Fetal Akinesia Deformation Sequence (FADS) with Compound Heterozygous Variants in MuSK: a Case Report

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Abstract

Fetal Akinesia Deformation Sequence (FADS) is a hereditary disease with a very low incidence, which mainly involves the abnormal development of the musculoskeletal system. we detected a novel compound heterozygous mutation in MuSK related to FADS, which may help to provide more information to the affected couples

1. Introduction

Fetal akinesia deformation sequence syndrome (FADS)(OMIM 208150) with alternative nomenclature include multiple congenital contractures(MCC)and arthrogryposis multiplex congenital(AMC), is a recessive single gene disease with genetic heterogeneity and was reported by Pena and Shokeir in 1974 for the first time[Pena & Shokeir, 1974]. The affected fetus appears a series of abnormal ultrasonographic manifestations, including reduced fetal motion (fetal akinesia), intrauterine growth restriction, joint flexion, and other deformities such as cystic edema disease, pulmonary hypoplasia, cleft palate, and cryptorchidism, which may be caused by exogenous (extra-fetal) or endogenous (fetal) disease. Extrinsic etiologiesof FADS include uterine abnormalities, a maternal disease or multifetal pregnancy, and the inherent aetiology mainly is from the genetic factor. For FADS, the genetic factor is primarily caused by genetic variation associated with the neuromuscular junction (NMJ). It has been suggested FADS most often inherited as an autosomal recessive trait[Prontera et al.,2006], and X-linked or dominant inheritance [Tolmie et al.,1987; Mckeown,&Harris, 1988]. Several genes involved in the NMJhave been previously reported associated with FADS, such as *DOK7* (OMIM 610285) [Vogt et al.,2009], *RAPSN* (OMIM601592)[Vogt et al.,2008], *MuSK*(OMIM601296)[Wilbe et al.,2015] and *AGRN*(OMIM103320) [Geremek et al.,2020].

Since two homozygous and a compound heterozygous mutations in *MuSK* (Muscle-specific tyrosine kinase receptor) have been reported to cause FADS: a homozygous mutation c.40dupA[Wilbe et al.,2015], a missense variant c.1724T4C; p. (Ile575Thr) [Tan-Sindhunata et al.,2015] and a compound heterozygous c.220C>T and c.421delC [Li et al.,2019], in this report, by using the whole exon sequencing upon the latest affected fetus and the couples from a family with thrice unfavourable pregnancy, we detected a novel compound heterozygous mutation in *MuSK* that caused a series clinical features of FADS in a fetus.

2. Medical Record

A 30-year-old Chinese pregnant woman, gravida 3, para 0, abortus 2, came to our centre for consultation due to twice adverse birth histories at the 23rd gestational week. The first pregnancy was in 2015, without any fetal movement at 24th gestational weeks yet. A detailed ultrasound examination revealed the fetal joint

contracture, stomach unvisualized, fetal hydrops and left equinovarus. Karyotype analysis was performed on amniotic fluid cells, with a negative result. The second pregnancy was in 2017, ultrasound examination of the fetus at 20th week of gestation showed that the fetal stomach unvisualized, akinesia, pulmonary hypoplasia, double equinovarus and heart shifting to the left. Both of the last two pregnancies were terminated.

A detailed ultrasound examination was performed for this pregnancy, and the results were shown as follow:lack of limb movements, leftpulmonary hypoplasia, right pleural effusion, atrial septal defects, small stomach, subcutaneousoedema, clenched fingers, all limbs contracture, and polyhydramnios (Figure 1). Then Amniocentesis was subjected to chromosome karyotype analysis and chromosomal microarray analysis, and the results were negative. Though, the couples chose to terminate the pregnancy, because of the fetus showing multiple abnormalities with poor prognosis. Furthermore, we carried out the whole exon sequencing on the family trio (maternal blood, paternal blood and fetal musculus gastrocnemius) (Figure 2).

Personal and family history

The pregnant woman and her husband were healthy. They are not inbred, but they were from the same geographical area and couldn't be ruled out to be a common ancestor.

3. Methods

3.1 Sample collection

Blood samples from the couples and the gastrocnemius muscle from the fetus corpse were collected, and genomic DNA was extracted from these tissue using the QIAamp DNA Blood Mini kit (Qiagen, 51106) following the manufacturer's protocol. Informed consent was obtained from the couples.

3.2 Next-generation sequence

The genomic DNA (5μg) was randomly fragmented and purified by magnetic beads.DNA fragments were ligated with adaptor and captured by oligo probes from the IDT XGen Exome Research Panel (IDT, Lowa,USA) with targeting 19,396 genes approximately. The captured DNA libraries were subjected to NovaSeq6000 sequencer for sequencing according to the manufacturer's instructions (Illumina, San Diego, USA). All reads were mappedagainstthe hg19 from UCSC database by Burrows-Wheeler Aligner (BWA, v0.5.9-r16) [Li, & Durbin, 2010]. Follow by data annotation, variants were called by PriVar toolkit [Lu et al.,2013], then the clinical significance of the variants were annotated[Yang et al.,2013]. The candidate variants were verified using PCR amplification, and the products were sequenced using 3500XL Genetic Analyzer (Applied Biosystems, Foster City, USA) according to the manufacturer's instructions.

3.3PCR and Sanger sequencing

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The specific amplification primers of c.790C>T (Foward primer: TCCTCATTAACAAGTCATCGG, Reverse primer: GTGAACCGAGATCATGCC) and c.296G>T (Foward primer: TCCTCATTAACAAGTCATCGG, Reverse primer: GTGAACCGAGATCATGCC) were designed using Primer Premier v5.0.

The target segments were amplified applying 2X PCR MasterMix kits (Tiangen Biotech) on ABI9700 PCR instrument (Life technology, USA), and then sequenced on ABI3500 Genetic Analyzer (Life technology, USA).

3.4 Mutation prediction.

Potential disease-causing was predicted using mutation prediction tool Mutation Taster (*http://www.mutationtaster.org/).*

3.5 Results

From the Sanger sequencing results, we revealed a novel compound heterozygous variant contained a nonsense mutation(c.790C > T:p.(Arg264*))and a missense mutation(c.296G > T:p.(Cys99Phe)), which was absent in relevant databases. Meanwhile, the pregnant woman carries a missense mutationc.296G >T in exon 3, and the husband carries a nonsense mutation c.790C > T in exon 7. With Mutation Taster, we found that the two mutations were nonsense-mediated mRNA decay and missense mutation respectively, and their corresponding protein might be affected.

4. Discussion

*MuSK*is located in Cytogenetic location:9q31.3, Genomic coordinates*(GRCh38):* chr9:110,668,188-110,806,632. A mutation in *MuSK* associated with autosomal recessive FADS was firstly reported in 2015, which presented a family trio in inheritance mode with an insertion mutation in exon 1 of *MuSK* . It resulted in prematurely terminate translation [Wilbe et al.,2015]. MuSK is a receptor tyrosine kinase expressed on the muscle cell membrane [DeChiara et al.,1996; Glass et al.,1996]. Acetylcholine(ACh) is a medium for signal transmission. The combination of ACh and acetylcholine receptors (AChR) can cause muscle contractions. MuSK is a component of the AChR pathway and a major regulator of neuromuscular connection formation and maintenance[Wilbe et al.,2015]. The mutation of *MuSK* gene can lead to the decreased expression of mRNA level and stability of Musk protein level. When AChR was decreased, it led to muscle contractility disorder. Up to December 31, 2019, four families with 24 patients with FADS had been reported carrying *MuSK* mutation(Table1). Two families carried homozygous mutations, and one carried a compound heterozygous mutation [Wilbe et al.,2015; Tan-Sindhunata et al.,2015; Li et al.,2019]. Of the 24 patients, 45.8% (11/24) selected termination of pregnancy, 4.1%(1/24) developed intrauterine fetal death, one was stillborn, and 45.8% (11/24) died after birth. The longest survival time was five days. No survival cases were reported. These pregnancy outcomes were consistent with previous studies. Wible et al. knocked out the Mouse *MuSK*gene and conducted animal experiments. They identified that loss of function mutations occur in *MuSK* is lethal. In this case, the nonsense mutation(NM-005592.3) c.790C > T is expected to cause the encoded protein to truncate and lose its normal function. The missense mutation (NM-005592.3) c.296G >T is expected to change the amino acid at position 99 of the encoded protein from Cys to Phe. Various bioinformatics tools predict this amino acid change with potential pathogenic effects. *MuSK* has an important biological function, and it is highly conserved across most species. MuSK protein contains IgG-like domains, extracellular domain,Frizzled-like cysteine-rich domain,atransmembrane domain and a cytoplasmic domain[Stiegler et al.,2009]. In this case, the two mutations located in Ig-like1/3 in an extracellular domain. The extracellular domain of MuSK plays an important role in AChR cluster, which can lead to muscle contractility disorder. Based on the fetus' ultrasound examination, we identified the two mutations are pathogenic variation. The fetal ultrasound manifestations of the previous twice pregnancies of this patient were very similar to this one, so we speculated that the previous twice fetuses perhaps carried the same pathogenic *MuSK*gene.

5. Conclusion

We described a novel compound heterozygous mutation in *MuSK* gene which can lead to FADS, and described the genotype-phenotype relationship between *MuSK* and FADS, and further expanded the genes associated with FADS. These would offer a new opportunity for prenatal genetic testing. Mutation of *MuSK*gene can cause FADS, which is related to acetylcholine receptor disruption and inherited by autosomal recessive genetic disease. Considering Karyotype analysis and CMA were unable to detect single base variation, whole exon sequencing could provide a new diagnostic method for this type of mutation. A prenatal investigation is essential to not only identify an underlying FADS but also provide information regarding its prognosis and inheritance. Preimplantation genetic diagnosis (PGD) or early prenatal diagnosis is recommended for the next pregnancy to the affected couples.

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Contributors

Every authors had contribution to this research. Zhihua Li planned and organized the study. Zhen Sui evaluated the clinical manifestations of the cases. Yinong Xie and Tingting Yuan performed ultrasonography of the fetus. Hongzhou Liu collated the experimental data. Lina Ding performed studies about molecular genetics, analyzed the data and wrote the manuscript. All authors read and agree to the final manuscript.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of the Third Affiliated Hospital of Guangzhou Medical University (Guangzhou, China). Obtain the patient's written informed consent.

Competing interests

None.

Reference

Pena, S. D.,&Shokeir, M.H.(1974). Syndrome of camptodactyly, multiple ankyloses, facial anomalies, and pulmonary hypoplasia: a lethal condition. Journal of Pediatrics, 85(3): 373-375.

Prontera, P., Sensi, A., Merlo, L., Garani, G., Cocchi, G., Calzolari E. (2006). Familial occurrence of multiple pterygium syndrome: expression in a heterozygote of the recessive form or variability of the dominant form? American Journal of Medical Genetics Part A, 140(20): 2227-2230.

Tolmie, J.L., Patrick, A., Yates, J. R. W. (1987). A lethal multiple pterygium syndrome with apparent x-linked recessive inheritance. American Journal of Medical Genetics, 27(4): 913–919.

Mckeown, CM., &Harris, R. (1988). An autosomal dominant multiple pterygium syndrome. Journal of Medical Genetics, 25(2): 96-103.

Vogt, J., Morgan, NV., Marton, T., Maxwell, S., Harrison, B.J,... Maher, E. R.(2009). Germline mutation in DOK7 associated with fetal akinesia deformation sequence. Journal of Medical Genetics, 46(5): 338-340. Vogt, J., Harrison, BJ., Spearman, H., Cossins, J., Vermeer, S., ... Maher, E.R. (2008).Mutation analysis of CHRNA1, CHRNB1, CHRND, and RAPSN genes in multiple pterygium syndrome/fetal akinesia patients.The American Journal of Human Genetics, 82(1): 222-227 Wilbe, M., Ekvall, S., Eurenius, K., Ericson, K., Ericson, K., ... Bondeson, M. (2015).Musk: a new target for lethal fetal akinesia deformation sequence (FADS). Journal of Medical Genetics, 52(3): 195-202. Geremek, M., Dudarewicz, L., Obersztyn, E., Baas, F.,Paczkowska, M.,... Nowakowska, B. (2020). Null variants in AGRN cause lethal fetal akinesia deformation sequence. Clinical Genettics, 97(4): 634-638 Tan-Sindhunata, M. B., Mathijssen, I. B., Smit, M., Vries, J., Voorn, J.,... Groffen, A. (2015).Identification of a Dutch founder mutation in MUSK causing fetal akinesia deformation sequence. European Journal of Human Genetic, 23(9): 1151-1157. Li, N., Qiao, C., Lv, Y., Yang, T., Liu, H., ... Liu, C. (2019). Compound heterozygous mutation of MUSK causing fetal akinesia deformation sequence syndrome: a case report. World Journal of Clinical Cases, 7(21): 655-3661. Li, H.,& Durbin, R. (2010). Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics, 26(5): 589-95. Lu, Z., Jing, Z., Jing, Y., Dingge, Y., Lung, L.Y., Wanling, Y. (2013). PriVar: a toolkit for prioritizing SNVs and indels from next-generation sequencing data. Bioinformatics, 29(1): 124-125. Yang, Y., Muzny, D.M., Reid, J. G., Bainbridge, M. N., Willis, A., ... Eng, C.M. (2013). Clinical whole-exome sequencing for the diagnosis of mendelian disorders. New England Journal of Medicine, 369(16):1502-1511. Stiegler, A. L., Burden, S. J., Hubbard, S. R. (2009). Crystal structure of the frizzled-like cysteine-rich domain of the receptor tyrosine kinase MuSK. Journal of Molecular Biology, 393(1): 1-9.

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Table1 : Clinical features of 24 fetuses related with fetal akinesia deformation sequence syndrome in the database. Present +; Absent**-**, Not assessed NA; TOP: Termination of pregnancy; IUFD: Intrauterine fetal death.

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	Sweden					Dutch														China		China			
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	>A	pA	pA	pA	pA	T>C	T>C		T>C	T>C	T>C	T>C	T>C	T>C		T>C	T>C		T>C		C>T			C > T;	
																					c.421d			296G	
	27W	30W	18W	18W	17W	41W	33+4W	34W	38+1W	38+4W	33+1W	35W	23W	37W	32+2W	22W	31W	35+6W	23W	25W	24+5W	26W	21W	25W	
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