHFM6* mutation to the *WNT10B* locus on 12q13 [1]. *SHFM1* is associated with hearing loss in 35% of patients, while SHFM with deafness is a unique clinical condition, referred to as SHFM1D, which is very complex because of the variable clinical manifestations, irregular patterns of genetic inheritance, and unique heterogeneous genetic and molecular alterations among affected individuals [1,10].

SHFM is related to other limb abnormalities, as observed in SHFLD, which commonly involves the tibia, and in Gollop-Wolfgang complex, which is characterized by bifurcation of the femur [1,7,11]. Fibular agenesis with SHFM has been explained as a discrete clinical entity characterized by failure of fibular development and central clefts of the hands and/or feet [12]. Fibular aplasia with SHFM is a rare disorder that shares several characteristics with the better known tibial agenesis-SHFM syndrome [3].

SHFLD is a genetically heterogeneous disorder with variable clinical manifestations within the same family and differing degrees of penetrance among individuals [13]. Most often, the inheritance of SHFLD is autosomal dominant with reduced penetrance [2]; however, autosomal recessive inheritance has been confirmed in some cases [14] and digenic inheritance is possible [15].

At least three loci, namely SHFLD1, SHFLD2, and SHFLD3, are known to be associated with SHFLD, although specific genetic causes have not yet been identified. In a retrospective study of a large consanguineous Arabic family, both 1q42.2-q43 and 6q14.1, designated as loci SHFLD1 and SHFLD2, were found to be associated with SHFLD [15,16]. Moreover, SHFLD3 was mapped to an 861-kb interval at 17p13.1-17p13.3 in a Brazilian family [5]. Although no causative mutation is currently known, screening for copy number variations revealed diverse genomic micro-duplications on 17p13.3 [2].

The smallest area of duplication overlap markedly narrowed the critical area for the SHFLD phenotype and includes only one

complete gene (*BHLHA9*), along with a portion of a second gene (*ABR*) and an assumed processed transcript [1]. *BHLHA9* was recently proposed as a major candidate gene in developmental limb abnormalities. *BHLHA9* duplications have been identified in clinically normal family members as well as patients with SHFM and SHFLD [11]. Variable phenotypic expression is observed in patients with SHFLD harboring a 17p13.3 duplication. The most common site of long bone involvement in 17p duplication carriers is reportedly the tibia [2]. Among these carriers, male-specific bias has been associated with increased susceptibility and the clinical manifestations of males appear to be more severe than those of females [2]. However, no sex-specific bias was observed in another study [14]. Although the reason for this sex-specific bias remains unknown, the presence of intra-individual variability among affected and unaffected carriers may be due to unidentified genetic factors. Hormone-responsive promoters/enhancers or the presence of a modifier on the X chromosome together with 17p duplication are important factors in the expression and development of SHFLD [1,2].

Once fetal structural anomalies are identified by ultrasonography, prenatal chromosomal microarray analysis is useful to identify major chromosomal aneuploidy as well as submicroscopic abnormalities that could go undetected by conventional karyotyping [17]. Although the use of chromosomal microarray analysis in prenatal diagnosis remains controversial, array CGH is a useful genetic testing method for the detection of these changes as well as other unbalanced chromosomal aberrations, such as submicroscopic rearrangements affecting different foci [18]. Chromosomal microarray analysis, in addition to the ability to detect almost all abnormalities that are identifiable by fetal karyotyping, allows clinicians to identify specific genetic diseases, such as SHFM [17]. In a prenatal setting, aCGH is informative and beneficial in most cases [18]. However, the genetic and clinical heterogeneity of SHFM render genetic testing and counseling especially challenging [1]. Informed consent and genetic counseling should be addressed before the patients undergo testing.

In this report, we identified a 17p13.3 duplication containing *BHLHA9* in a patient with ectrodactyly that was inherited from an unaffected parent.

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