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CASE REPORT

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Novel Mutation *chrX:110644366 C>A* of the DCX Gene in 4-year-old Girl with Sporadic Double Cortex Syndrome

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Abstract

Subcortical band heterotopia (SBH), also known as double cortex syndrome (DC), is listed as a "rare disease" by the Genetic and Rare Diseases Information Center of the National Institutes of Health with an incidence of 1 to 200,000 people in the population. The cause of the disease is mutation in the DCX gene (also known as DBCN, XLIS) on chromosome Xq22.3-q23. SBH is an X-linked dominant disorder. Traditionally, genetic testing for SBH has been done in the order of the probability of detection of mutation according to the radiologic features, but the success rate could be variable with this time-consuming approach.

In this study, novel mutation *chrX: 110644366C>A* in the DCX gene was identified in a 4-year-old Russian girl with sporadic SBH. The present report demonstrates that whole exome sequencing may be a useful tool for the identification of previously known and *de novo* mutations in children with SBH as well as malformations of cortical development. **(Int J Biomed. 2017;7(1):67-70.)**

Key Words: Double cortex syndrome • absence seizures • DCX gene • *de novo* mutations

Introduction

Dysfunction of mechanisms regulating the migration of neuronal precursors leads to neuronal heterotopia.[1] Two common neuronal migration disorders, SBH and the X-linked isolated lissencephaly sequence, have been linked to mutations such as missense, nonsense, aberrant splicing, deletion, and insertions in the X-chromosomal gene of doublecortin (DCX).[2]

Case Presentation

A 4-year-old Russian girl was admitted to the Department of Medical Genetics and Clinical Neurophysiology of our Institute with symptomatic epilepsy and developmental delay. She had atypical absences with eye upward rolling (duration: 30-60 sec; frequency: 1-3 times per day), complex focal seizures with disturbance of the balance, swallowing disorder, and hiccough (duration: 1–5 min; frequency: 1-2 times per day; usually immediately after waking from sleep). She was able to sit and walk. She had normal growth but abnormal development. Her speech was limited to a few words. A physical exam was unremarkable. Lab data including CBC, blood biochemical, and urinalysis results were all within normal limits, but the electroencephalography (EEG) revealed generalized poly spike-wave discharges. Family history was negative for febrile seizures and epilepsy. No similar illness with developmental delay was seen in the family.

The girl was the only child of nonconsanguineous parents. She was born from a planned first pregnancy when the mother was 27 years old. There were no occupational hazards or exposure to mutagens and teratogens during pregnancy. During the first trimester of pregnancy, preeclampsia of moderate severity was identified; during the second trimester - oligohydramnios, fetal malnutrition, increased tone of the uterus, threat of pregnancy termination, treatment in a maternity hospital, stitches on the cervix to prolong pregnancy; during the third trimester - oligohydramnios, fetal malnutrition, breech presentation.

A planned delivery was performed by cesarean section with epidural anesthesia at the gestational age of 38 weeks.

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The newborn's body length was 52 cm, weight of 2400 g. The infant was discharged from the hospital a week later. She was subsequently hospitalized to the Krasnoyarsk Perinatal Center. After discharge, she continued to be monitored by a neurologist with regular follow-ups. Rare atypical absence seizures with eyes rolling back debuted at the age of 3 months, but antiepileptic drugs were not prescribed; neither brain MRI nor EEG video monitoring was performed. At the age of 13 months, she had a cluster of complex adversely afebrile seizures with turning of the head and eyes to the left. This episode developed a week after acute respiratory infection with febrile fever. Isolated seizures in this cluster lasted up to 20 seconds, and the cluster up to 20 minutes. First aid was provided by emergency medical care, but the girl was not admitted to hospital. She was seen by a consultant neurologist the following day. A routine EEG in wakefulness was conducted and epileptiform activity was not registered, but antiepileptic therapy was prescribed: Depakine chronosphere (valproic acid) 50 mg in the morning and 50 mg in the evening. Suspected diagnosis was cryptogenic epilepsy (ICD - 10: G40.9). The therapeutic effect was insufficient: short, rare, atypical absence persisted with a frequency up to once per month. However, antiepileptic drugs were canceled after 6 months from start of the treatment. EEG video monitoring was not performed. The patient had a rare atypical absence seizures after discontinuation of treatment. Her parents consulted different neurologists, but antiepileptic drugs were not prescribed.

A brain MRI (Epilepsy Protocol at 1.5 Tesla) revealed SBH for the first time at age 4 years. At this age, complex focal seizures with disturbance of balance, swallowing disorder and hiccough debuted (June 2016). Frequency of seizures increased in August 2016. However, the girl did not take anti-epileptic drugs. The child's parents asked for consultation with a neurologist-epileptologist and neurogeneticist at the Neurological Center of Epileptology, Neurogenetics and Brain Research of the University Clinic of Krasnoyarsk State Medical University named after Prof. V. F. Voino-Yasenetsky. The purpose of consultation was to specify the hereditary nature of the disease in their daughter, select antiepileptic therapy, and specify genetic risk of the disease**.** The girl's parents wanted to have a second child who would be healthy.

DNA was extracted from peripheral blood using in-house operating procedures. Mutations were identified through whole exome sequencing using the next-generation Illumina NextSeq 500 DNA capture method, with an average coverage of at least 70-100x, performed at the Sequencing Facility of GENOMED laboratory (Moscow). After excluding lissencephaly-related genes, novel mutation *chrX:110644366C>A* in the DCX gene was identified. We recommended Sanger sequencing of the patient and her parents to validate this new mutation, not registered in OMIM, and to specify the nature of the mutation (sporadic cases or X-linked dominant). Further Sanger sequencing (GENOMED laboratory, Moscow) validated the variant in the patient but not in both parents, indicating the *de novo* mutation *chrX:110644366C>A* the DCX gene. It was concluded that the results of Sanger sequencing in peripheral blood lymphocytes cannot exclude the germinal and somatic mosaicism. It has been observed that patients with less that 30% mosaicism are clinically unaffected, whereas those with more than 30% of the cells with the mutated allele are symptomatic with SBH.[3] Since the pathogenic mutation has been identified in the family, prenatal testing for pregnancies at increased risk is possible.

Thus, clinical diagnosis of DCX-related SBH (OMIM: 300067; ICD - 10: Q04.8) and symptomatic focal epilepsy (ICD - 10: G40.2) was updated at 4 years of age in December 2016. Currently, the girl is taking the anti-epileptic drug Depakine chronosphere 100 mg per day. The level of valproic acid in serum is in the middle band (65–85 μg/mL) of the therapeutic range. No adverse drug reactions are noted. EEG video monitoring has not detected focal epileptiform activity. Atypical absences have not been registered. Complex focal seizures with hiccoughs have become very short (3-5 seconds) and rare (1-2 per month). The girl's behavior and speech development have improved. She has become interested in toys, and she likes her mother to read children's books to her.

Discussion

Gleeson et al. (1998) and des Portes et al. (1998) determined that the DCX gene spans over 100 kb of DNA and contains 9 exons with 6 coding exons (OMIM:300121). The structure of the gene is unusual because only 16% of the sequence is coding, and the 3-prime untranslated region, which is contained in 1 exon, is 7.9 kb long.[4] Using in situ hybridization, des Portes et al.[5] observed DCX gene expression in human cerebral cortex at 21 weeks' gestational age. There was strong labeling in the ventricular zone and cortical plate and moderate labeling of the intermediate zone. In the intermediate zone, labeled cells were organized as oriented chains, suggestive of migrating neurons.

To date, 40 various mutations have been identified resulting in nonsense, splice site, and missense mutations throughout the DCX gene,[6] but no clear correlation has been observed between the clinical severity and mutation profiles.[7] The proportion of cases caused by *de novo* mutations of DCX gene is unknown.

DCX-related SBH (OMIM: 300067) is a rare neuronal migration disorder deriving from mutations in DCX gene located on chromosome Xq22.3-q23 [4,8,9] in most patients, and is usually associated with medically intractable epilepsy. About one third of patients with SBH have an association of tonic–clonic and myoclonic seizures with atypical absences and drop attacks.[10-13] However, focal clinical signs such as head deviation or clonic movements of one limb at seizure onset observed in some individuals suggest the diagnosis of focal partial epilepsy.[10-12] Infantile spasms and Lennox–Gastaut syndrome also have been reported.[14] About 60% of the patients have focal lobar or multifocal epileptic abnormalities,[10,11,14] but EEG findings are usually characterized by generalized slow spike-and-wave or polyspike and wave, and multifocal spiking.[11-13] In individuals with SBH, cognitive abilities range from normal to learning disabilities and/or severe intellectual disability. Behavior problems may also be observed. The severity of the clinical symptoms correlates with the degree of the underlying brain malformation.[10-12]

Based on the MRI findings,[18] DCX-related SBH is characterized by symmetric bands of gray matter within the white matter between and parallel to the cortex and the lateral ventricles, which appears as an isointense second cortical structure beneath the cortex (double cortex). The cerebral cortex in SBH may appear normal and/or thickened with or without simplified gyration.[14-16] DCX-related SBH is predominantly located in the frontoparietal lobe and is grade 6 (complete band heterotopia). Grade 5, a more severe malformation that overlaps with classic lissencephaly and band heterotopia, is characterized by SBH in the occipital regions and pachygyria in the frontal regions.[17]

Children diagnosed with a SBH may either have inherited the DCX pathogenic variant from their asymptomatic or only mildly affected mother or have the disorder as the result of a *de novo* DCX pathogenic variant. A detailed family history should be obtained. Special attention should be paid to epilepsy, miscarriages, stillbirths, children who died at a young age without obvious birth defects, and cognitive impairment or developmental delay.[18] Penetrance in females heterozygous for DCX pathogenic variants is greater than 90%; however, heterozygous females with missense and nonsense variants may have no obvious brain malformation or seizures.[19] Approximately 10% of unaffected mothers of children with a DCX pathogenic variant were reported to have somatic mosaicism or germline mosaicism.[18] Somatic mosaicism should, whenever possible, be further explored or confirmed by analysis of DNA from different tissues (e.g., hair roots, buccal swabs).[20] If the DCX pathogenic variant is not identified in the mother, neurologic and/or clinical examination of the mother is warranted. If cerebral MR imaging reveals SBH in the mother, additional maternal tissues should be examined for the DCX pathogenic variant identified in her offspring.[18] Cerebral MRI of the mother can be helpful because some heterozygous females with SBH can be asymptomatic.[18,20,21]

If the pathogenic variant has been identified in the family, carrier testing for at-risk family members and prenatal testing for pregnancies at increased risk are possible.. The optimal time for determination of genetic risk, clarification of carrier status, and discussion of the availability of prenatal testing is before pregnancy. During fetal development, first gyri appear around the 20th week of gestation and a reduced gyration pattern when compared to postnatal images remains physiologic until late gestation. Therefore, in the absence of a positive family history, DCX-related SBH may not be recognized until birth by fetal ultrasonography. However, Zhagn K. et al.[22] identified 11 reports of fetal gray matter heterotopias from 1998 to 2015, involving 43 cases with prenatal diagnoses. Of the total of 44 cases (including one case of the authors), 32 cases that had been confirmed postpartum had prenatal ultrasound and MRI data, which showed a significantly lower detection rates of fetal gray matter heterotopias by prenatal ultrasound than by MRI (43.8% vs 93.8%, $P<0.001$).

Prenatal ultrasound can only detect subependymal heterotopia with characteristic manifestations, and the detection of other types of fetal gray matter heterotopias relies on MRI, which is currently the best option for prenatal diagnosis of fetal gray matter heterotopias, including SBH. [23]

Summary, the diagnosis of *DCX*-related SBH is suspected on MRI findings and confirmed by molecular genetic testing. For patients with suspected SBH, sequence analysis is recommended as the first step in mutation identification. Sequence analysis of the DCX gene has been shown to identify 100% of mutations in families with more than one affected family member. However, mutations in the promoter region, some mutations in the introns, other regulatory element mutations, and large deletions cannot be detected by this analysis.

Conclusion

SBH or double cortex syndrome is a rare neuronal migration disorder, classically present with seizures and intellectual impairment and is seen almost exclusively in females.[24-26] It is an X-linked genetic disorder with DCX gene mutation being the causative factor in most of the cases, [24,27] but the proportion of cases caused by *de novo* mutations of DCX gene is unknown.

We are presenting the first case of the novel mutation DCX-related SBH from the city of Krasnoyarsk (the Russian Federation). As described in our case, a girl with developmental delay and symptomatic epilepsy was treated with antiepileptic medications without any specific diagnosis, and the result was that the parents did not adhere to therapy because they were not given sufficient information. However, by using MRI (Epilepsy Protocol at 1.5 Tesla) and new methods of molecular genetics (whole exome sequencing and Sanger sequencing) on this patient, a specific diagnosis was made, information regarding the management and prognosis was provided to parents, and as a result, her seizures were more effectively controlled. In the case of a female patient with SBH, this usually means two possibilities: a *de novo* mutation or an inherited mutation from a heterozygous asymptomatic mother. In the family presented here a female patient showed typical clinical and imagiological phenotype of SBH. We identified the novel mutation *chrX:110644366C>A* in the DCX gene in the patient, but not in either parent.

The present report demonstrates that whole exome sequencing may be a useful tool for the identification of previously known and *de novo* mutations in children with SBH as well as malformations of cortical development.

The proper use of imaging and molecular genetics modalities leads to a clear diagnosis and a management plan; the use of prenatal testing and genetic counseling offers great benefits on preventing such syndromes in future offspring.

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Competing interests

The authors declare that they have no competing interests.

References

1. Couillard-Despres S, Winkler J, Uyanik G, Aigner L. Molecular mechanisms of neuronal migration disorders, quovadis? Curr Mol Med. 2001;1(6):677–88.
2. Couillard-Despres S, Uyanik G, Ploetz S, Karl C, Koch H, Winkler J, Aigner L. Mitotic impairment by doublecortin mutations found in patients. Neurogenetics. 2004;5(2):83-93.
3. Bahi-Buisson N1, 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;136(Pt 1): 223–44. doi: 10.1093/brain/aws323.
4. Gleeson JG, Allen KM, Fox JW, Lamperti ED, Berkovic S, Scheffer I, et al. Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. Cell 1998;92(1):63-72.
5. des Portes V, Pinard JM, Billuart P, Vinet MC, Koulakoff A, Carrié A, et al. A novel CNS gene required for neuronal migration and involved in X-linked subcortical laminar heterotopia and lissencephaly syndrome. Cell 1998;92(1):51-61.
6. Sapir T, Horesh D, Caspi M, Atlas R, Burgess HA, Wolf SG, et al. Doublecortin mutations cluster in evolutionarily conserved functional domains. Hum Mol Genet. 2000;9(5):703-12.
7. des Portes V, Francis F, Pinard JM, Desguerre I, Moutard ML, Snoeck I, et al. Doublecortin is the major gene causing X-linked subcortical laminar heterotopias (SHLH). Hum Mol Genet. 1998;7(7):1063–70.
8. Dobyns WB, Andermann E, Andermann F, Czapansky-Beilman D, Dubeau F, Dulac O, et al. X-linked malformations of neuronal migration. Neurology. 1996;47(2):331–9.
9. Ross ME, Allen KM, Srivastava AK, Featherstone T, Gleeson JG, Hirsch B, et al. Linkage and physical mapping of X-linked lissencephaly/SBH (XLIS): a gene causing neuronal migration defects in human brain. Hum Mol Genet. 1997;6(4):555–62.
10. Palmini A, Andermann F, Aicardi J, Dulac O, Chaves F, Ponsot G, et al. Diffuse cortical dysplasia, or the "double cortex" syndrome: the clinical and epileptic spectrum in 10 patients. Neurology. 1991;41(10):1656–62.
11. Granata T, Battaglia G, D'Incerti L, Franceschetti S, Zucca C, Savoiardo M, Avanzini G. Double cortex syndrome: electroclinical study of three cases. Ital J Neurol Sci. 1994;15(1):15–23.
12. Parmeggiani A, Santucci M, Ambrosetto P, Amadi A, Baioni E, Rossi PG. Interictal EEG findings in two cases with "double cortex"syndrome. Brain Dev. 1994;16(4):320–4.
13. Ricci S, Cusmai R, Fariello G, Fusco L, Vigevano F. Double cortex. A neuronal migration anomaly as a possible cause of Lennox-Gastaut syndrome. Arch Neurol. 1992;49(1):61–4.
14. Barkovich AJ, Guerrini R, Battaglia G, Kalifa G, N'Guyen T, Parmeggiani A, et al. Band heterotopia: correlation of outcome with magnetic resonance imaging parameters. Ann Neurol. 1994;36(4):609–17.
15. Guerrini R, Filippi T. Neuronal migration disorders, genetics, and epileptogenesis. J Child Neurol. 2005;20(4):287–99.
16. Shnayder NA, Dmitrenko DV, Govorina YB, Kantimirova EA, Alekseeva OV, Molgachev AA, Makarkin AA. The late diagnosis of double cortex syndrome in a 36-year-old woman with resistant atonic seizures. Neurology, Neuropsychiatry, Psychosomatics. 2015;7(3):40-45. doi: 10.14412/2074-2711-2015-3-40-45. [Article in Russian].
17. Dobyns WB, Truwit CL, Ross ME, Matsumoto N, Pilz DT, Ledbetter DH, et al. Differences in the gyral pattern distinguish chromosome 17-linked and X-linked lissencephaly. Neurology. 1999;53(2):270–7.
18. Hehr U, Uyanik G, Aigner L, Couillard-Despres S, Winkler J. *DCX*-Related Disorders. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Ledbetter N, Mefford HC, Smith RJH, Stephens K, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2017. 2007 Oct 19 [updated 2011 Mar 24].
19. Aigner L, Uyanik G, Couillard-Despres S, Ploetz S, Wolff G, Morris-Rosendahl D, et al. Somatic mosaicism and variable penetrance in doublecortin-associated migration disorders. Neurology. 2003;60(2):329–32.
20. Demelas L, Serra G, Conti M, Achene A, Mastropaolo C, Matsumoto N, et al. Incomplete penetrance with normal MRI in a woman with germline mutation of the DCX gene. Neurology. 2001;57(2):327–30.
21. Moreira I, Bastos-Ferreira R, Silva J, Ribeiro C, Alonso I, Chaves J. Paternal transmission of subcortical band heterotopia through DCX somatic mosaicism. Seizure. 2015;25:62-4. doi: 10.1016/j.seizure.2014.12.005.
22. Zhagn K, Li S, Wen H, Yuan Y. Prenatal diagnosis of fetal gray matter heteropia in one case and literature review. Nan Fang Yi Ke Da Xue Xue Bao. 2015;35(12):1770-4. [Article in Chinese].
23. Levine D. Fetal magnetic resonance imaging. J Matern Fetal Neonatal Med. 2004;15(2):85-94.
24. Bahi-Buisson N, Souville I, Fourniol FJ, Toussaint A, Moores CA, Houdusse A, et al; SBH-LIS European Consortium. New insights into genotype-phenotype correlations for the doublecortin-related lissencephaly spectrum. Brain. 2013;136(Pt 1):223-44. doi: 10.1093/brain/aws323.
25. Parisi P, Miano S, Mei D, Paolino MC, Castaldo R, Villa MP. Diffuse subcortical band heterotopia, periodic limb movements during sleep and a novel "de novo" mutation in the DCX gene. Brain Dev. 2010;32(6):511–5. doi: 10.1016/j.braindev.2009.06.007.
26. Dericioglu N, Oguz KK, Ergun EL, Tezer FI, Saygi S. Ictal/interictal EEG patterns and functional neuroimaging findings in subcortical band heterotopia: Report of three cases and review of the literature. Clin EEG Neurosci. 2008;39(1):43–9.
27. Kaur S, Ghuman MS, Devarajan LJ. A pediatric epilepsy classic: "Double cortex" syndrome. J Pediatr Neurosci. 2015;10(2):125-6. doi: 10.4103/1817-1745.159201.