Case Report

Prenatal Diagnosis of Xq26.1-q26.3 Duplication in Two Fetuses of a Woman with Gonadal Mosaicism

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We report prenatal diagnosis of two affected males with an Xq26.1-q26.3 duplication from a mother with gonadal mosaicism. The first affected pregnancy was referred by ultrasound observed intrauterine growth retardation and abnormal cerebellar anatomy. Chromosome analysis on cultured amniocytes found a normal male karyotype but array comparative genomic hybridization (aCGH) detected a 4.808 Mb duplication at Xq26.1-q26.3. The proband was born at 35 weeks gestation. At age of three years, he presented with growth retardation, scoliosis, short stature and micropenis. Follow up aCGH analysis on a peripheral blood sample from the mother found a normal result. The second pregnancy was referred due to advanced maternal age and previous history of an affected child. Prenatal chromosome analysis on chorionic villus sampling found a normal male karyotype and aCGH detected the same Xq26.1-q26.3 duplication. The recurrence of this duplication indicated the presence of gonadal mosaicism in the mother. Of the 28 refseq genes within the duplicated region, mutations in genes AIFM1, IGSF1, FRMD7, GPC3, PHF6 and HPRT1 are known to be associated with syndromic phenotypes in the Online Mendelian Inheritance in Man (OMIM). It was reported that deletions of the GPC3 gene in males causes Simpson-Golabi-Behmel syndrome featuring an overgrowth phenotype and a deletion involving IGSF1 results in gonadal enlargement. The duplication of these two genes in our cases is associated with growth retardation and micropenis.

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INTRODUCTION

Cytogenetic analysis on amniotic fluid and chorionic villus sampling (CVS) has been the gold standard for prenatal diagnosis of chromosomal abnormalities. However, given the limited resolution of G-banding analysis, most of currently known microdeletion/duplication syndromes cannot be prenatally diagnosed by the conventional cytogenetic technology. Recently, the integration of array comparative genomic hybridization (aCGH) analysis into prenatal diagnosis has significantly improved the analytical capacity for delineating genomic imbalances resulting from structural chromosomal abnormalities and detecting submicroscopic abnormalities.[1] It is estimated that approximately 10% of pregnancies with ultrasound-detected fetal anomalies and normal cytogenetic findings may have submicroscopic genomic abnormalities.[2] Since 2009, aCGH analysis has been an integral part of prenatal genetic evaluation in Yale's Clinical Cytogenetics and Genomics Laboratory.[3-4] Genomic DNA samples extracted from cultured amniocytes, CVS and peripheral blood were used for aCGH or aCGH/SNP using Agilent Human Genome CGH microarray 180K kit. This aCGH procedure can achieve 99% sensitivity and 99% specificity using a sliding window of five to seven contiguous oligonucleotides, indicating an analytical resolution of 100–150 Kb covering the whole human genome.[5] In this report, we present prenatal and postnatal findings in a male with a submicroscopic duplication at Xq26.1-q26.3. Follow up aCGH analysis on the mother showed absence of this duplication. However, the recurrence of the same duplication in the second pregnancy indicated the presence of gonadal mosaicism in the mother. This case demonstrated the necessity and capability of aCGH analysis in prenatal diagnosis of submicroscopic abnormalities and its impact on follow up family study and genetic counseling.

CASE REPORT

The woman was at age 33 with her first pregnancy conceived by in vitro fertilization with intra-cytoplasmic sperm injection (IVF/ICSI) due to blocked fallopian tube. Ultrasound examination at 18 weeks gestation noted intrauterine growth retardation, lagging long bones and keyhole cerebellum. Amniocentesis was conducted. Chromosome analysis was performed on cultured amniocytes and aCGH analysis on DNA extracted from cultured amniocytes was performed as previously described.[3-4] Routine chromosome analysis showed a normal male karyotype while oligonucleotide aCGH analysis using SurePrint G3 Human CGH 4x180K Oligo Microarray Kit (Agilent Technologies Inc, Santa Clara, CA) detected a male

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genome with a 4.808 Mb duplication at Xq26.1-q26.3 (chrX:129,262,608-134,070,544) (**Figure 1B**). The base pair designation was described according to the UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) (http://genome.ucsc.edu/). Re-examination of G-band pattern on chromosome X implied an enlarged light-stained region of Xq26.1-q26.3. The proband was born at 35 weeks gestation. At age three years, he presented with scoliosis, short stature, and micropenis. His height was that of an average one-year-old and his weight was that of an average 5½-month-old. A high caloric diet was commenced. He displayed apparently normal cognition.

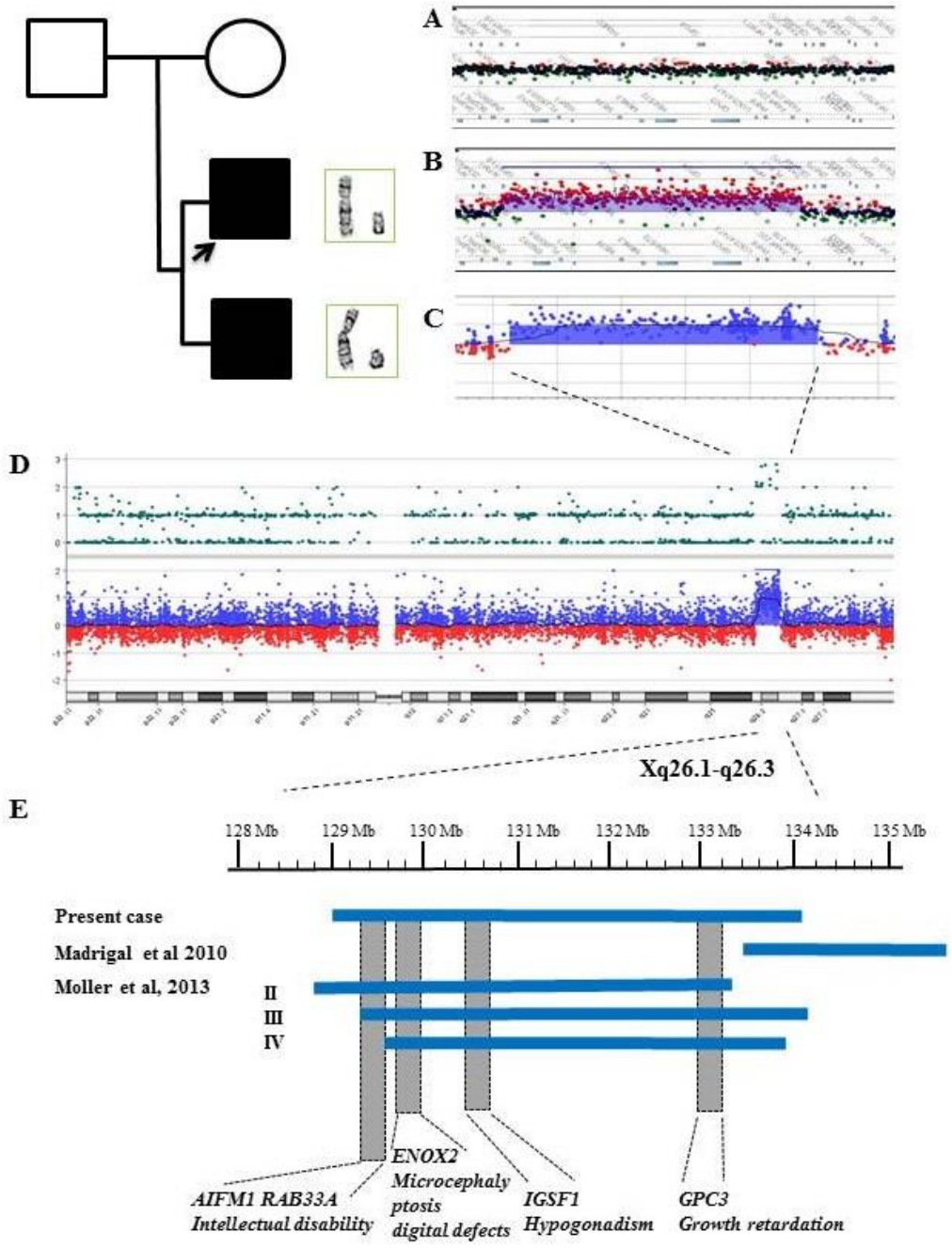

Figure 1. Cytogenomic findings in the family with the mother and two affected fetuses. The gene views from the aCGH analysis show a normal pattern in the mother (A) and identical Xq26.1-q26.3 duplications in the proband (B) (arrow in the pedigree) and second fetus (C). A chromosome view of aCGH/SNP result from the second fetus observed single copy and monoallelic pattern for the chromosome X except the homozygous biallelic pattern in the duplication region (bottom panel) (D). For Agilent RsaI/AluI SNP calling, line 0 represents uncut homozygous alleles, line 1 represents uncut/cut heterozygous alleles, line 2 represents cut homozygous alleles, and line 3 represents duplicated copy of cut homozygous alleles. The XY sex chromosome images for the two fetuses are included. A genomic map shows critical intervals and candidate genes for syndromic phenotypes (E).

Follow up aCGH analysis performed on the peripheral blood sample from the pregnant woman revealed a normal female pattern (**Figure 1A**). She was recommended to undertake maternal serum AFP screening at 16 weeks gestation and a sonogram at 18 weeks gestation for any subsequent pregnancies. There is no developmental delay, thyroid problems or diabetes in family history. A maternal first cousin has history of seizures and the patient's father has factor V Leiden.

The patient returned at age 37 with her second pregnancy also conceived through IVF/ICSI. She was referred due to advanced maternal age and history of an affected child. Prenatal chromosome and aCGH analyses were performed on chorionic villus sampling. Routine chromosome analysis showed a normal male karyotype while aCGH using SurePrint G3 ISCA CGH+SNP 4x180K Microarray Kit (Agilent Technologies Inc, Santa Clara, CA) revealed a male genome with a 4.808 Mb duplication at Xq26.1-q26.3 which is the same variant as observed in her first child (**Figure 1C**, **Figure 1D**). The homozygous biallelic SNP pattern in the duplication region indicated an intrachromosomal tandem duplication (**Figure 1D**). The recurrence of this abnormality was most likely due to mosaicism in the mother's gonads.

DISCUSSION

Intrachromosome Xq duplications in males have been reported as functional disomy by the presentation of abnormal phenotype associated with over-expression of duplicated genes.[6-8] Most female carriers of Xq duplications will exhibit normal or very mild phenotypes due to skewed inactivation of the abnormal X chromosome, however, functional mosaicism of Xq duplication presenting abnormal phenotypes by skewed inactivation of the normal X chromosome has also been reported in females.[9-10] X chromosome inactivation is a random process on a cell-by-cell basis, resulting in half of the cells with one X activated and the other half of cells with the other X activated. Skewing and extreme skewing are arbitrarily defined as greater than or equal to 75% and 95% of cells expressing the same X chromosome, respectively.[8] The duplication in our cases includes 28 refseq genes from *AIFM1* to *MOSPD1,* mutations in genes *AIFM1*, *IGSF1*, *FRMD7*, *GPC3*, *PHF6* and *HPRT1* have been associated with syndromic phenotypes in Online Mendelian Inheritance in Man (OMIM) (**Supplemental Table 1**). Detailed genomic information and predicted clinical outcome were presented in a post-testing genetic counseling and the parents decided to continue the pregnancy. Duplications at Xq25-q26 similar to our case have been reported in three families (II, III and IV) and a syndromic phenotype of growth retardation, congenital ptosis, microcephaly, cleft palate, digital and genital abnormalities and feeding difficulties in childhood was noted.[10] All affected males inherited the Xq25-q26 duplication from a carrier mother and extreme skewed inactivation of the duplicated X chromosome was noted in all three mothers. Genes found in the duplicated region of our patient and the above three families include *AIFM1, RAB33A, FAM45B, ENOX2, IGSF1* and *GPC3*. A 2.8 Mb microduplication at Xq26.2-q26.3 was reported in two brothers with severe mental retardation, hypotonia, growth delay, craniofacial disproportion and dental malocclusion. The carrier mother showed a complete skewed X-inactivation on the duplicated X chromosome.[11] Our case and reported cases with genomic coordinates defined by aCGH analysis provided a genomic map of critical intervals with potential candidate genes within the Xq26.1-26.3 region (**Figure 1E**).[10-11] Intellectual disability is likely linked to *AIFM1* and *RAB33A* due to the fact that the coded proteins are highly expressed in the brain. *ENOX2* encodes for a growth-related cell surface protein that is essential in the growth of early embryos. Mutations in this gene have been associated to microcephaly, ptosis and digital defects. A deletion involving *IGSF1* results in gonadal enlargement in males. Conversely, hypogonadism may be caused by overexpression or duplication of this gene. *GPC3* encodes for a protein that plays a role in cell division and growth regulation. Loss of function mutations and deletions in this gene cause a gigantism syndrome known as Simpson-Golabi-Behmel syndrome. A duplication of this gene may predict an opposite effect, as shown by our case who presents with intrauterine growth retardation, and delayed postnatal growth. A study has shown that *GPC3* transgenic mice with induced overexpression are significantly smaller at birth than their wild type littermates, and their growth remained delayed throughout postnatal development.[10] The opposite phenotypes observed for the deletion and duplication of the *GPC3* and *IGSF1* genes may be explained by nullisomic loss of function and disomic over expression mechanisms for the X chromosome. Additional clinical cases and further function study are needed for this X-linked condition.

Gonadal mosaicism is an interesting finding in our case which raises questions on the incidence and mechanisms of somatic mosaicism for submicroscopic abnormalities. Chromosomal mosaicism, caused by mitotic non-disjunction or anaphase lag, is prevalent through human pre-and post-implantation development and can be detected in cytogenetic analysis of miscarriage and stillbirths.[12] Depending on the time that an imbalance occurred, a mosaicism can have varying levels of severity. If a non-disjunction or anaphase lag event has occurred early in embryogenesis, the mosaic cell lines can be found globally throughout the body. On the other hand, if the event occurred later in embryogenesis then the mosaic cell lines would only be seen in certain tissue types. This phenomenon is referred to as confined mosaicism or tissue specific mosaicism. It is estimated that confined placental mosaicism exists in roughly 6% of all pregnancies.[13] The application of aCGH analysis has also detected mosaicism of chromosomal and submicroscopic abnormalities in periperphal blood samples.[14-15] Somatic and gonadal mosaicism of a 539 Kb duplication at 10q24.32 was detected in a female mother following aCGH analysis of the duplication in two affected children.[16] In the case of our patient, gonadal mosaicism was indicated by recurrence of the Xq duplication. The difficulty in accessing gonadal tissue hindered further investigation of this mosaic pattern. The occurrence of an Xq duplication is likely resulting from a replication error in the gonadal tissue. Currently there are only a few cases for tissue-specific mosaicism of submicroscopic copy number aberrations. The underlying

mechanisms of somatic/tissue-specific mosaicisms for gene abnormalities will be investigated as more cases being accumulated.

In summary, this case demonstrated that aCGH analysis is imperative for prenatal diagnosis of submicroscopic abnormalities. The 4.808 Mb duplication of Xq26.1-q26.3 is in the light-stained G-band region; therefore the slightly enlarged G-band pattern cannot be detected by routine chromosome analysis. Initially, the normal aCGH result on the mother's peripheral blood concluded a de novo duplication in the first child but the unexpected recurrence in the second child indicated the presence of gonadal mosaicism in the mother, although low-level tissue specific mosaicism cannot be ruled out. Therefore, the possibility of gonadal mosaicism should be taken into consideration when interpreting normal results of follow up parental study on peripheral blood samples. Furthermore, genomic mapping of critical intervals within the Xq25-q26 region and identification of opposite phenotypes from nullisomic loss of function and disomic over-expression could define the candidate genes for syndromic phenotypes.

CONFLICT OF INTEREST

None.

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