Brain & Development 41 (2019) 29–35

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

Identification of a novel *PAFAH1B1* missense mutation as a cause of mild lissencephaly with basal ganglia calcification

Chang-he Shi [a,1], Shuo Zhang [a,b,1], Zhi-hua Yang [a,1], Yu-tao Liu [a], Yu-sheng Li [a] Zhuo Li [a], Zheng-wei Hu [a,b], Yu-ming Xu [a,*]

[a] *Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou University, Zhengzhou, 450000 Henan, China*
[b] *Academy of Medical Sciences, Zhengzhou University, Zhengzhou 450052, Henan, China*

Received 14 April 2018; received in revised form 8 July 2018; accepted 17 July 2018

Abstract

Purpose: To investigate the genetic and clinical features of a Chinese family exhibiting an autosomal dominant inheritance pattern of lissencephaly.

Methods: Clinical examinations and cranial imaging studies were performed for all members of the family (two unaffected members and three surviving members from a total of four affected members). In addition, whole-exome sequencing analysis was performed for DNA from an affected patient to scan for candidate mutations, followed by Sanger sequencing to verify these candidate mutations in the entire family. A total of 200 ethnicity-matched healthy controls without neuropsychiatric disorder were also included and analyzed.

Results: We identified a novel missense mutation, c.412G > A, p.(E138K), that cosegregated with the disease in exon 6 of the platelet activating factor acetylhydrolase 1b regulatory subunit 1 (*PAFAH1B1*) gene in the affected members; this mutation was not found in the 200 controls. Multiple sequence alignments showed that codon 138, where the mutation (c.G412A) occurred, was located within a phylogenetically conserved region. Brain magnetic resonance imaging revealed calcification within the bilateral globus pallidus in all three affected members.

Conclusions: We identified a novel missense mutation, c.412G > A, p.(E138K), in the *PAFAH1B1* gene of a Chinese family with lissencephaly. In addition, our findings suggest that basal ganglia calcification is a novel clinical feature of *PAFAH1B1*-related lissencephaly.

Keywords: Lissencephaly; *PAFAH1B1*; Missense mutation; Basal ganglia calcification

1. Introduction

Lissencephaly, which includes classical lissencephaly (LIS) and subcortical band heterotopia (SBH), is a rare brain malformation caused by impaired neuroblast migration during embryonic development due to mutations in various genes associated with neuronal migration, such as *LIS1*, *DCX*, *RELN*, *ARX*, and *YWHAE* [1,2]. Patients with lissencephaly are characterized by a smooth cerebral surface, intellectual disability, and seizures [3]. LIS presents with a four-layered and thickened cortex with decreased gyration [4], while SBH is a related disorder wherein abnormal neuroblast migration

* Corresponding author at: Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou University, 1 Jian-she East Road, Zhengzhou 450000, Henan, China.
E-mail address: xuyuming@zzu.edu.cn (Y.-m. Xu).
[1] The authors contributed equally to this work.

https://doi.org/10.1016/j.braindev.2018.07.009
0387-7604/

results in the formation of a band of heterotopic gray matter between the cortex and ventricular walls, with an overlying six-layered cortex exhibiting shallow sulcation [4].

The platelet activating factor acetylhydrolase 1b regulatory subunit 1 (*PAFAH1B1*; NM_000430.3) gene encodes a 45-kDa protein designated as LIS1 or PAFAH1B1, which exhibits an N-terminal homodimerization domain and seven C-terminal tryptophan aspartic acid 40 repeats [5,6]. PAFAH1B1 binds to cytoplasmic dynein, a microtubule minus end-directed motor protein, and plays a key role in cell division and motility [7]. Research in mice suggests that *PAFAH1B1* is necessary for nuclear movement during neuronal migration [8]. The majority of described mutations in *PAFAH1B1* are deletions, splice site mutations, and nonsense mutations. Missense mutations reportedly account for <20% of all *PAFAH1B1* mutations [9,10]. Till date, missense mutations have been described to result in mild lissencephaly phenotypes and SBH [3,11]. Furthermore, no studies have reported calcification within the bilateral globus pallidus in patients with lissencephaly.

The aim of the present study was to investigate the genetic and clinical features of a Chinese family exhibiting an autosomal dominant inheritance pattern of LIS with seizures, severe intellectual disability, and calcification within the bilateral globus pallidus.

2. Material and methods

2.1. Subjects and ethics approval

A three-generation family comprising two unaffected members (I:1 and II:1) and four affected members (I:2, II:2, III:1 and III:2) with LIS characterized by seizures and mental retardation was recruited for this study. Blood samples were obtained from all participants, who also underwent cranial magnetic resonance imaging (MRI) studies. The severity of LIS was graded using an established system [10,12]. A total of 200 ethnicity-matched healthy controls without neuropsychiatric disorder were also included. Genomic DNA was extracted using the QIA-amp DNA Blood Mini Kit (QIAGEN, Germany) according to the manufacturer's protocol.

This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University, China. Written informed consent to participate in the study was obtained from all subjects.

3. Whole-exome sequencing

The proband in the family was selected for exome sequencing. Targeted exon enrichment was performed using the SureSelect Human All Exon V4 (Agilent Technologies, Santa Clara, CA, USA). The exon-enriched DNA libraries were subjected to paired-end sequencing with the Hiseq2000 platform (Illumina, San Diego, CA, USA). Sequence data were mapped onto the hg19 human genome, used as a reference, with Burrows Wheeler Aligner (BWA) and SAMTOOLS [13]; calls with a variant quality of less than 20 were filtered out, and 95% of the targeted bases were sufficiently covered to pass our thresholds for calling single-nucleotide polymorphisms (SNPs) and small indels. The remaining variants were annotated using ANNOVAR, the details of which are described in previous studies [14,15].

4. Polymerase chain reaction (PCR) and Sanger sequencing

On the basis of the results of exome sequencing, Sanger sequencing was performed to verify the identified genetic defects. Primers flanking the mutation area in *PAFAH1B1* (NM_000430.3) were designed on the basis of reference genomic sequences in the human genome obtained from GenBank at the National Center for Biological Information (NCBI) and synthesized by Sangon, Shanghai, China. The forward primer was 5′-CTGAGA CAGGGAGCGGACTATG-3′ and the reverse primer was 5′-GCAGAACAGGAAGCCAGAAGC-3′. PCR amplification and subsequent DNA sequencing were performed. Sequence data comparisons and analyses were performed using CHROMS software. Cosegregation analysis was subsequently performed for the DNA samples from available family members.

5. Bioinformatics analysis

DNA sequencing results were compared with the human reference sequence (GRCh37/hg19) using the BLAT tool from the UCSC Genome Browser. The Single Nucleotide Polymorphism Database, 1000 Genome project database, ExAC (http://exac.broadinstitute.org), dbSNP build 132, Hapmap, YH project, and the National Heart, Lung, and Blood Institute Exome Sequencing Project were used for the mutation exclusion analysis. Four online bioinformatics tools were used for the analysis of missense mutations; Mutation Taster (http://www.mutationtaster.org), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), and SIFT (http://sift.jcvi.org) were used to predict the biological impact of the variant in silicon, while I-Mutant (http://folding.biofold.org/i-mutant/) was used to predict protein stability. The closest homologs of the PAFAH1B1 protein were aligned using the program Clustal-X.

6. Results

6.1. Clinical features

We initially observed a lissencephaly pedigree consistent with an autosomal dominant inheritance pattern

and identified four affected members across three generations (Fig. 1A). However, one of the four affected members was deceased, and the remaining three were available for analyses.

The proband (III-1) was born at 38 weeks of gestation after a normal pregnancy. She developed symptoms at the age of 6 years, following the onset of seizures with episodes of confusion and decreased responsiveness. Neurological examination at that time only revealed mild clumsiness. The findings of general physical examinations were normal, and there was no facial dysmorphism. Brain MRI at the age of 24 years showed grade 4b LIS with pachygyria restricted to the bilateral frontal lobes (Fig. 2A). T1-weighted images showed low-signal intensities in the bilateral globus pallidus (Fig. 2D), CT (Computed Tomography) scan showed high-density calcification in the same area (Fig. 2G). Meanwhile, the common causes of basal ganglia calcification, such as hyperparathyroidism; hypoparathyroidism; and excessive serum levels of calcium, magnesium, phosphorus, and other metals, were ruled out. The rest of the brain was normal. However, she exhibited severe intellectual disability and could only perform simple manual tasks that did not require any skills. Furthermore, she needed supervision for seizures during some activities. Cognitive impairment was remarkable, and she was unable to cope in the intelligence test. Her epilepsy was well controlled with carbamazepine and piracetam.

The proband's younger sister (III-2) was born at 36 weeks of gestation after an uncomplicated pregnancy. Her early development was marked by a mild speech delay, and she began walking at 16 months. Symptoms of seizures and psychomotor retardation manifested at the age of 3 years, following a high fever. The findings of general physical examination were normal. Neurological examination only revealed mild clumsiness, and there was no facial dysmorphism. Brain MRI performed at 21 years of age showed grade 4b LIS with generalized pachygyria that was most severe in the frontal regions (Fig. 2B). Interestingly, basal ganglia calcification was also observed in this patient (Fig. 2E, H), and all common causes were ruled out. The rest of the brain was

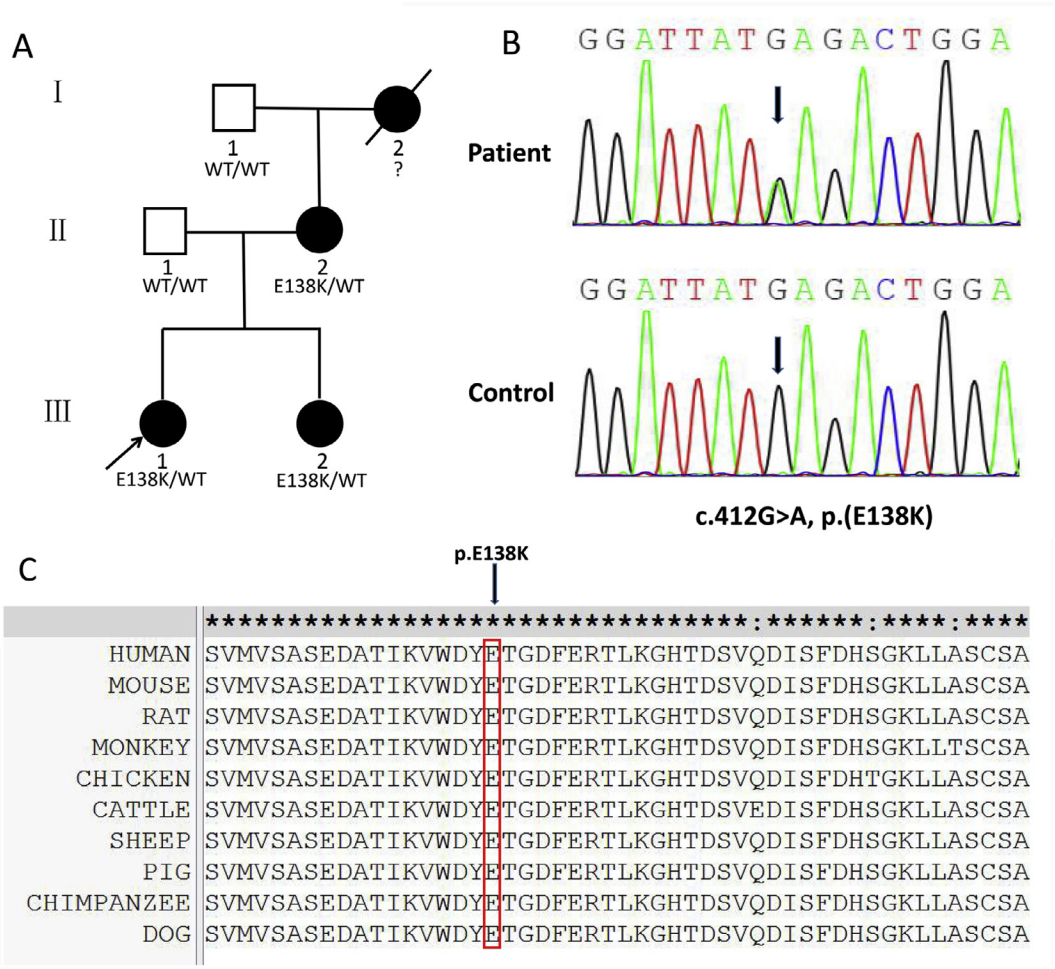

Fig. 1. Findings of genetic analysis for the pedigree of a Chinese family with classical lissencephaly (LIS). (A) The pedigree of the Chinese family. (B) Sequences of the *PAFAH1B1* c.G412A mutation. (C) Conservation of the PAFAH1B1 protein residues targeted by the mutation identified in the affected family members.

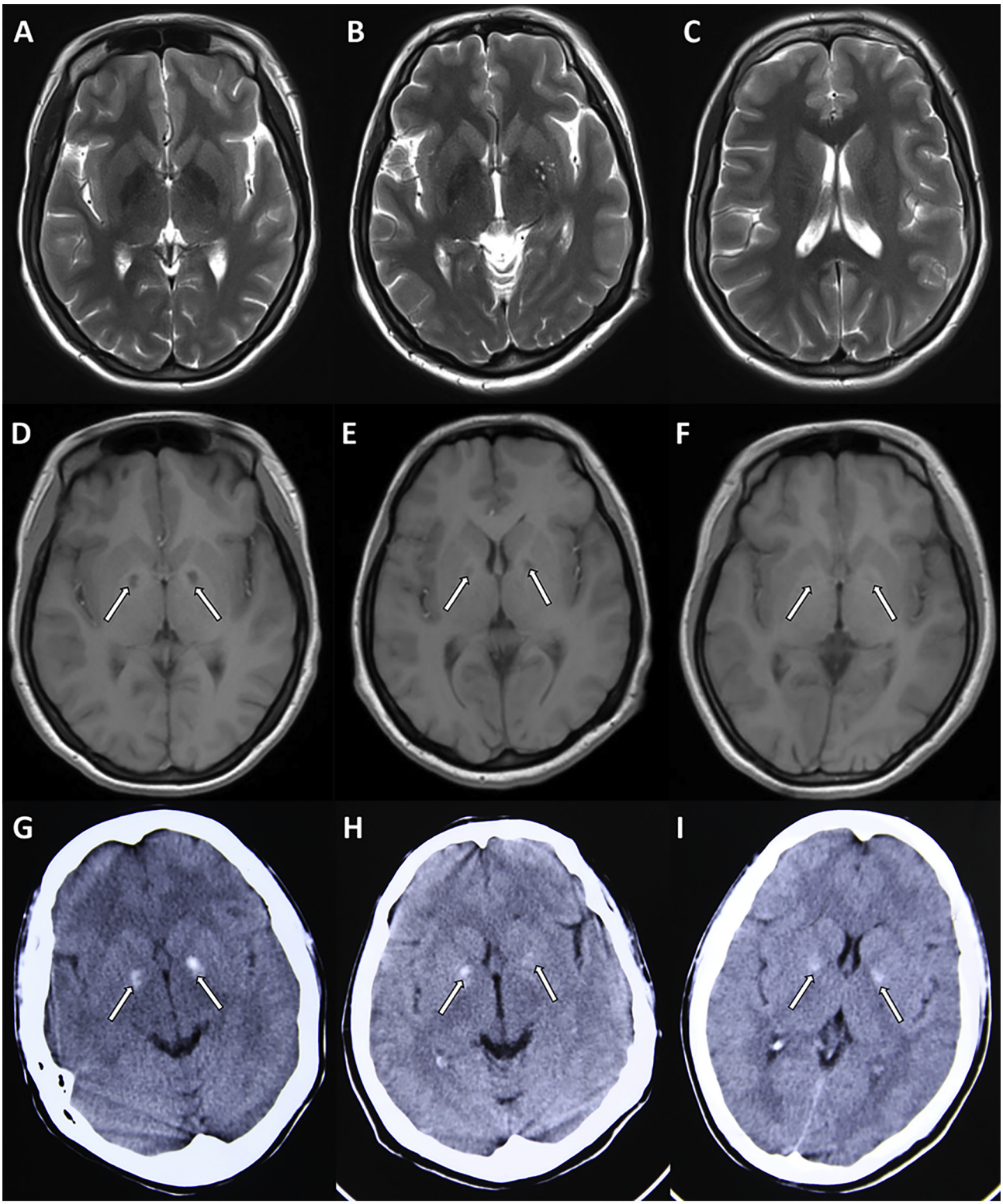

Fig. 2. Neuroimaging findings for the pedigree of a Chinese family with classical lissencephaly (LIS). (A) T2-weighted magnetic resonance (MR) images for the proband (III-1) show grade 4b LIS with pachygyria in the frontal lobe. (B) T2-weighted MR images for Patient III-2 show grade 4b LIS with pachygyria in the frontal lobes. (C) T2-weighted MR images for Patient II-2 show grade 4b LIS, similar to that in the proband. (D, E, F) T1-weighted MR images for the proband and patients III-2 and II-2 showing long T1 signals in the basal ganglia. (G, H, I) CT of the proband and patients III-2 and II-2, showing high density calcification in basal ganglia. The arrows indicate calcification.

normal. She never attended school because of severe intellectual disability and specific weaknesses in the comprehension and use of language. Along with her sister, she underwent back surgery at the age of 12 years, although the outcomes were poor. Her intellectual disability was as severe as that exhibited by the proband. Similar to the proband, she response well to drug therapy with dilantin sodium, valproate sodium and piracetam.

The proband's mother (II-2) was born at 40 weeks of gestation to nonrelated parents. The pregnancy was normal. She developed seizures at the age of 8 years and was diagnosed with epilepsy. Her epileptic symptoms did not relapse after acupuncture and moxibustion therapy at the age of 8 years, although she was left with a crooked mouth. Brain MRI at 49 years of age revealed grade 4b LIS, similar to that in the proband (Fig. 2C). In addition, basal ganglia calcification was observed (Fig. 2F, I) without any common causes. The rest of the brain was normal. At present, she can understand simple commands and communicates by gestures, limited sign language, and occasional words. The proband's deceased grandmother (I-2) also developed seizures during her childhood. Her grandfather and father (I-1 and II-1) were healthy without any neuropsychiatric symptoms, and brain MRI showed no basal ganglia calcification or other abnormalities.

The proband's head circumference is 48 cm, the head circumference of the proband's younger sister is 46 cm, the head circumference of the proband's mother is 49 cm.

7. Whole exome sequencing

Using previously described methods [14–18], after quality control and coverage criteria were met, one heterozygous exonic variant on chromosome 17, c.412 G > A, p.(E138K), in the *PAFAH1B1* gene was highlighted. Because *PAFAH1B1* mutations have been reported as major causative factors for lissencephaly, we considered that this mutation was the most likely cause of LIS in the assessed pedigree.

8. *PAFAH1B1* mutation and bioinformatics analyses

A previously undescribed missense mutation, c.412G > A, p.(E138K), was identified in exon 6 of the *PAFAH1B1* gene in the three affected patients (Fig. 1B), but not in the two unaffected family members. This confirmed that the genotype was cosegregated with the phenotype in this pedigree. The identified mutation was not found in the 200 controls and was also absent in the Single Nucleotide Polymorphism database, 1000 Genome project database, ExAC (http://exac.broadinstitute.org), dbSNP build 132, Hapmap, YH project, and the National Heart, Lung, and Blood Institute Exome Sequencing Project. Multiple sequence alignments revealed that codon 138, where the mutation (c. G412A) occurred, was located within a phylogenetically conserved region (Fig. 1C).

The mutation was predicted as potentially damaging by Polyphen-2, disease causing by Mutation Taster, and deleterious by SIFT. In addition, I-Mutant predicted that the mutation decreased the stability of the PAFAH1B1 protein.

We have also searched on possibilities of familial brain calcification and other genetic brain calcification disorders, including significant mutations in *SLC20A* [19], *XPR1* [20], *IBGC2* [21], *PDGFRB* [22], *PDGFB* [23], and candidate genes of Aicardi-Goutieres syndrome [24]. We found some SNPs such as rs72611914 in *SLC20A1*, rs4589068, rs4439326, rs3002117, rs2271668, rs2271667, rs10914123 in *XPR1*, rs4821874, rs9622979, rs879180, rs1800817 in *PDGFB*, all these SNPs are located in the intron region and the clinical significance are NA. We also found some INDELs such as rs5833454 in *SLC20A1*, rs34984251 in *XPR1*, rs369019315, rs370356342 in *PDGFRB*, rs150503603, rs138673005 in *PDGFB*, all these INDELs are located in the intron region and the clinical significance are NA.

9. Discussion

In the present study, we described the clinical features of a Chinese family with LIS along with the findings of neuroimaging and genetic analyses. Molecular genetic analysis identified a novel missense mutation, c.412G > A, p.(E138K), in exon 6 of the PAFAH1B1 gene in all three affected patients. With regard to the clinical phenotype, our patients presented with milder seizures and imaging findings, in accordance with previous reports about *PAFAH1B1*-mutated patients [10,11]. However, all patients suffered from severe intellectual disability and exhibited basal ganglia calcification.

Lissencephaly is generally considered a rare neurodevelopmental disorder characterized by intractable seizures, spasticity, profound intellectual disability, and even early death [4]. More than 50% cases of lissencephaly, including LIS and SBH, are caused by *PAFAH1B1* mutations [25]. Deletions, duplication, and nonsense mutations are the most frequent types of mutations in *PAFAH1B1* [10,26]. In recent years, some other genes have been found to be associated with lissencephaly, such as *RELN, TUBA1A, ACTG1, ARX,* and *YWHAE* [1,2,10].

In the present study, MRI and CT revealed symmetrical calcium accumulation in the globus pallidus of all affected family members, but not in the unaffected ones. Our imaging findings cosegregated with the pedigree, and all alternative causes of basal ganglia calcification, such as hyperparathyroidism; hypoparathyroidism; and excessive serum levels of calcium, magnesium, phosphorus, and other metals; were ruled out. More-

over, mutations in SLC20A [19], XPR1 [20], IBGC2 [21], PDGFRB [22], PDGFB [23], and candidate genes of Aicardi-Goutieres syndrome [24] were searched in this family, without no significant mutations was found. The *PAFAH1B1* mutation c.412G > A, p.(E138K) has never been reported previously in several databases including dbSNP build 132, the 1000 Genomes Project, Hapmap, YH project and so on, so we suggest it is a novel mutation, not a founder mutation. Furthermore, the variant was not found in the controls, which indicated that the variant might be a mutation rather than a rare polymorphism in the Chinese population. Accordingly, the identified *PAFAH1B1* mutation was suggested as a novel genetic cause for basal ganglia calcification in patients with lissencephaly. However, more evidence is required to support this finding.

Several functional studies have revealed the role of *PAFAH1B1* in lissencephaly [27–29]. *PAFAH1B1* is important for neuronal migration throughout the nervous system, and heterozygous mutations or deletions in *PAFAH1B1* are found in the majority of patients with lissencephaly [10]. *PAFAH1B1* mutations or knockdown has been identified in association with cortical malformations in humans and mice [10,30,31]. In bioinformatics analysis in the present study, the missense mutation was predicted to be potentially damaging and the cause for a decrease in the stability of the PAFAH1B1 protein.

In summary, we identified a novel missense mutation, c.412G > A, p.(E138K), in the *PAFAH1B1* gene of individuals affected with lissencephaly in a Chinese family. In addition, our findings suggest that basal ganglia calcification is a novel clinical feature of *PAFAH1B1*-related lissencephaly. Further genetic and functional studies are required to elucidate the pathogenicity of *PAFAH1B1* mutations with regard to lissencephaly.

Conflict of interest

The authors report no conflict of interest.

Funding

This work was supported by the National Natural Science Foundation of China to Dr. Chang-he Shi [grant number 81771290, 2017]; the National Natural Science Foundation of China to Dr. Yu-ming Xu [grant numbers 81471158, 2014; grant number 81530037, 2015].

Acknowledgements

We would like to thank all the participating family members for their cooperation. This work was supported by the National Natural Science Foundation of China to Dr Changhe Shi [grant number 81771290, 2017]; the National Natural Science Foundation of China to Dr Yuming Xu [grant number 81471158, 2014; grant number 81530037, 2015].

References

[1] Kato M, Dobyns WB. Lissencephaly and the molecular basis of neuronal migration. Hum Mol Genet 2003;12 Spec No 1:R89-96.
[2] Kerjan G, Gleeson JG. Genetic mechanisms underlying abnormal neuronal migration in classical lissencephaly. Trends Genet 2007;23:623–30.
[3] Leventer RJ. Genotype-phenotype correlation in lissencephaly and subcortical band heterotopia: the key questions answered. J Child Neurol 2005;20:307–12.
[4] Dobyns WB, Reiner O, Carrozzo R, Ledbetter DH. Lissencephaly: A human brain malformation associated with deletion of the LIS1 gene located at chromosome 17p13. JAMA 1993;270:2838–42.
[5] Reiner O, Carrozzo R, Shen Y, Wehnert M, Faustinella F, Dobyns WB, et al. Isolation of a Miller-Dieker lissencephaly gene containing G protein beta-subunit-like repeats. Nature 1993;364:717–21.
[6] Hattori M, Adachi H, Tsujimoto M, Arai H, Inoue K. Miller-Dieker lissencephaly gene encodes a subunit of brain platelet-activating factor acetylhydrolase [corrected]. Nature 1994;370:216–8.
[7] Moon HM, Wynshaw-Boris A. Cytoskeleton in action: lissencephaly, a neuronal migration disorder. Wiley Interdiscip Rev Dev Biol 2013;2:229–45.
[8] Tanaka T, Serneo FF, Higgins C, Gambello MJ, Wynshaw-Boris A, Gleeson JG. Lis1 and doublecortin function with dynein to mediate coupling of the nucleus to the centrosome in neuronal migration. J Cell Biol 2004;165:709–21.
[9] Cardoso C, Leventer RJ, Matsumoto N, Kuc JA, Ramocki MB, Mewborn SK, et al. The location and type of mutation predict malformation severity in isolated lissencephaly caused by abnormalities within the LIS1 gene. Hum Mol Genet 2000;9:3019–28.
[10] Fry AE, Cushion TD, Pilz DT. The genetics of lissencephaly. Am J Med Genet C Semin Med Genet 2014;166c:198–210.
[11] Leventer RJ, Cardoso C, Ledbetter DH, Dobyns WB. LIS1 missense mutations cause milder lissencephaly phenotypes including a child with normal IQ. Neurology 2001;57:416–22.
[12] Dobyns WB, Truwit CL. Lissencephaly and other malformations of cortical development: 1995 update. Neuropediatrics 1995;26:132–47.
[13] Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009;25:1754–60.
[14] Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucl Acids Res 2010;38 e164.
[15] Shi CH, Mao CY, Zhang SY, Yang J, Song B, Wu P, et al. CHCHD2 gene mutations in familial and sporadic Parkinson's disease. Neurobiol Aging 2016;38:217.e9–217.e13.
[16] Li R, Yu C, Li Y, Lam TW, Yiu SM, Kristiansen K, et al. SOAP2: an improved ultrafast tool for short read alignment. Bioinformatics 2009;25:1966–7.
[17] Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and SAMtools. Bioinformatics 2009;25:2078–9.
[18] Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 2010;26:589–95.
[19] Wang C, Li Y, Shi L, Ren J, Patti M, Wang T, et al. Mutations in SLC20A2 link familial idiopathic basal ganglia calcification with phosphate homeostasis. Nat Genet 2012;44:254–6.

[20] Legati A, Giovannini D, Nicolas G, López-Sánchez U, Quintáns B, Oliveira JR, et al. Mutations in XPR1 cause primary familial brain calcification associated with altered phosphate export. Nat Genet 2015;47(6):579–81.
[21] Grütz K, Volpato CB, Domingo A, Alvarez-Fischer D, Gebert U, Schifferle G, et al. Primary familial brain calcification in the 'IBGC2' kindred: all linkage roads lead to SLC20A2. Mov Disord 2016;31(12):1901–4.
[22] Arts FA, Velghe AI, Stevens M, Renauld JC, Essaghir A, Demoulin JB. Idiopathic basal ganglia calcification-associated PDGFRB mutations impair the receptor signalling. J Cell Mol Med 2015;19(1):239–48.
[23] Vanlandewijck M, Lebouvier T, Andaloussi Mäe M, Nahar K, Hornemann S, Kenkel D, et al. Functional characterization of germline mutations in PDGFB and PDGFRB in primary familial brain calcification. Plos One 2015;23;10(11):e0143407.
[24] Hsu SC, Sears RL, Lemos RR, Quintáns B, Huang A, Spiteri E, et al. Mutations in SLC20A2 are a major cause of familial idiopathic basal ganglia calcification. Neurogenetics 2013;14(1):11–22.
[25] Pilz DT, Matsumoto N, Minnerath S, Mills P, Gleeson JG, Allen KM, et al. LIS1 and XLIS (DCX) mutations cause most classical lissencephaly, but different patterns of malformation. Hum Mol Genet 1998;7:2029–37.
[26] Cardoso C, Leventer RJ, Dowling JJ, Ward HL, Chung J, Petras KS, et al. Clinical and molecular basis of classical lissencephaly: mutations in the LIS1 gene (PAFAH1B1). Hum Mutat 2002;19:4–15.
[27] Yamada M, Toba S, Yoshida Y, Haratani K, Mori D, Yano Y, et al. LIS1 and NDEL1 coordinate the plus-end-directed transport of cytoplasmic dynein. EMBO J 2008;27:2471–83.
[28] Morris NR. Nuclear migration. From fungi to the mammalian brain. J Cell Biol. 2000;148:1097–101.
[29] Gambello MJ, Darling DL, Yingling J, Tanaka T, Gleeson JG, Wynshaw-Boris A. Multiple dose-dependent effects of Lis1 on cerebral cortical development. J Neurosci 2003;23:1719–29.
[30] Cahana A, Escamez T, Nowakowski RS, Hayes NL, Giacobini M, von Holst A, et al. Targeted mutagenesis of Lis1 disrupts cortical development and LIS1 homodimerization. Proc Natl Acad Sci USA 2001;98:6429–34.
[31] Fleck MW, Hirotsune S, Gambello MJ, Phillips-Tansey E, Suares G, Mervis RF, et al. Hippocampal abnormalities and enhanced excitability in a murine model of human lissencephaly. J Neurosci 2000;20:2439–50.