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DCX variants in two unrelated Chinese families with subcortical band heterotopia: Two case reports and review of literature

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ABSTRACT

Introduction: Subcortical band heterotopia (SBH) is a rare brain developmental malformation caused by deficient neuronal migration during embryogenesis. Published literature on pediatric SBH cases caused by *DCX* mutations is limited.
Methods: The detailed clinical and genetic features of two pediatric SBH with *DCX* mutations were analyzed. The available literature on *DCX* mutations was reviewed.
Results: Both patients were girls with varying degrees of developmental delay. Patient 1 was short in stature with peculiar facial features. Patient 2 had an early seizure onset and developed drug-resistant epilepsy. Whole-exome sequencing (WES) revealed two de novo heterozygous variants of *DCX* (NM_178153.3), including a novel missense variant of c.568A > G (p.K190E) in P1 and a reported nonsense variant of c.814C > T (p.R272*) in P2. We reviewed all the available literature regarding *DCX* mutations. A total of 153 different mutations have been reported, with the majority of 99 (64.7 %) being missense mutations.
Conclusion: Our study expanded the mutational spectrum of *DCX*, which has important implications for the study of genotype-phenotype correlations. Furthermore, it provided insights to better understand SBH and genetic counseling.

1. Introduction

Lissencephaly (X-linked lissencephaly, OMIM #300067) is a rare malformation of cortical development caused by impaired neuronal migration during embryonic development with a prevalence of 1/100,000 to 4/100,000 newborns [1]. The spectrum of lissencephaly includes agyria, pachygyria, and SBH. SBH, also known as double cortex, is the mildest form of malformation in the lissencephaly spectrum. The majority of lissencephaly is caused by heterozygous pathogenic variants in *PAFAH1B1* (*LISI*) and *TUBA1A*, which can also cause SBH. For *PAFAH1B1*-associated lissencephaly/SBH, GeneReview reports a prevalence of 11.7–40/1,000,000 births. The prevalence of *TUBA1A*-associated disease is less than 1/1,000,000. *DCX* is located in q22.3-q23 on the X chromosome and encodes the protein doublecortin (DCX). Mutations in *DCX* cause lissencephaly in hemizygous male patients, whereas

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lead to SBH in heterozygous female patients [2,3]. *DCX* mutations are found in 53–84 % of patients with SBH, making *DCX* more common than *PAFAH1B1* and *TUBA1A*. *PAFAH1B1* SBH tends to cause posterior malformations (parietal and occipital lobes), while *DCX* causes anterior disease. The actual incidence of SBH caused by *DCX* pathogenic variants is not known. In this study, we report the detailed clinical and genetic features of two SBH patients carrying *DCX* mutations. Our new findings expand the mutational spectrum of *DCX*.

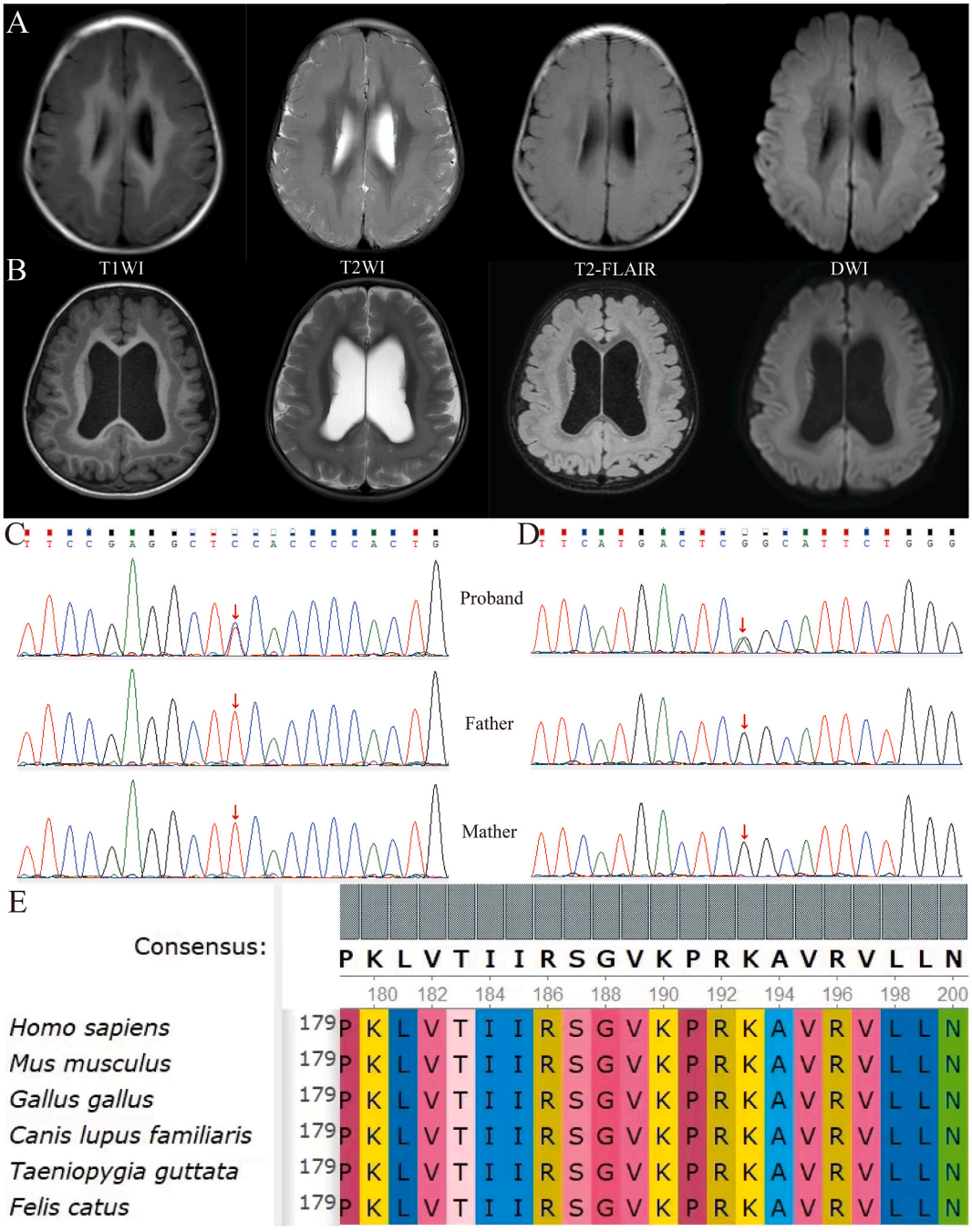

Fig. 1. Clinical data of two patients. (A) Brain MRI of P1 showed partial cortical thickening and increased regional gyri. The banded cortical signal shadows were seen in the medulla. (B) Brain MRI of P2 showed local gyri widening and broad bands of gray matter signals in the white matter. (C–D) Heterozygous mutations of c.568A > G and c.814C > T were found in P1 and P2, respectively. (E) Conservative analysis showed the Lysine 190 is highly conserved in different species.

2. Materials and methods

2.1. Patients

Two patients from unrelated families were examined at our hospital. The clinical features, physical examinations, electroencephalogram (EEG), brain magnetic resonance image (MRI), treatments, and genetic characteristics were collected and reviewed. All available literature regarding *DCX* mutations was analyzed.

2.2. Next-generation sequencing and variant discovery

Peripheral blood samples were collected from two patients and their parents for genomic DNA extraction. Mutations were tested by WES. Target gene fragments were enriched and sequenced. NextGeneV2.3.4 software was used to obtain all genetic mutations. SIFT, PolyPhen-2, Mutation Taster, and GERP were then applied to predict pathogenicity. Conservative analysis was performed using ClustalX. All variants were classified according to the 2015 American College of Medical Genetics and Genomics (ACMG) guidelines [4]. The potential pathogenic mutations were validated by Sanger sequencing.

3. Results

3.1. Clinical characteristics of two patients

Patient 1 was a 21-month-old girl suffering from global developmental delay. She could sit independently at 9 months and crawl at 18 months. By 21 months, she can only speak in monosyllables and cannot stand independently. The child was found to be short in stature at the age of 2 years. The girl was born at 39 weeks, with a birth length of 50 cm and a birth weight of 2.75 kg. Her parents and brother were in good health. Her father was 170 cm tall, and her mother was 154 cm, with no history of spontaneous pregnancy losses. Physical examination revealed low muscle tone in four limbs and peculiar facial features, including hypertelorism, a slightly high ear position, and a slightly narrow jaw. Brain MRI showed partial cortical thickening, enlarged regional gyri, and reduction of medulla in bilateral cerebral hemispheres. The medulla showed banded cortical signal shadows with multiple microcephalic gyrus malformations and gray matter heterotopia (Fig. 1A). She went to the rehabilitation department regularly for physical therapy and had ongoing interactions with the physical therapists. She was diagnosed with SBH caused by the de novo *DCX* mutation after detection. By 49 months at the last follow-up, her language, cognitive, motor, and intellectual development had significantly improved. She can speak simple sentences, communicate with others, walk independently, and jump. However, her height of 94.3 cm was shorter than the third percentile of normal height for the same sex and age, with height growth slowing to less than 6.5 cm per year and the growth curve declining from −1.91 SD to −2.4 SD.

Patient 2 was a 5m20d-old girl who was admitted to our hospital for “10 days of seizures”. Her seizures were characterized by frequent blinking, upward gaze of both eyes, nodding, and abduction of both upper limbs with shaking. The girl was born at 39 weeks with a birth weight of 3.1 kg. She doesn’t have siblings. Both parents were in good health. Her mother has no history of spontaneous pregnancy losses. EEG after admission showed spike-slow and multi-spike-slow complex wave emissions in the bilateral occiput. Infantile spasms were diagnosed and treated with phosphocreatine, prednisone, topiramate, and nitrazepam for 22 days. Treatment with antiepileptic drugs was continued after discharge. The epilepsy was well controlled; however, she showed global developmental delay with increasing age. EEG was abnormal on multiple follow-up examinations. Brain MRI at 9 and 18 months showed local gyri widening, cortical thickening, and broad bands of gray matter signals in the white matter between the cortex and the lateral ventricles (Fig. 1B). WES at 19 months showed a de novo *DCX* mutation. She was able to speak some simple words by 2 years and began to walk independently at 3 years. She experienced two recurrences of seizures at 45 and 63 months, which were gradually controlled with the addition of antiepileptic medications. At the last follow-up by 74 months, her language, motor, communication, and intellectual development continued to improve. The child is currently being treated with four antiepileptic medications.

3.2. Genetic findings and pathogenicity analysis

WES showed two de novo heterozygous variants of *DCX*, including a novel missense variant of c.568A > G (p.K190E) in P1(Fig. 1C) and a reported [5] nonsense variant of c.814C > T (p.R272*) in P2 (Fig. 1D). The mutation sites were distributed in exons 3 and 5, individually. Conservative analysis showed the Lysine 190 is highly conserved in different species (Fig. 1E). According to the ACMG guidelines, the mutation in P1 was determined as likely pathogenic (PS2+PM1+PM2+PP3), and the mutation of P2 was pathogenic (PVS1+PS4+PM6).

4. Discussion

SBH is a rare severe brain developmental disorder characterized by bilateral bands of gray matter located beneath the cortex and separated from it by a thin zone of normal white matter [6]. SBH cases caused by *DCX* mutations are not commonly described in pediatric patients. In this study, we presented the detailed clinical and genetic features of two pediatric SHB with *DCX* mutations.

DCX interacts with microtubules through its C-DC and N-DC domains to regulate neuronal migration [7]. Mutations in *DCX* impair neuronal migration, leading to pathogenic alterations. Fig. 2 shows a review of genetically confirmed *DCX* mutations published before

January 2023. A total of 153 different mutations (including a novel variant in this study) have been reported, and mutation characteristics revealed 99 (64.7 %) missense mutations, followed by 13 (8.5 %) small deletions, 12 (7.8 %) gross deletions, 11 (7.2 %) nonsense mutations, 7 (4.6 %) small insertions, 7 (4.6 %) splicing variants, 2 (1.3 %) gross insertions, 1 (0.7 %) small indel, and 1 (0.7 %) complex rearrangement (Supplementary Tables S1–S9).

Both *DCX* mutations in our study are de novo mutations, which is consistent with the report by Bahi-Buisson [8]. In his work, several recurrent mutations defining potential hot spots in multiple unrelated individuals were found, with the most common mutations affecting Arg186 (p.R186C, p.R186H, or p.R186L). Other recurrent missense (p.R78C, p.R78H, p.R78L, or p.R192W) or nonsense (p.R39X or p.R303X) mutations were found in 2–3 cases each. Overall, these hot spot mutations accounted for 38.7 % of de novo *DCX* SBH mutations. They also showed that approximately 11 % of female patients had inherited *DCX* sequence variants. Although the frequency of familial SBH is lower than that of sporadic SBH and accounts for one-third of the female population with *DCX* mutations, they cannot rule out the possibility that these individuals are underdiagnosed [8]. Furthermore, it has been known that some patients with *DCX* pathogenic variants are speculated to have germline mosaicism with or without somatic mosicism, thus it's possible for both female probands in this study, inherited the variants from germline mosaicism of their respective parents though we didn't find any *DCX* pathogenic variants in their respective parents' leukocyte DNA.

The clinical manifestation of SBH has a wide heterogeneity, and the severity depends on the location of mutation and the degree of potential brain malformation [9]. One of the most common clinical manifestations is epilepsy. It could occur at any age, often during the first decade but sometimes in the second or third decade and become therapy resistant [10]. Heterozygous females with germline missense or nonsense *DCX* variants may have no obvious brain malformations or seizures [11,12]. Both patients in our study showed developmental delay. Additionally, P1 was short in stature with peculiar facial features, which is consistent with Chou's report [13]. In P2, seizures occurred at age 5 months and progressed to drug-resistant epilepsy, which is consistent with patients from Kato et al. [5]. In their report, patients 2 and 3 were female with diffuse SBH. Patient 2 had intractable multiple epileptic seizures. Patient 3 showed infantile spasms at the age of 9 months and a complex partial seizure at 7 years. Both of them were mentally retarded. Matsumoto et al. [14] divided SBH into three subgroups: diffuse thick bands, diffuse thin bands, and frontal predominant thin bands, with diffuse thick bands being the most common. Although many females with nonsense mutations, including P2 in our study, have diffuse thick bands, not all females with nonsense mutations produce diffuse thick bands of gray matter interposed in the white matter. For example, a mother is clinically unaffected and shows no heterotopic gray matter on MRI scan [11].

Treatment of SBH patients with *DCX* mutations is symptomatic and supportive. Individualized treatment strategies should be made according to the type and severity of seizures, EEG findings, and responsiveness for patients with seizures. Antiepileptic drug therapy is the main treatment. Besides, deep brain stimulation has been utilized [15]. More recently, mainly corpus callosotomy and formal temporal lobectomy have been considered as non-pharmacological treatment for refractive epilepsy in SBH patients [6].

5. Conclusion

In conclusion, our study described the detailed clinical features and genetic analysis of two patients with SBH and discovered a novel pathogenic variant in *DCX* by WES with a comprehensive review of publications regarding *DCX* mutations. A total of 153 different *DCX* variants have been reported with the majority of 99 (64.7 %) being missense mutations. Our data further expanded the mutational spectrum of *DCX* and provided insights to better understand SBH and genetic counseling.

Ethics statement

This study was approved by the Medical Ethics Committee of Children's Hospital affiliated to Shandong University (No.: QLET-IRB/

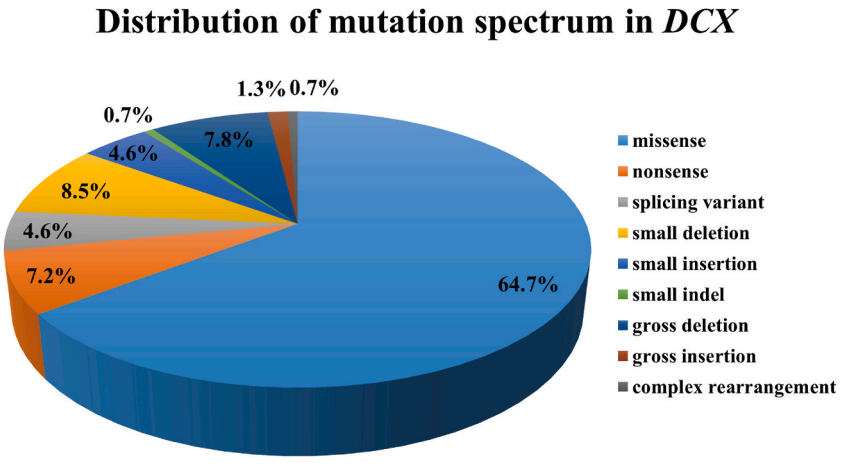

Fig. 2. Distribution of mutation spectrum in *DCX* reported in our study and literature.

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Informed consent

The parents of the child provided their written informed consent to participate in this study. Written informed consent was obtained from the parents of the child for the anonymous publication of the images and data contained in this article.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Chunlai Gao: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Ning Liu:** Methodology. **Jian Ma:** Methodology. **Jianshe Zhao:** Data curation. **Bing Zhao:** Data curation. **Fengling Song:** Data curation. **Rui Dong:** Data curation. **Zilong Li:** Data curation. **Yuqiang Lv:** Data curation. **Yi Liu:** Writing – review & editing. **Zhongtao Gai:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22323.

References

[1] S.M. Herbst, C.R. Proepper, T. Geis, et al., LIS1-associated classic lissencephaly: a retrospective, multicenter survey of the epileptogenic phenotype and response to antiepileptic drugs, Brain & development 38 (4) (2016) 399–406, https://doi.org/10.1016/j.braindev.2015.10.001.
[2] J.R. Lin, J.F. Cheng, Y.T. Liu, et al., Novel lissencephaly-associated DCX variants in the C-terminal DCX domain affect microtubule binding and dynamics, Epilepsia 63 (5) (2022) 1253–1265, https://doi.org/10.1111/epi.17198.
[3] S. Kolbjer, D.A. Martin, M. Pettersson, et al., Lissencephaly in an epilepsy cohort: molecular, radiological and clinical aspects, Eur. J. Paediatr. Neurol. : EJPN : official journal of the European Paediatric Neurology Society 30 (2021) 71–81, https://doi.org/10.1016/j.ejpn.2020.12.011.
[4] S. Richards, N. Aziz, S. Bale, et al., 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. : official journal of the American College of Medical Genetics 17 (5) (2015) 405–424, https://doi.org/10.1038/gim.2015.30.
[5] M. Kato, T. Kimura, C. Lin, et al., A novel mutation of the doublecortin gene in Japanese patients with X-linked lissencephaly and subcortical band heterotopia, Hum. Genet. 104 (4) (1999) 341–344, https://doi.org/10.1007/s004390050963.
[6] A.R. Kurzbuch, B. Cooper, A. Israni, et al., Non-pharmacological treatment options of drug-resistant epilepsy in subcortical band heterotopia: systematic review and illustrative case, Child's nervous system : ChNS : official journal of the International Society for Pediatric Neurosurgery 39 (2) (2023) 451–462, https://doi.org/10.1007/s00381-022-05638-w.
[7] A. Delprato, E. Xiao, D. Manoj, Connecting DCX, COMT and FMR1 in social behavior and cognitive impairment, Behav. Brain Funct. : BBF 18 (1) (2022) 7, https://doi.org/10.1186/s12993-022-00191-7.
[8] N. Bahi-Buisson, I. Souville, F.J. Fourniol, et al., New insights into genotype-phenotype correlations for the doublecortin-related lissencephaly spectrum, Brain : a journal of neurology 136 (Pt 1) (2013) 223–244, https://doi.org/10.1093/brain/aws323.
[9] R. Mahmud, Subcortical band heterotopia presented with refractory epilepsy and reversible aphasia, Cureus 13 (8) (2021), e16990, https://doi.org/10.7759/cureus.16990.
[10] K. Gall, E. Izzo, E.H. Seppälä, et al., Next-generation sequencing in childhood-onset epilepsies: diagnostic yield and impact on neuronal ceroid lipofuscinosis type 2 (CLN2) disease diagnosis, PLoS One 16 (9) (2021), e0255933, https://doi.org/10.1371/journal.pone.0255933.
[11] L. Aigner, G. Uyanik, S. Couillard-Despres, et al., Somatic mosaicism and variable penetrance in doublecortin-associated migration disorders, Neurology 60 (2) (2003) 329–332, https://doi.org/10.1212/01.wnl.0000042091.90361.d2.
[12] R. Guerrini, F. Moro, E. Andermann, et al., Nonsyndromic mental retardation and cryptogenic epilepsy in women with doublecortin gene mutations, Ann. Neurol. 54 (1) (2003) 30–37, https://doi.org/10.1002/ana.10588.

[13] A. Chou, C. Boerkoel, C. du Souich, et al., Phenotypic and molecular characterization of a novel DCX deletion and a review of the literature, Clin. Genet. 76 (2) (2009) 214–218, https://doi.org/10.1111/j.1399-0004.2009.01206.x.
[14] N. Matsumoto, R.J. Leventer, J.A. Kuc, et al., Mutation analysis of the DCX gene and genotype/phenotype correlation in subcortical band heterotopia, Eur. J. Hum. Genet. : EJHG (Eur. J. Hum. Genet.) 9 (1) (2001) 5–12, https://doi.org/10.1038/sj.ejhg.5200548.
[15] A. Franco, J. Pimentel, A.R. Campos, et al., Stimulation of the bilateral anterior nuclei of the thalamus in the treatment of refractory epilepsy: two cases of subcortical band heterotopia, Epileptic Disord. : international epilepsy journal with videotape 18 (4) (2016) 426–430, https://doi.org/10.1684/epd.2016.0878.