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CASE REPORT

EPHA4 GENETIC VARIANT IN A PATIENT WITH EPILEPSY, OPHTHALMOLOGICAL ANOMALIES, AND NEURODEVELOPMENTAL DELAY

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ABSTRACT

We present the findings of a Whole Exome Sequencing in a 2-year-old boy, conceived via *In Vitro* Fertilization with donor sperm, who suffers from an undiagnosed neurological syndrome. The following heterozygous variant in the *EPHA4* gene was identified and classified as likely pathogenic: c.1655_1656, p.(Ser552CysfsTer23). Subsequent segregation analysis showed that the variant was not inherited from the mother and the sperm donor is not accessible for genetic testing. The presented results can further expand upon the genetic variants considered when diagnosing complex neurological syndromes and shows the importance of access to biological samples from donor banks in genetically ambiguous cases.

Keywords: *EPHA4*, Whole Exome Sequencing, Epilepsy, Novel genetic variant, Ophthalmological Anomalies, Neurodevelopmental Delay

INTRODUCTION

The clinical presentation of most early-onset neurological disorders is ambiguous due to their heterogeneous manifestation and symptom non-specificity (1). In recent years, genetic testing has become a useful diagnostic tool for identifying genetic mutations associated with rare neurological disorders. However, even Whole Exome Sequencing (WES) – a technique which allows for analysis of all exons in a patient's genome – often results in the identification of multiple genetic variants which may potentially explain a patient's complex clinical picture. In such cases, segregation analysis becomes an indispensable method for clarifying the significance of the variants. The diagnostic process is further complicated if one of the parents is not available for segregation analysis, which is the case in *In Vitro* Fertilization (IVF) with donor material.

In the following case study, we present a patient with a complex neurological syndrome with accompanying facial abnormalities, who was conceived through IVF with donor sperm. Via analysis of the WES data one heterozygous genetic variant in the *EPHA4* gene was selected as a target.

The Ephrin Receptor A4 (*EPHA4*) gene, located on the long arm of human chromosome 2 (2q36.1), is a protein encoding gene producing a Protein Tyrosine Kinase (PTK) receptor. Although within the Central Nervous System (CNS) *EPHA4* has been implicated in processes such as neural migration, axonal proliferation, and synaptic plasticity (2), its pathogenicity in clinical practice is not well understood. In humans, thus far, only one germline likely pathogenic missense point mutation has been reported in a male patient with atypical cerebral palsy (3) and Van Hoecke *et al.* (4) showed that decreased *EPHA4* expression was significantly correlated with later onset of Amyotrophic Lateral Sclerosis (ALS). Light *et al.* (2) have reported several somatic genetic variants in relation to melanoma tumors. On the other hand, studies with animal models, ranging from rodents to primates, have shown that EphA4 expression plays a role in various severe CNS disorders. Fu *et al.* (5) showed that blocking EphA4 activity

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in mice had a positive effect on the hippocampal plasticity, typically ravaged by Alzheimer's disease. Goldshmit and Bourne (6) found that astrocytic upregulation of EphA4 in non-human primates has an indirect inhibitory effect on axonal regrowth and regeneration following a Traumatic Brain Injury (TBI).

CASE PRESENTATION

A 2-year-old Caucasian male was referred to our laboratory for genetic clarification of a non-specified neurological syndrome with developmental delay and facial abnormalities. The case history revealed that the patient was conceived via IVF with sperm from an unidentified Caucasian donor. The mother was healthy with no neurological disorders or genetic abnormalities, and there were no complications during pregnancy and birth (patient birth weight and height – 3490g and 50cm, respectively).

Anamnesis revealed that at approximately 4 months of age the patient's condition started deteriorating as indicated by delayed psychomotor, cognitive, and visual development. At around the same time, the patient started suffering from grand-mal seizures. At approximately 7 months of age, the patient already exhibited severe drug-resistant epilepsy, significant psychomotor retardation despite physiotherapy, limb hypertonia, loss of pupillary light response, and nearly complete loss of visual acuity, which rendered him effectively blind. He also exhibited peculiar dysmorphic facial features with hypertelorism, micrognathia, and unusually low auricles. During the following 5 months he suffered a number of additional medical complications including abnormal elevation of Vitamins B1 and B12, pneumonia, and anemia, some of these complications led to hospitalization.

Although the patient's epilepsy and eye abnormalities have continued to aggravate, multiple neurological and ophthalmological examinations have revealed no apparent cause of the patient's complex medical state. Furthermore, subsequent metabolic and biochemical blood tests were negative for lysosomal enzymes, Very Long Chain Fatty Acids (VLCFA), amino acids and acylcarnitines, and 3-O-Methyldopa (3-OMD). A dry blood spot test for Neuronal Ceroid Lipofuscinosis (NCL) was also negative. Initial genetic testing has shown a normal karyotype, no mutations in a targeted 341 gene retinal degeneration-related panel, and no mutations in the mitochondrial genome. Following from the patient's increasingly worsening condition and the absence of any effective treatment, he was referred for WES in hopes of determining his diagnosis.

METHODS

After a detailed explanation of all procedures, a written informed consent was obtained from the patient's mother, and all described medical procedures and analyses were conducted in accordance with the Declaration of Helsinki and the ethical guidelines of Medical University Sofia. The Ethics Committee of Medical University Sofia has approved this study.

A blood sample was taken from the patient and subjected to DNA extraction by standard salting-out procedure (7). WES was performed and the patient's genetic profile was analyzed via the GenesearchNGS software (Phenosystems). The detected variants were interpreted with respect to their pathogenicity following the recommendations of the American College of Medical Genetics and Genomics/ Association for Molecular Pathology (ACMG/AMP) (8). Direct Sanger sequencing was performed with BigDye® Terminator cycle sequencing kit v.3.1 (Applied Biosystems, Foster City, CA, USA) for confirmation of the WES findings and for Segregation analysis in order to determine the variants' inheritance. Due to the ethical standards of IVF with donor sperm, no genetic analyses of the biological father were possible.

RESULTS

We identified a heterozygous frameshift variant c.1655_1656del, p.(Ser552CysfsTer23) in the *EPHA4* gene (NM_004438.5). This variant is classified as likely pathogenic (categories: PVS1 and PM2) (8). Segregation analysis showed that the patient's mother is not a carrier of the genetic variant. The biological father is not accessible for genetic testing due to ethical and legislative issues as well as the anonymous process of sperm donation for IVF procedures.

DISCUSSION

Mutations in the *EPHA4* gene are compatible with optic nerve defects and severe abnormalities in the central nerve system. The reasons behind our focus on the genetic variant c.1655_1656del, p.(Ser552CysfsTer23) in the *EPHA4* gene are described below.

MetaDome (9) indicates that the identified *EPHA4* genetic variant is located in the beginning of the protein's transmembrane domain (IPR027936; Figure 1). This type of domain is characteristic for ephrin receptors and is responsible for the oligomerization of these receptors and for successful signaling (IPR027936) (10). Due to this

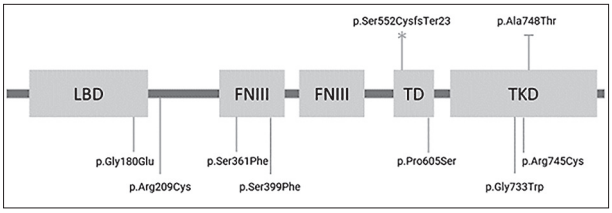

Figure 1. The genetic variant found in our patient and reported genetic variants presented in relation to the Epha4 protein domains. Above the protein domains are shown the genetic variant found in our patient (marked with *) and the likely pathogenic variant reported in ClinVar (marked with -), and below the protein domains - the somatic genetic variants reported by Light *et al.* (2). Domain names are the following: LBD – Ligand Binding Domain; FNIII – Fibronectin type III domain; TD – Transmembrane Domain and TKD – Tyrosine Kinase Domain. The protein domain structure is created based on MetaDome (9).

localization, the frameshift variant could affect the protein's ability to integrate into the cell membrane and function as an ephrin receptor in the CNS. To the best of our knowledge, this is the first identification of a frameshift genetic variant in the transmembrane domain of the EphA4 protein. The only reported likely pathogenic variant in ClinVar (3) falls within the kinase region of the protein.

Moreover, the genetic variant is not found in the gnomAD v2.1.1 controls and *EPHA4*'s pLI score is 1, which indicates that the gene is highly intolerant to loss of function variants (11,12). As the genetic variant does not abide the 50-55 nt boundary rule (13), we can assume that the produced mRNA undergoes nonsense-mediated decay. It has been suggested previously that mutations in ephrin receptor genes, causative of nonsense-mediated decay, lead to pathogenesis (14).

Recently, a number of *EPHA4*-cases emerged in the GeneMatcher platform (15), helping understand the pathophysiology of severe neurological cases having *EPHA4* gene variants in common. Based on this new understanding, the variation in *EPHA4* seems highly compatible with our patient's clinical manifestation. Unfortunately, we cannot confirm that the genetic variant has occurred *de novo*, due to the lack of a paternal sample. Furthermore, Oliver *et al.* (16) have published a comprehensive list of epilepsy-related genes, wherein *EPHA4* is not included. With this study we bring attention to the *EPHA4* gene as a potential target for additional functional studies in association with neurodevelopmental disorders, including epilepsy. Moreover, we emphasize on the difficulties in classifying genetic variants when DNA from donors is not available for genetic testing.

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CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

DATA AVAILABILITY STATEMENT

The genetic variant reported in this study is openly available in ClinVar with accession number: SCV003925746.

REFERENCES

1. Helbig, I. and Abou Tayoun, A. N. (2016) 'Understanding Genotypes and Phenotypes in Epileptic Encephalopathies', *Molecular Syndromology*, 7(4), pp. 172–181.
2. Light, T. P., Gomez-Soler, M., Wang, Z., Karl, K., Zapata-Mercado, E., Gehring, M. P., Lechtenberg, B. C., Pogorelov, T. V., Hristova, K. and Pasquale, E. B. (2021) 'A cancer mutation promotes EphA4 oligomerization and signaling by altering the conformation of the SAM domain', *Journal of Biological Chemistry*, 297(1), p. 100876.
3. National Center for Biotechnology Information. ClinVar (no date) *[VCV000431727.1]*.
4. Van Hoecke, A., Schoonaert, L., Lemmens, R., Timmers, M., Staats, K. A., Laird, A. S., Peeters, E., Philips, T., Goris, A., Dubois, B., *et al.* (2012) 'EPHA4 is a disease modifier of amyotrophic lateral sclerosis in animal models and in humans', *Nature Medicine*, 18(9), pp. 1418–1422.
5. Fu, A. K. Y., Hung, K.-W., Huang, H., Gu, S., Shen, Y., Cheng, E. Y. L., Ip, F. C. F., Huang, X., Fu, W.-Y. and Ip, N. Y. (2014) 'Blockade of EphA4 signaling ameliorates hippocampal synaptic dysfunctions in mouse models of Alzheimer's disease', *Proceedings of the National Academy of Sciences*, 111(27), pp. 9959–9964.

6. Goldshmit, Y. and Bourne, J. (2010) 'Upregulation of EphA4 on Astrocytes Potentially Mediates Astrocytic Gliosis after Cortical Lesion in the Marmoset Monkey', *Journal of Neurotrauma*, 27(7), pp. 1321–1332.
7. Miller, S. A., Dykes, D. D. and Polesky, H. F. (1988) 'A simple salting out procedure for extracting DNA from human nucleated cells', *Nucleic Acids Research*, 16(3), pp. 1215–1215.
8. Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., *et al.* (2015) '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', *Genetics in Medicine*, 17(5), pp. 405–424.
9. Wiel, L., Baakman, C., Gilissen, D., Veltman, J. A., Vriend, G. and Gilissen, C. (2019) 'MetaDome: Pathogenicity analysis of genetic variants through aggregation of homologous human protein domains', *Human Mutation*, p. humu.23798.
10. Paysan-Lafosse, T., Blum, M., Chuguransky, S., Grego, T., Pinto, B. L., Salazar, G. A., Bileschi, M. L., Bork, P., Bridge, A., Colwell, L., *et al.* (2023) 'InterPro in 2022', *Nucleic Acids Research*, 51(D1), pp. D418–D427.
11. Fuller, Z. L., Berg, J. J., Mostafavi, H., Sella, G. and Przeworski, M. (2019) 'Measuring intolerance to mutation in human genetics', *Nature Genetics*, 51(5), pp. 772–776.
12. Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alföldi, J., Wang, Q., Collins, R. L., Laricchia, K. M., Ganna, A., Birnbaum, D. P., *et al.* (2020) 'The mutational constraint spectrum quantified from variation in 141,456 humans', *Nature*, 581(7809), pp. 434–443.
13. Nagy, E. and Maquat, L. E. (1998) 'A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance', *Trends in Biochemical Sciences*, 23(6), pp. 198–199.
14. Arvanitis, D. N. and Davy, A. (2012) 'Regulation and misregulation of Eph/ephrin expression', *Cell Adhesion & Migration*, 6(2), pp. 131–137.
15. Sobreira, N., Schiettecatte, F., Valle, D. and Hamosh, A. (2015) 'GeneMatcher: A Matching Tool for Connecting Investigators with an Interest in the Same Gene', *Human Mutation*, 36(10), pp. 928–930.
16. Oliver, K. L., Scheffer, I. E., Bennett, M. F., Grinton, B. E., Bahlo, M. and Berkovic, S. F. (2023) 'Genes4Epilepsy: An epilepsy gene resource', *Epilepsia*, 64(5), pp. 1368-1375.