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Case report

DOI:10.2478/rrlm-2022-0031

Novel DCX pathogenic variant in a girl with subcortical band heterotopia

Sorina Mihaela Papuc[1*], Magdalena Budisteanu[2,3], Alina Erbescu[1], Virgil Ionescu[4], Catrinel Iliescu[2,5], Carmen Sandu[2], Aurora Arghir[1]

1.Medical Genetics Laboratory, Victor Babes National Institute of Pathology, Romania

2. Department of Pediatric Neurology, Prof. Dr. Alex. Obregia Clinical Hospital of Psychiatry, Romania

3. Department of Genetics, Faculty of Medicine, Titu Maiorescu University, Bucharest, Romania

4. Radiology and Medical Imaging Department, Monza Metropolitan Hospital, Bucharest, Romania

5. Pediatric Neurology Discipline, Carol Davila University of Medicine and Pharmacy, Romania

Abstract

*Subcortical band heterotopia (SBH), is a brain malformation defined by symmetrical and bilateral heterotopic gray matter bands localized deep within the white matter, between the cortex and lateral ventricles. SBH is the result of abnormal neuronal migration, with improper positioning of the cortical neurons. DCX gene (doublecortin), a microtubule-associated protein with essential roles in neuronal migration and differentiation during brain development, is one of the main contributors to the X-linked Lissencephaly spectrum pathogenesis (OMIM #300067). DCX variants are responsible for SBH in females and isolated lissencephaly in males. Herein, we present a 7-year-old girl with a de novo frameshift variant in DCX gene, unreported by date. The patient has focal complex seizures with onset at 23 months of age, fully controlled with medication, mild tremor and coordination impairment of fine movements and some learning difficulties, otherwise with normal development. The brain magnetic resonance imaging revealed the presence of thick SBH. Direct sequencing of DCX gene revealed a pathogenic heterozygous cytosine duplication in exon 3; this frameshift variant leads to a premature stop codon in position 164 (p.Gln160Profs*5). The variant type and its predicted consequence at protein level correlates with the severity of radiological findings. The clinical presentation of our patient is, however, milder than expected. Our research expands the mutational spectrum of DCX gene in SBH females and provides a detailed clinical and imagistic description of the patient. This paper highlights the utility of single gene sequencing as a first-tier diagnostic test of patients with gene-specific phenotypic features.*

Keywords*: Sanger sequencing, seizures, band heterotopia, brain magnetic resonance, abnormal cortical development*

Received: 10th December 2021; Accepted: 22nd June 2022; Published: 8th July 2022

[*] **Corresponding author**: Papuc Sorina Mihaela, Medical Genetics Laboratory, Victor Babes National Institute of Pathology, Romania. E-mail: ela_papuc@yahoo.com

Introduction

Subcortical band heterotopia (SBH), is a brain malformation defined by symmetrical and bilateral heterotopic gray matter bands localized deep within the white matter, between the cortex and lateral ventricles (1,2). The presence of a normal cortical layer and a subjacent heterotopic cortical layer separated by white matter gives the appearance of a "double cortex". This brain malformation is the result of abnormal neuronal migration, with improper positioning of the cortical neurons (2). The phenotype varies widely from nearly unaffected to severe neurodevelopmental problems (3). A vast majority of patients develop seizures in early childhood, SBH being often detected when brain imaging is performed following epilepsy onset. Other clinical features are intellectual disability, behavioural and learning problems, language impairment, hypotonia, visual perception difficulties, and fine motor deficits (1,3).

DCX gene (*XLIS*, doublecortin) is one of the main contributors to the X-linked Lissencephaly spectrum pathogenesis (OMIM #300067), variants in this gene being responsible for SBH in females, while the affected males display isolated lissencephaly (2,4,5). DCX is a microtubule-associated protein (MAP) that plays an essential role in the neuronal migration and differentiation during brain development (6). The mutational spectrum includes all type of variants from large deletions of *DCX* gene to intragenic variants (frameshift, nonsense, splice-site and missense variants). The truncating variants leading to loss of function of the affected allele are frequently reported in sporadic forms of SBH, while missense variants are more prevalent in familial cases with Lissencephaly spectrum disorder (3,7).

Herein, we present a 7-year-old girl with a *de novo* frameshift variant in *DCX* gene unreported to date. The patient has seizures, mild tremor and mild coordination impairment of fine movements and some learning difficulties, with an otherwise normal development. The brain magnetic resonance imaging (MRI) revealed the presence of diffuse, thick subcortical band heterotopia.

This study was approved by the Ethics Committee of institutions where the study took place. Written informed consent for participation in the study and for data publication was obtained from the parents of the patient, before inclusion in the study.

Case presentation

Patient description

The patient is the first child of healthy, nonconsanguineous parents. She was born at term after an uncomplicated pregnancy. Family history is otherwise unremarkable. The psychomotor development was normal. The epileptic seizures first occurred at the age of 23 months - she exhibited the first seizure with hypotonia, unresponsiveness, perioral cyanosis, vomiting, with a duration of 5 minutes, and postictal sleep. At the age of 3 years and 7 months she presented a new seizure with stertorous breathing, left limbs hypertonia, followed by generalized hypertonia, unresponsiveness, head and eyes deviated to the left, with a duration of a few minutes. She continued to present focal seizures (eyes deviation to the left, left limb hypertonia) with secondary generalization from sleep, duration of about 5 minutes, some of them with vomiting. Neurological examination was normal. Wake and Sleep electroencephalogram (EEG) showed spike and spike-wave with high amplitude, predominantly on temporal-occipital derivations, especially in right posterior regions. Antiepileptic treatment with levetiracetam was initiated. Under this treatment she continued to present focal complex seizures with a frequency of 2-5 seizures at every 3-6 months. At the age of 5 years and 9 months the treatment with valproate was initiated, and levetiracetam was withdrawn.

At the last evaluation, at the age of 7 years, she was seizure free (in the last five months), and the clinical and neurological examination was normal except for a mild tremor and mild coordination impairment of fine movements. She is integrated in a regular school, but with some learning difficulties. Sleep EEG showed isolated diffuse spikes.

Brain imaging

MRI (3T) showed symmetric, diffuse thick subcortical band heterotopia which involves both hemispheres; all cerebral lobes are affected, except for the temporal poles (Figure 1.A, B). The cortical thickness was slightly decreased. The thickness of the band was 5-9 mm and the ventricular system was symmetrically disposed with size at the upper limit of the normal range.

Sequence variant analysis

Genomic DNA (gDNA) was isolated from peripheral blood samples using PureLink™. Genomic DNA Mini Kit (Invitrogen) according to manufacturer's protocol. Variant analysis of the coding sequence and the exon-intron boundaries of *DCX* was performed using Sanger sequencing. The PCR amplicons were generated using primers described previously (4). Each PCR reaction was performed in a final volume of 25 µl, containing: 50 ng gDNA, 2.5 µl reaction buffer 10X, 0.75 µl MgCl2 (50 mM), 0.5 µl dNTP (10 mM), 0.5 µl of each primer (10 µM), 0.1 µl Invitrogen Taq Polymerase Recombinant (5 U/µl) (Thermo Fisher Scientific, Waltham, MA) and nuclease free water. Thermocycler parameters were: denaturation at 94°C for 3 minutes, 35 cycles of denaturation 94°C for 45 seconds, primers annealing 56°C for 45 seconds, elongation 72°C for 1 minute, and the final elongation at 72°C for 5 minutes. After amplicons purification with ExoSap-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific), sequencing reaction was performed using BigDye Terminator v3.1 Cycle sequencing kit (Thermo Fisher Scientific) according to manufacturer's protocol. Both forward and reverse products were further direct sequenced on ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The quality evaluation of sequencing products was made using Sequencing Analysis Software (SeqA6) (Thermo Fisher Scientific). The sequences obtained were compared with the wild-type *DCX* (RefSeq NM_001195553.2).

Direct sequencing of *DCX* gene revealed a frameshift variant consisting of a cytosine duplication in exon 3, NG_011750.1:g.15962dup, NM_001195553.2:c.478dup (Figure 1 C); this variant is predicted to create a premature stop codon in position 164 (NP_001182482.1:p.Gln160Profs*5).

This sequence change was not detected in the general population (GnomAD, 1000 Genomes, dbSNP); also, no previous *DCX* lissencephaly

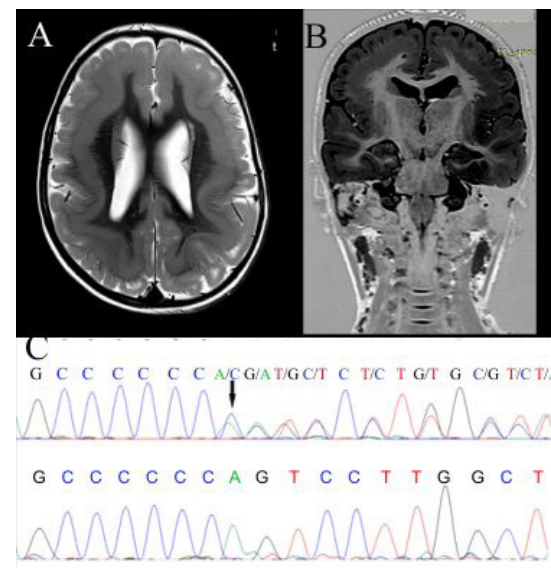

Fig. 1. Brain imaging and genetic data of the patient. Brain MRI showing thick subcortical band heterotopia: T2 - weighted sequence – axial section (A), T1 - weighted sequence - coronal section (B). Sanger sequencing electropherograms of *DCX* showing heterozygous frameshift variant in the child (C, upper sequence) and normal alleles in the mother (C, bottom sequence)

spectrum patients bearing this variant were reported, to the best of our knowledge (Human Gene Mutation Database - http://www.hgmd.org/ or literature). Sanger sequencing of exon 3 of *DCX* gene in the mother showed the normal sequence.

Discussion

We report on a new *de novo* pathogenic frameshift variant in *DCX* gene, in a girl with seizures, mild neurologic features, and SBH.

Subcortical heterotopia (HP:0032391) includes SBH - the most frequent form, and other rare anomalies such as nodular, diffuse ribbon-like, mesial-parasagittal, curvilinear subcortical heterotopia. To date, several genes associated with these conditions were identified, including *ARX, DCX, EML1, KIF2A, NDE1, PAFAH1B1 (LIS1), RELN, TUBA1A, TUBG1, VLDLR, CEP85L* (8,9,10,11).

Based on the overlapping genetic etiology, SBH is classified as part of the lissencephaly spectrum (8). Overall, the majority of SBH patients are females, although rare male patients were also reported. Most females with SBH show an anomaly of *DCX*, both sporadic and familial cases being described. Several studies evaluating DCX variants in SBH females showed a prevalence of 100% in familial cases and varying from 38% to 90% in sporadic cases, due to different inclusion criteria (3,7).

DCX is an important member of MAP family with significant contribution in the organization and stability of microtubules (MT) (12). DCX is predominantly expressed in developing brain, playing a crucial role in neurodevelopment, especially in neuronal migration (6,13). By direct interactions with MT and indirect association with actin filaments, DCX regulates the cytoskeleton dynamics, a critical process in normal formation and development of axons and dendrites of neurons (13).

DCX presents two homologous globular doublecortin domains: the N-terminal DC (N-DC, residues 45–150) and C-terminal DC (C-DC, residues 170–275) connected by a flexible, unstructured linker (residues 151-169) (14). These two DC domains are flanked by an N-terminal unstructured region (residues 1–44) and a serine/proline-rich region at C-terminal end (residues 275–366). Due to 27% sequence identity, N-DC and C-DC have the same folding pattern (ubiquitin-like) (15). Further structural and functional studies showed some differences between these two DC domains, which indicate possible distinct roles in organization and stabilization of MT. Thus, both DC domains bind to assembled MT, but C-DC binds also to unpolymerized tubulin (16). The experimental results indicate that C-DC seems to be important for MT nucleation and for stabilization of tubulin-tubulin contact, while N-DC is essential for MT stabilization (17**)**. The function of the unstructured regions which flank the DC domains has to be elucidated, although these regions are considered to be important regulators of DCX. While no information is currently available regarding the role of N-terminal end of DCX, the C-terminus, which comprises a serine/proline-rich region, is involved in multiple protein-protein interactions (18).

The analysis of pathogenic variant types and their location on *DCX* gene revealed a wide variety of functional consequences at protein level associated with a large spectrum of clinical presentation in SBH females. Missense variants are mainly clustered in the N- and C-DC domains and the affected females present a high variability of their clinical presentations based on the changed residues position in the DCX protein tridimensional structure (3,7). Thus, protein 3D structural studies showed that missense variants in N- and C-DC domains affecting residues which fall at protein surface lead to deficient interactions with microtubules and are associated with a milder

phenotype; missense variants affecting the buried residues of these domains impair the protein stability and lead to more severe clinical presentations. In some particular cases, skewed X-inactivation can explain for phenotype variability in females carrying the same variants (3). Missense variants located in the unstructured N-terminal region were reported in unaffected carrier females. No missense variants were reported in the linker or serine/proline rich regions, in SBH females, indicating an increased tolerance of these regions to this type of sequence variation. Missense variants are more frequently reported in inherited cases, while truncating variant are more prevalent in sporadic cases (3,7).

Truncating variants (gene deletion, frameshift, nonsense, and splice-site variants) seem to occur throughout the entire gene with a higher density in C-DC and usually lead to a severe clinical and imagistic phenotype in SBH females (3,19,20). Our patient has a frameshift variant located in the linker region between N-DC and C-DC, with a predicted stop codon in position 164 and protein truncation with loss of the entire C-DC domain and serine/proline-rich region. The variant occurred *de novo*, which is in agreement with the majority of previous reports.

The degree of protein function impairment generally correlates with the severity of imagistic and clinical presentation. Thus, truncating variants and missense variants which lead to destabilization of protein structure were often reported in females with thick or diffuse thin band heterotopia, while missense variants with minor effect on protein function are reported in females with frontal thin heterotopia or normal carriers. The presence of thick or diffuse thin band heterotopia is associated with moderate to severe intellectual disability, severe language delay, behavioural problems, and epilepsy in SBH females (3).

A limitation of our study is that we report a single case; however, taking into account that SBH is a rare disorder, we consider that each patient brings valuable insights. Moreover, we have compared the genetic and clinical data of our patient with previously published reports in order to interpret it within the larger context of existing knowledge on this disorder.

In conclusion, we report on a 7-year-old girl SBH bearing a novel frameshift variant in *DCX* gene. The type of variant and its predicted consequence at protein level correlates with the severity of radiological findings, the presence of diffuse thick subcortical band heterotopia. The clinical presentation of our patient is, however, milder than expected. The patient had focal complex seizures fully controlled with medication, learning difficulties with mild tremor and mild coordination impairment of fine movements.

Thus, our research expands the mutational spectrum of *DCX* gene in SBH females and provides a detailed clinical and imagistic description of the patient. This paper highlights the utility of single gene sequencing as a first-tier diagnostic test of patients with gene-specific phenotypic features.

Abbreviations

SBH - subcortical band heterotopia
MAP - microtubule-associated protein
MRI - magnetic resonance imaging
EEG - electroencephalogram
gDNA - genomic DNA
MT – microtubules

Acknowledgments

This work was partially supported by grants of the Romanian National Authority for Scientific Research and Innovation CCCDI – UEFISCDI, Projects COFUND-ERANET E-RARE 3-HET-ER-OMICS-2 Number 87/2019 and 88/2019, within PNCDI III, and Ministry of Research and Innovation in Romania, Grant Number PN 19.29.01.03.

Authors' contribution

Substantial contribution to the conception and design of the work (SMP, AA, MB);
Substantial contribution to the acquisition, analysis, or interpretation of data for the work (SMP, AA, MB, AE, VI, CI, CS)
Drafting the manuscript (SMP, AE, VI, CS)
Critically revising the manuscript for important intellectual content (AA, MB, CI).
Final approval of the version to be published (SMP, AA, MB, AE, VI, CI, CS).
Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved (SMP, AA, MB, AE, VI, CI, CS)

Conflict of interest statement

The authors declare that they have no conflict of interest.

References

1. Barkovich AJ, Jackson DJ, Boyer RS. Band heterotopias: a newly recognized neuronal migration anomaly. Radiology. 1989 May;171(2):455-8. DOI: 10.1148/radiology.171.2.2468173
2. Dobyns WB, Andermann E, Andermann F, Czapansky-Beilman D, Dubeau F, Dulac O, et al. X-linked malformations of neuronal migration. Neurology. 1996 Aug;47(2):331-9. DOI: 10.1212/WNL.47.2.331
3. Bahi-Buisson N, Souville I, Fourniol FJ, Toussaint A, Moores CA, Houdusse A, et al. New insights into genotype-phenotype correlations for the doublecortin-related lissencephaly spectrum. Brain. 2013 Jan;136(Pt 1):223-44. DOI: 10.1093/brain/aws323
4. 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 Dec;7(13):2029-37. DOI: 10.1093/hmg/7.13.2029
5. Gleeson JG, Luo RF, Grant PE, Guerrini R, Huttenlocher PR, Berg MJ, et al. Genetic and neuroradiological heterogeneity of double cortex syndrome. Ann Neurol. 2000 Feb;47(2):265-9. DOI: 10.1002/1531-8249(200002)47:2<265::AID-ANA22>3.0.CO;2-N
6. Reiner O. LIS1 and DCX: Implications for Brain Development and Human Disease in Relation to Microtubules. Scientifica. 2013 Mar;2013:1-17. DOI: 10.1155/2013/393975
7. Matsumoto N, Leventer RJ, Kuc JA, Mewborn SK, Dudlicek LL, Ramocki MB, et al. Mutation analysis of the DCX gene and genotype/phenotype correlation in subcortical band heterotopia. Eur J Hum Genet. 2001 Jan;9(1):5-12. DOI: 10.1038/sj.ejhg.5200548
8. Dobyns WB. The clinical patterns and molecular genetics of lissencephaly and subcortical band heterotopia. Epilepsia. 2010 Feb;51(Suppl. 1):5-9. DOI: 10.1111/j.1528-1167.2009.02433.x
9. Bakircioglu M, Carvalho OP, Khurshid M, Cox JJ, Tuysuz B, Barak T, et al. The essential role of centrosomal NDE1 in human cerebral cortex neurogenesis. Am J Hum Genet. 2011 May; 88(5):523-35. DOI: 10.1016/j.ajhg.2011.03.019
10. Watrin F, Manent JB, Cardoso C, Represa A. Causes and Consequences of Gray Matter Heterotopia. CNS Neurosci Ther. 2015 Feb;21(2):112-22. DOI: 10.1111/cns.12322
11. Tsai MH, Muir AM, Wang WJ, Kang YN, Chao NH, Wu MF, et al. Pathogenic variants in CEP85L cause sporadic and familial posterior predominant lissencephaly. Neuron. 2020 Apr;106(2):237-245.e8. DOI: 10.1016/j.neuron.2020.01.027
12. Moslehi M, Ng DCH, Bogoyevitch MA. Dynamic microtubule association of Doublecortin X (DCX) is regulated by its C-terminus. Sci Rep. 2017 Jul;7(1):5245. DOI: 10.1038/s41598-017-05340-x
13. Tint I, Jean D, Baas PW, Black MM. Doublecortin associates with microtubules preferentially in regions of the axon displaying actin-rich protrusive structures. J Neurosci. 2009 Sep;29 (35):10995-1010. DOI: 10.1523/JNEUROSCI.3399-09.2009
14. Moslehi M, Ng DCH, Bogoyevitch MA. Doublecortin X (DCX) serine 28 phosphorylation is a regulatory switch, modulating association of DCX with microtubules and actin filaments. Biochim Biophys Acta Mol Cell Res. 2019 Apr;1866(4):638-49. DOI: 10.1016/j.bbamcr.2019.01.003
15. Burger D, Stihle M, Sharma A, Di Lello P, Benz J, D'Arcy B, et al. Crystal Structures of the Human Doublecortin C- and N-terminal Domains in Complex with Specific Antibodies. J Biol Chem. 2016 Jul;291(31):16292-306. DOI: 10.1074/jbc.M116.726547
16. Kim MH, Cierpicki T, Derewenda U, Krowarsch D, Feng Y, Devedjiev Y, et al. The DCX-domain tandems of doublecortin and doublecortin-like kinase. Nat Struct Biol. 2003 May;10(5):324-33. DOI: 10.1038/nsb918
17. Manka SW, Moores CA. Pseudo-repeats in doublecortin make distinct mechanistic contributions to microtubule regulation. EMBO Rep. 2020 Dec;21(12):e51534. DOI: 10.15252/embr.202051534
18. Tanaka T, Serneo FF, Tseng HC, Kulkarni AB, Tsai

LH, Gleeson JG. Cdk5 phosphorylation of doublecortin ser297 regulates its effect on neuronal migration. Neuron. 2004 Jan;41(2):215-27. DOI: 10.1016/S0896-6273(03)00852-3
19. Haverfield E, Whited A, Petras K, Dobyns W, Das S. Intragenic deletions and duplications of the LIS1 and DCX genes: a major disease-causing mechanism in lissencephaly and subcortical band heterotopia. Eur J Hum Genet. 2009 Jul;17(7):911-8. DOI: 10.1038/ejhg.2008.213
20. Jamuar SS, Walsh CA. Genomic variants and variations in malformations of cortical development. Pediatr Clin North Am. 2015 Apr;62(3):571-585. DOI: 10.1016/j.pcl.2015.03.002