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Case report

Novel *SASS6* compound heterozygous mutations in a Chinese family with primary autosomal recessive microcephaly

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ABSTRACT

Primary autosomal recessive microcephaly (MCPH) is a rare hereditary disease characterized by congenitally small with brain circumference of the head below 3 standard deviations (SD). By far, 18 MCPH genes have been reported to be associated with the disease. SASS6 gene functioned in assembly of centrioles that the majority of MCPH genes present at the centrosome. There was only research reporting a homozygous missense mutation in SASS6 gene detected in a consanguineous Pakistani family. By conducting Whole-exome sequencing (WES) and Sanger sequencing on the family trio, we identified two novel splice site mutations c.127-13A > G and c.1867 + 2T > A in compound heterozygous hereditary form in the *SASS6* gene. The two mutations were confirmed to alter mRNA splicing by RT-PCR assay. Our finding supported the role of *SASS6* in the pathogenesis of microcephaly, expanding mutation spectrums and contributing to understanding of molecular mechanisms of MCPH.

1. Introduction

Primary autosomal recessive microcephaly (MCPH; OMIM# 251200) is a rare hereditary disease characterized by congenitally small brain, resulting from inadequate prenatal production of neurons, which results in a occipitofrontal circumference of the head more than 3 standard deviations (SD) below the age, sex and ethnically matched mean [1,2]. The global incidence of this rare disease has been estimated to be 1/10,000–1/100,000 depending on ethnicity and rate of consanguinity [3,4].

By far, 18 MCPH genes have been reported to be, namely *Microcephalin, WDR62, CDK5RAP2, CASC5, ASPM, CENPJ, STIL, CEP135, CEP152, ZNF335, PHC1, CDK6, CENPE, SASS6, MFSD2A,ANKLE2, CIT* and *WDFY3* [5]. The encoding proteins of the vast majority of these genes are localized at the centrosome, acting as basal body and anchor for cilia or spindle pole during cell division, and playing a key role in the brain development [6]. Mutations of genes mentioned above were speculated to lead to malfunction of proteins thus perturbing neurogenesis and causing primary autosomal recessive microcephaly [2].

SASS6 (OMIM# 609321) encodes centriolar assembly protein HsSAS-6 which is critical for the formation of new centrioles during the cell cycle [7]. Because of the rare condition of the disease, the mutations were easily to be detected in endogamy areas. The only report of pathogenic variants in the *SASS6* gene in MCPH was a homozygous missense mutation found in a large consanguineous Pakistani family [8]. Here, we reported two *SASS6* splice-site mutations in compound heterozygous hereditary form in a Chinese non-consanguineous MCPH family.

2. Materials and methods

2.1. Study participants

A 25-year-old, gravida 2, para 0, woman received routine prenatal care until 24 weeks of gestation when the prenatal ultrasound was observed that her fetus had microcephaly (−4 SD). She underwent an amniocentesis for detailed genetic testing. The karyotype of the fetus revealed 46,XX, and the copy number variation sequencing (CNV-seq) did not find abnormity. The situation was unimproved indicated by prenatal ultrasound and fetal MRI at 30 weeks of gestation. The prenatal ultrasound showed that fetus had microcephaly (−5 SD) with no sulci or gyri observed and a small thalamus. The fetal MRI corroborated the microcephaly and no gyral or sulcal development. Bilateral frontal

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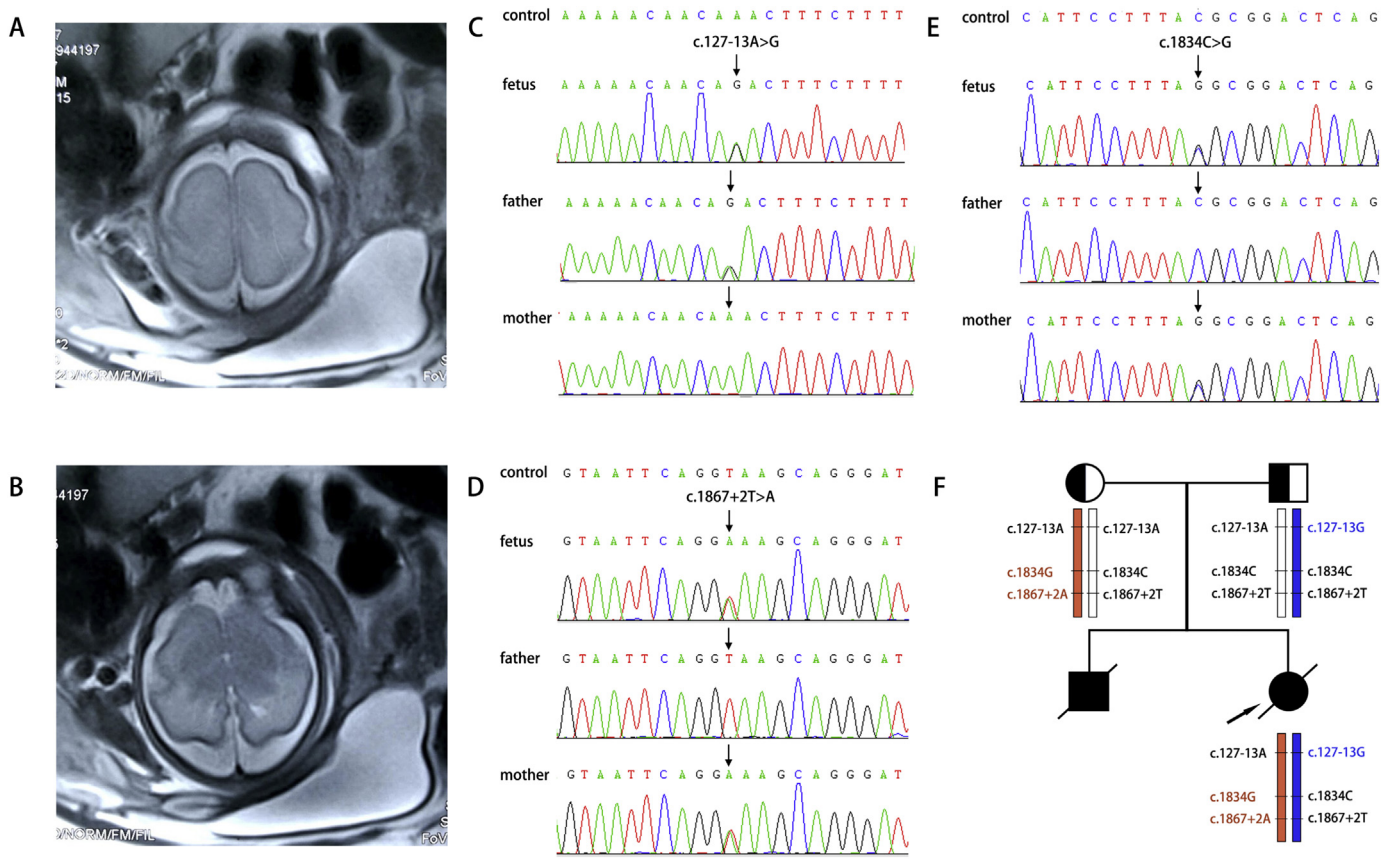

Fig. 1. Clinical and genetic data of the family. (A) Fetal MRI. No gyral or sulcal present. (B) Fetal MRI. No bilateral frontal horns or cavum septi pellucidi present. (C-E) Sanger sequencing of the family. (F) Family tree. c.127-13A > G was inherited from the father. c.1834C > G and c.1867 + 2T > A were two linked variants inherited from the mother.

horns and cavum septi pellucidi were unrevealed (Fig. 1). In addition, two imaging modalities both indicated a left duplex kidney existed. The parents elected to terminate the pregnancy, and a 1007-g female fetus was delivered at 30 weeks of gestation. The first pregnancy of this woman was terminated with no genetic testing because of microcephaly, as in the case of this gestation.

2.2. Genetic analysis

Genomic DNA was extracted from skin tissue of aborted fetus and peripheral blood leucocytes of parents on the basis of the phenol/chloroform method. Whole-exome sequencing (WES) in family trio was performed on the Illumina HiSeq XTen platform (Illumina, San Diego, California, USA) using the IDT xGen exome capture panel v1.0 (Integrated DNA Technologies, Coralville, Iowa, USA). The analysis of the high-throughput sequencing data was performed using the GATK software package (Broad Institute, Cambridge, MA, USA).

Sanger sequencing was performed for candidate variants. We further performed reverse transcription-polymerase chain reaction (RT-PCR) to see if the splice site mutations had effect on RNA expression from the patients' cDNAs.

3. Results

WES analysis yielded three heterozygous candidate variants in SASS6 (NM_194292). c.127-13A > G in intron 2 was inherited from the father, besides c.1834C > G in exon 16 and c.1867 + 2T > A in intron 16 were inherited from the mother (Fig. 1). All three variants were absent from all public databases (minor allele frequency (MAF) = 0.00), as well as in the inhouse database of Chigene (Joy Orient) translational medicine research center including 80,000 Chinese exomes. c.127-13A > G and c.1834C > G/c.1867 + 2T > A were co-segregated with the disease in the family which were confirmed by Sanger sequencing. No pathogenic variants in other MCPH-related genes were observed in proband.

Variants c.127-13A > G and c.1867 + 2T > A were classified as pathogenic and c.1834C > G was classified as likely pathogenic following the standards and guidelines for the interpretation of sequence variants proposed by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) [9].

Because of the variant positions, it was necessary to perform the reverse transcription PCR of c.127-13A > G and c.1867 + 2T > A to confirm their effect on splicing. Using reverse transcription PCR technology, both variants were confirmed to be splice site mutations. Mutation c.127-13A > G caused exon 3 skipping and c.1867 + 2T > A caused exon 16 skipping (Fig. 2).

4. Discussion

MCPH is a rare hereditary disease characterized by significant decreasing size of the brain with or without other symptoms and 18 MCPH genes have been reported as stated above. A majority of microcephaly proteins encoding by MCPH-related genes were related to centrosomal proteins directly or indirectly [2]. The centrosomal-related proteins encoded by relevant microcephaly mutated genes including *SASS6, CPAP, STIL, CEP63, CEP135, CEP152* functioned uniquely in brain development [8]. These genes indicated a link between centrosome function and neurogenesis in pathogenesis of MCPH. Through affecting the major role of centrosome in regulating mitosis, these centrosome-

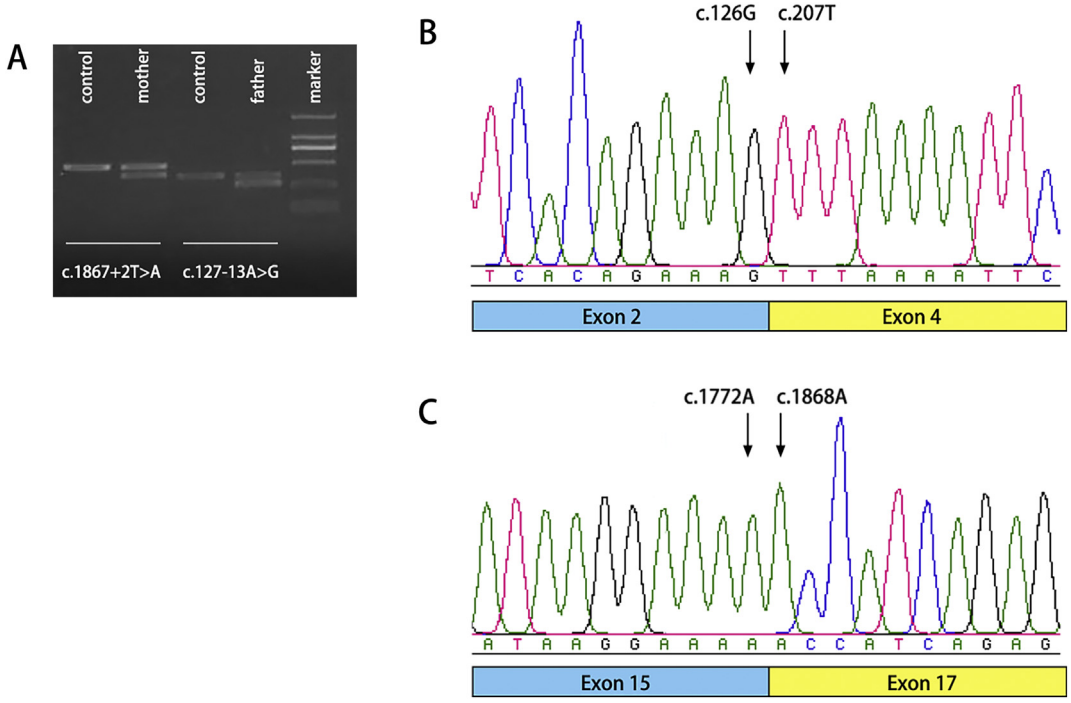

Fig. 2. Results of RT-PCR of two splice site mutations. (A) Agarose gel electrophoresis(AGE). (B) c.127-13A > G caused exon 3 skipping to form a mutant of p.D43FfsX49. (C) c.1867 + 2T > A caused exon 16 to form a mutant of p.N591KfsX54.

related microcephaly (CRM) mutants could increase apoptosis and differentiation and reduce neural progenitor cells (NPCs), thus causing reduced neurons and brain size [10].

SASS6 gene is one of the pathogenic gene in MCPH. As the formation of centriole is the fundamental function of its encoding protein, *SASS6* is also one of CRM genes [11]. A homozygous c.185T > C missense mutation in *SASS6* gene was firstly detected in a Pakistani consanguineous family in 2014 by Muzammil et al. The homozygous mutation of the gene was proved to impair the procentriole formation and thus causing abnormal neuronal cell division and microcephaly [8]. Since then, there was no case report on this microcephaly pathogenic gene until our present research. In our study, we observed two novel splice site mutations in compound heterozygous hereditary form in a Chinese non-consanguineous MCPH family and confirmed their effect on RNA expressions by RT-PCR assay.

The HsSAS-6 could function in formation of centriole by interaction with various proteins including STIL,γ-tubulin, Plk4, CPAP, Cep135 in different pathways. Among them, STIL, CPAP and Cep135 were CRM proteins [5]. For example, HsSAS-6 could directly bind to Cep135 and functioned in centriole duplication [12]. HsSAS-6 and STIL were cartwheel component for centriole assembly and Plk4 phosphorylates STIL to recruit HsSAS-6 to cartwheel [13]. The mutations in *SASS6* genes could cause malfunctions of HsSAS-6 and its related pathways thus affecting centriole assembly, reducing the number of neuroprogenitor cells and causing MCPH [2].

5. Conclusion

In conclusion, we have identified two novel splice site mutations c.127-13A > G and c.1867 + 2T > A in compound heterozygous hereditary form in the *SASS6* gene in a Chinese family with MCPH and the two mutations were confirmed to alter mRNA splicing by RT-PCR assay. Our finding broadened the mutation spectrum of *SASS6* which contributed to understanding of molecular mechanisms of MCPH.

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References

[1] S. Duerinckx, M. Abramowicz, The genetics of congenitally small brains, Semin. Cell Dev. Biol. 76 (2018) 76–85.
[2] M. Barbelanne, W.Y. Tsang, Molecular and cellular basis of autosomal recessive primary microcephaly, Biomed. Res. Int. (2014) (2014) 547986.
[3] C.G. Woods, J. Bond, W. Enard, Autosomal recessive primary microcephaly (MCPH): a review of clinical, molecular, and evolutionary findings, Am. J. Hum. Genet. 76 (5) (2005) 717–728.
[4] M. Sajid Hussain, S. Marriam Bakhtiar, M. Farooq, I. Anjum, E. Janzen, M. Reza Toliat, H. Eiberg, K.W. Kjaer, N. Tommerup, A.A. Noegel, P. Nurnberg, S.M. Baig, L. Hansen, Genetic heterogeneity in Pakistani microcephaly families, Clin. Genet. 83 (5) (2013) 446–451.
[5] M. Naveed, S.K. Kazmi, M. Amin, Z. Asif, U. Islam, K. Shahid, S. Tehreem, Comprehensive review on the molecular genetics of autosomal recessive primary microcephaly (MCPH), Genet. Res. (Camb.) 100 (2018) e7.
[6] A. Vertii, H. Hehnly, S. Doxsey, The centrosome, a multitalented renaissance organelle, Cold Spring Harb. Perspect. Biol. 8 (12) (2016).
[7] P. Strnad, S. Leidel, T. Vinogradova, U. Euteneuer, A. Khodjakov, P. Gonczy, Regulated HsSAS-6 levels ensure formation of a single procentriole per centriole during the centrosome duplication cycle, Dev. Cell 13 (2) (2007) 203–213.
[8] M.A. Khan, V.M. Rupp, M. Orpinell, M.S. Hussain, J. Altmuller, M.O. Steinmetz, C. Enzinger, H. Thiele, W. Hohne, G. Nurnberg, S.M. Baig, M. Ansar, P. Nurnberg, J.B. Vincent, M.R. Speicher, P. Gonczy, C. Windpassinger, A missense mutation in the PISA domain of HsSAS-6 causes autosomal recessive primary microcephaly in a large consanguineous Pakistani family, Hum. Mol. Genet. 23 (22) (2014) 5940–5949.
[9] S. Richards, N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W.W. Grody, M. Hegde, E. Lyon, E. Spector, K. Voelkerding, H.L. Rehm, A.L.Q.A. Committee, Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, Genet. Med. 17 (5) (2015) 405–424.
[10] R.S. O'Neill, T.A. Schoborg, N.M. Rusan, Same but different: pleiotropy in centrosome-related microcephaly, Mol. Biol. Cell 29 (3) (2018) 241–246.
[11] M. van Breugel, M. Hirono, A. Andreeva, H.A. Yanagisawa, S. Yamaguchi, Y. Nakazawa, N. Morgner, M. Petrovich, I.O. Ebong, C.V. Robinson, C.M. Johnson, D. Veprintsev, B. Zuber, Structures of SAS-6 suggest its organization in centrioles, Science 331 (6021) (2011) 1196–1199.

[12] Y.C. Lin, C.W. Chang, W.B. Hsu, C.J. Tang, Y.N. Lin, E.J. Chou, C.T. Wu, T.K. Tang, Human microcephaly protein CEP135 binds to hSAS-6 and CPAP, and is required for centriole assembly, EMBO J. 32 (8) (2013) 1141–1154.

[13] C.S. Fong, M. Kim, T.T. Yang, J.C. Liao, M.F. Tsou, SAS-6 assembly templated by the lumen of cartwheel-less centrioles precedes centriole duplication, Dev. Cell 30 (2) (2014) 238–245.