Check for updates

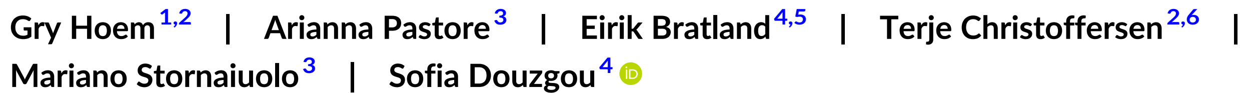
Received: 15 September 2023 | Revised: 16 January 2024 | Accepted: 30 January 2024

DOI: 10.1111/cge.14499

SHORT REPORT

CLINICAL GENETICS WILEY

Severe isolated exudative vitreoretinopathy caused by biallelic *FZD4* variants



Gry Hoem[1,2] | Arianna Pastore[3] | Eirik Bratland[4,5] | Terje Christoffersen[2,6] | Mariano Stornaiuolo[3] | Sofia Douzgou[4]

[1]Department of Medical Genetics, University Hospital of North Norway, Tromsø, Norway

[2]Department of Clinical Medicine, UiT – The Arctic University of Norway, Tromsø, Norway

[3]Department of Pharmacy, University of Naples Federico II, Naples, Italy

[4]Department of Medical Genetics, Haukeland University Hospital, Bergen, Norway

[5]Department of Clinical Science, University of Bergen, Bergen, Norway

[6]Department of Ophthalmology, University Hospital of North Norway, Tromsø, Norway

Correspondence
Gry Hoem, Department of Medical Genetics, University Hospital of North Norway, Tromsø, Norway and Department of Clinical Medicine, UiT – The Arctic University of Norway, Tromsø, Norway.
Email: gry.hoem@unn.no

Funding information
Norwegian National Advisory Unit on Rare Disorders, Grant/Award Number: 43066

Abstract

Familial exudative vitreoretinopathy (FEVR) is linked to disruption of the Norrin/Frizzled-4 signaling pathway, which plays an important role in retinal angiogenesis. Severe or complete knock-down of proteins in the pathway also causes syndromic forms of the condition. Both heterozygous and biallelic pathogenic variants in the *FZD4* gene, encoding the pathway's key protein frizzled-4, are known to cause FEVR. However, it is not clear what effect different *FZD4* variants have, and whether extraocular features should be expected in those with biallelic pathogenic *FZD4* variants. Biallelic *FZD4* variants were found in a young boy with isolated, severe FEVR. His parents were heterozygous for one variant each and reported normal vision. In-vitro studies of the two variants, demonstrated that it was *the combination* of the two which led to severe inhibition of the Norrin/Frizzled-4 pathway. Our observations demonstrate that biallelic *FZD4*-variants are associated with a severe form of FEVR, which does not necessarily include extraocular features. In addition, variants causing severe FEVR in combination, may have no or minimal effect in heterozygous parents as non-penetrance is also a major feature in dominant FZD4-FEVR disease. This underscores the importance of genetic testing of individuals and families with FEVR.

KEYWORDS

familial exudative vitreoretinopathy, genotype, heterozygote, human genetics, retinal disease

1 | INTRODUCTION

Familial exudative vitreoretinopathy (FEVR) is a rare disorder characterized by abnormal retinal angiogenesis and incomplete vascularization of the peripheral retina which can lead to visual loss.[1] FEVR is clinically variable, even within the same family and early diagnosis is crucial in improving visual outcomes. While mildly affected individuals may have asymptomatic, small, avascular retinal areas only visible by fluorescein angiography, severely affected ones present with congenital, profound visual impairment.[2] FEVR can also be progressive.[1] This is more common in children and adolescents, but apparently stable disease may suddenly advance and cause vision threatening complications at any age.[3]

FEVR is genetically heterogeneous and can be caused by pathogenic variants in over 12 genes resulting in both isolated and multisystem conditions and following all types of inheritance. Four of these

Mariano Stornaiuolo and Sofia Douzgou shared last authorship.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2024 The Authors. *Clinical Genetics* published by John Wiley & Sons Ltd.

genes (*FZD4*—OMIM* 604579, *NDP*—OMIM* 300658, *LRP5*—OMIM* 603506, and *TSPAN12*—OMIM* 613138) are part of the Norrin/Frizzled-4 and Wnt signaling pathways, play important roles in retinal angiogenesis[4] and represent the most frequent genetic forms of FEVR.[5]

A genotype–phenotype correlations has been postulated for variants in *NDP*, *FZD4*, *LRP5*, and *TSPAN12* based on their distinct roles in the norrin-*FZD4*- pathway.[6] Norrin binds to Frizzled-4 (FZD4), which then forms a complex with LRP5. TSPAN12 acts as a co-receptor, enhancing signaling transduction, but it is not mandatory for further signaling to occur. The complex must be located to the plasma membrane where Frizzled-4 binds Disheveled (DVL) and induces further signaling. The downstream effect is suppression of β-catenin degradation (phosphorylation) and increased β-catenin signaling.[5] Loss-of-function (LoF) *FZD4*-variants cause autosomal dominant FEVR with variable expressivity and reduced penetrance,[7] but have also been reported to cause recessive disease.[8] Recently, biallelic *FZD4*-variants were described as a possible cause of FEVR with extraocular features including hearing loss and developmental delay, because of complete knock-down of the Norrin/Frizzled-4 pathway.[9] This also appears to be the case for hemizygous *NDP*-variants causing Norrie disease.[10] In addition, heterozygous variants in *LRP5* are mainly linked to either FEVR or increased bone density, while biallelic LoF variants cause a syndromic form (OMIM*603506).

We describe an individual with isolated, severe FEVR and biallelic *FZD4* variants. Functional studies demonstrate that the combination of these two variants affects both the localization and signaling capacity of *FZD4*, thus inhibiting the entire pathway.

2 | RESULTS

2.1 | Severe isolated FEVR

A 4-month-old boy was referred to the ophthalmology clinic because of nystagmus and strabismus. He was the first child of non-consanguineous, healthy parents, born at term following a normal pregnancy. Delivery was through acute cesarean section because of umbilical cord prolapse. Eye examination under general anesthesia revealed a marked fibrovascular structure involving the maculae in the right eye, and abnormal retinal vascularization with incomplete vascularization of peripheral retina in the left eye (Figure 1). He underwent laser treatment of avascular retina in the left eye. The changes appeared stable over a 2-year period. At age 2.5 years he appears to

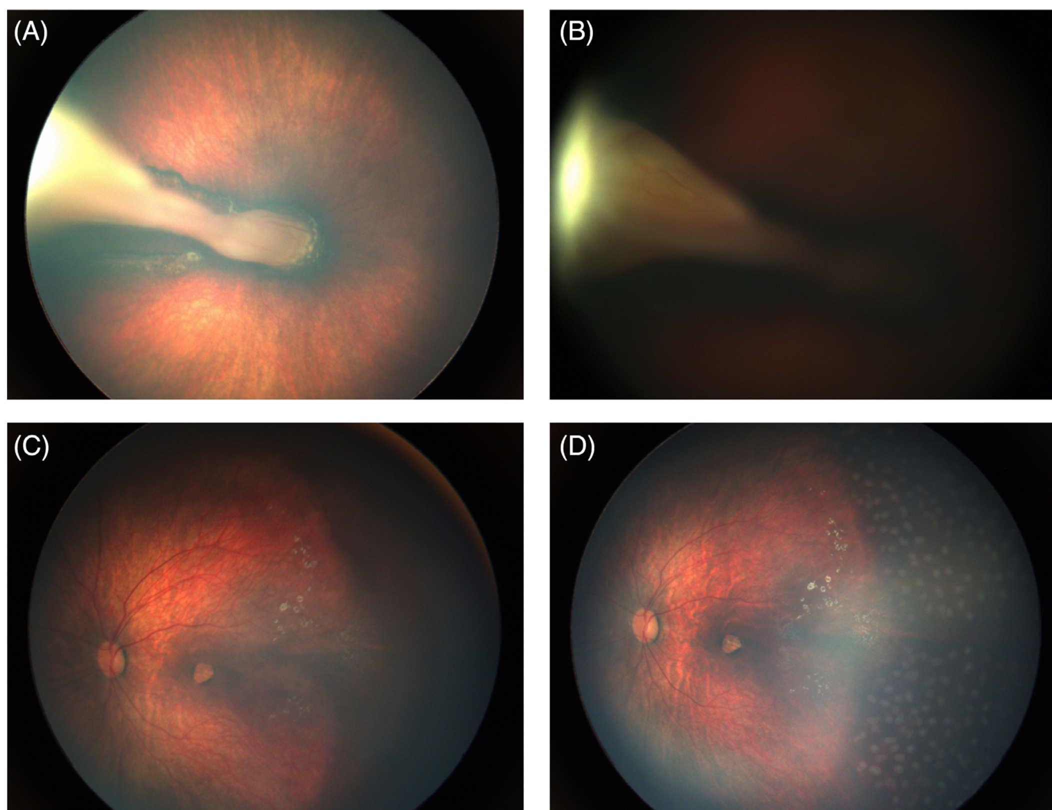


FIGURE 1 Fundus photographs of the proband. (A, B) Posterior pole of the right eye. A marked fibrovascular structure passes forward from the optic disc. The macula is involved in the structure. Small retinal vessels are seen nasal to the optic disc (A). The anterior part of the fibrovascular structure is attached temporal to the lens. The lens is clear (B). (C, D) Posterior pole of the left eye. Abnormal retinal vascularization in the temporal periphery with a retinal defect in the macula and several smaller retinal defects or exudates further temporal. Seemingly no retinal vessels in the periphery (C). Posterior pole of the left eye 7 months after laser treatment of avascular retina (D). [Colour figure can be viewed at wileyonlinelibrary.com]

13990004, 2024, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/cge.14499 by Arctic University of Norway - UIT Tromso, Wiley Online Library on [06/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

be blind in his right eye. In his left eye his preferential looking vision is 4.8 cyc/cm at 55 cm. His growth and development are age-appropriate. He walked by 12 months and spoke in full sentences by 2 years of age. Hearing is normal. General systemic examination did not reveal any significant findings. Both parents report normal vision. Fundus photographs of the asymptomatic mother and grandmother did not reveal any significant signs of FEVR (Supplementary Figure 1).

2.2 | Identification of FZD4 variants

WES revealed two heterozygous variants in the *FZD4* (NM_012193.3) gene: c.757C>T p.(Arg253Cys) and c.1607_1608delTG p.(Val536Glyf-sTer26). The first variant was located to the Frizzled/Smoothened family membrane region of Frizzled-4, while the latter variant was in the cytoplasmic tail.

The missense variant p.(Arg253Cys) is located in a highly conserved position. The Combined Annotation Dependent Depletion (CADD) v1.6 tool assigned the variant with a Phred score of 31.0, predicting that this variant is among the top 0.1% most deleterious missense variants in the human genome.[11] The variant has been found in three out of 251 156 alleles reported in gnomAD v.2.1.1. Several patients with FEVR have been reported to carry the variant, apparently as part of an autosomal dominant trait.[12] ClinVar includes an entry for the variant, interpreting it as likely pathogenic (Variation ID: 1068101). According to the American College of Medical Genetics and Genomics (ACMG) guidelines, PM1, PM2, PP3, and PP5 criteria were fulfilled, and we therefore classified the variant as likely pathogenic (see Supplementary Information).

The second *FZD4* variant, a deletion of two nucleotides, introduces a frameshift two amino acid codons prior to the normal stop codon, resulting in a prolonged C-terminus containing 24 additional amino acids (p.(Val536GlyfsTer26)). As the variant is located in the last coding exon of *FZD4*, nonsense-mediated decay (NMD) of mRNA is not expected. The far C-terminus of FZD4 have been shown to be crucial for the ability of FZD4 to recruit Disheveled (DVL) and to

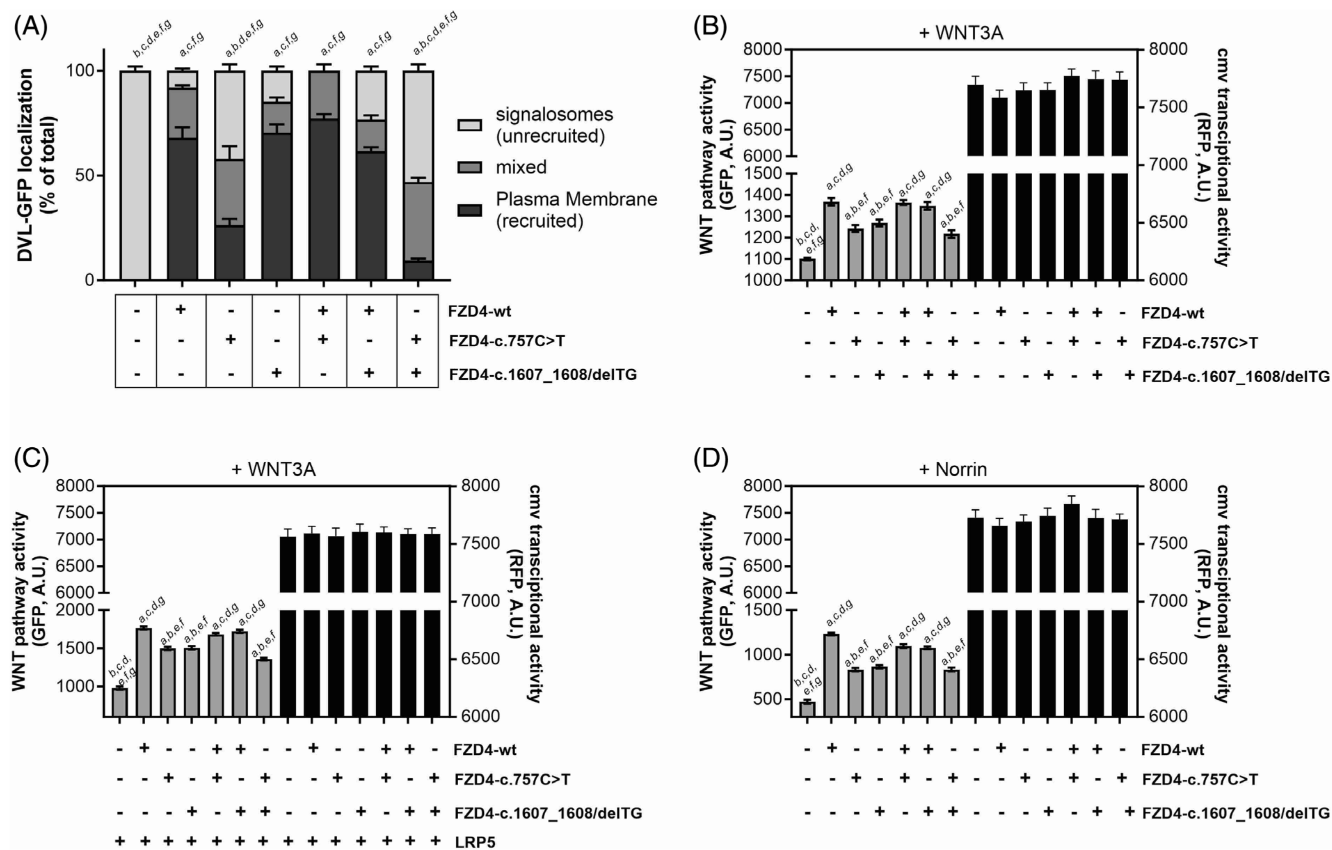


FIGURE 2 In vitro analysis of FZD4 variants. (A) Folding and DVL-recruitment. Efficiency of DVL recruitment at the PM by FZD4 variants. Recruitment is reported as percentage of cells showing DVL-GFP localized in signalosomes (unrecruited) or at PM (recruited by FZD4) in cells not expressing (−) or expressing (+) the indicated FZD4 variant(s). (B–D) WNT pathway activity. Transcriptional activity of the TCF/LEF promoter expressed as total GFP fluorescence produced by the reporter vector TCF/LEF-GFP in cells not expressing (−) or expressing (+) the indicated FZD4 variants. FZD4 is stimulated by WNT3A in (B and C), or by Norrin in (D). In (C), FZD4 variants are co-expressed with the co-receptor LRP5. In graphs (B–D), to control efficiency of transfection and cellular transcription/translation activity, TCF/LEF-dependent GFP expression is compared to cmv-dependent RFP expression (WNT-unrelated). In all graphs, results are shown as means ± SEM (p values <0.05: a vs. untransfected; b vs. HA-FZD4wt; c vs. HA-FZD4c.757C>T; d vs. HA-FZD4c.1607_1608/delTG; e vs. HA-FZD4 wt || HA-FZD4c.757C>T; f vs. HA-FZD4 wt || HA-FZD4c.1607_1608/delTG; g vs. HA-FZD4c.1607_1608/delTG ||HA-FZD4c.757C>T).

13990004, 2024, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/cge.14499 by Arctic University of Norway - UIT Tromso, Wiley Online Library on [06/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

maintain normal WNT pathway activity.[13] The variant has not been reported in gnomAD, nor has it been described in the literature in relation to *FZD4*-related disease. According to ACMG guidelines, PVS1_strong, PM2, and PM3 criteria were fulfilled, hence we classified the variant as likely pathogenic (see Supplementary Information).

Sanger sequencing of parental DNA confirmed compound heterozygosity for the *FZD4* variants in the proband; the mother and the maternal grandmother were carriers of the two-nucleotide deletion, while the father carried the missense variant (Supplementary Figure 5).

2.3 | Localization and activity of FZD4 variants

FZD4c.757C>T and FZD4c.1607_1608delTG variants were expressed in cultured cells in the presence of a fluorescent-tagged version of DVL (DVL-GFP). Intracellular localization and rate of DVL-GFP recruitment for each variant are quantitated in Figure 2A. FZD4-wt appears localized on the Plasma Membrane (PM) of the cell, confirming its proper folding.[11] In the presence of FZD4-wt, DVL-GFP moves from punctate signalosomes (Supplementary Figure 2A,B) to PM, where it colocalizes with FZD4.

Compared to FZD4-wt, FZD4c.757C>T localizes intracellularly, suggesting impaired transport of the receptor (Supplementary Figure 2C). Moreover, in cells expressing FZD4c.757C>T, DVL-GFP recruitment is impaired (Figure 2A). FZD4c.1607_1608delTG appears localized on the PM of the cells (Supplementary Figure 2D), and DVL recruitment by the FZD4 variant is similar to FZD4-wt.

To mimic heterozygosity, we co-expressed either FZD4c.757C>T or HA-FZD4c.1607_1608delTG with FZD4-wt. Both the combinations resulted in PM localization of the variants (Supplementary Figure 3A,B) and efficient DVL-GFP recruitment (Figure 2A).

Finally, to predict the phenotype of compound heterozygous FZD4c.757C>T||FZD4c.1607_1608delTG, we co-expressed the two variants. This promoted intracellular trapping of the variants and impaired DVL-GFP recruitment (Supplementary Figure 3C). Since FZD4c.1607_1608delTG presents proper PM localization, our data point toward FZD4c.757C>T exerting a negative effect on FZD4c.1607_1608delTG. In silico modeling of FZD4c.757C>T confirms that the point mutation might affect the folding and proper establishment of polar contacts in the juxta membrane region of the receptor (Supplementary Figure 4).

The effect of FZD4c.757C>T and FZD4c.1607_1608delTG variants on WNT signaling was then tested under different conditions (Figure 2B–D). Cells expressing either variant present reduced WNT pathway activity, while co-expression of FZD4-wt re-establishes proper WNT pathway activity. The double heterozygous FZD4c.757C>T||FZD4c.1607_1608delTG presents impaired WNT signaling (Figure 2B–D).

Analysis of WNT signaling extends and confirms results on intracellular DVL-1 recruitment of the variants and suggests: (i) impaired WNT pathway activity for both FZD4c.757C>T and FZD4c.1607_1608delTG variants; (ii) impaired PM localization and DVL-recruitment for FZD4c.757C>T; (iii) dominant positive/rescue effect of FZD4-wt on each of the variants; (iv) negative effect of FZD4c.757C>T on FZD4c.1607_1608delTG.

3 | DISCUSSION

We have identified two *FZD4* variants, one novel, in an individual with severe FEVR. We expand the spectrum of *FZD4*-mutations causing FEVR. Functional assays indicate that while each variant has impaired activity, it is the combination of the two which results in reduction of Norrin/Frizzled-4 signaling.

Van der Ende et al.[9] reported *FZD4* biallelic variants causing severe FEVR, hearing loss and developmental delay. They suggested that the variants resulted in dramatic loss of Norrin-*FZD4* signaling and therefore cause extraocular features.[9] The clinical presentation of the affected individual presented here is isolated, severe FEVR in childhood. This is in line with the findings of Khan et al.[8] who describe recessive FEVR without extraocular features. While it is important to bear in mind that loss of signaling is only assessed using in-vitro assays both by Van der Ende et al.[9] and in our study, our observation suggests that biallelic *FZD4*-variants do not necessarily cause extraocular features. In conclusion, while both studies are in line with the theory that autosomal recessive FEVR is associated with a more severe phenotype, our findings indicate that a severe defect in the Norrin/Frizzled-4 signaling may only cause the ocular phenotype.

A limitation to our study is the absence of a detailed ophthalmology review of the father, who is heterozygous for the missense variant but reports normal eyesight in adulthood.

Our report confirms that the severity of FEVR constitutes a spectrum which correlates with the underlying variants. Thus, genetic testing plays an important role in the diagnosis of FEVR and helps predict severity and family members at risk.

AUTHOR CONTRIBUTIONS

GH and TC examined, investigated, treated and cared for the individuals and the family; provided clinical information; obtained the family's informed consent. EB and SD reported and interpreted the variants. MS and AS performed the functional in-vitro experiments and data analysis. GH and SD wrote the manuscript. SD and MS conceived and designed the approach. All co-authors reviewed and approved the submitted version of the manuscript.

ACKNOWLEDGMENTS

We thank the family for consenting to share their story. We thank our colleague Hilde Monica Frostad Riise Stensland for her help with the segregation studies. This study was supported by the Norwegian National Advisory Unit on Rare Disorders through a grant to S. Douzgou (grant number 43066).

CONFLICT OF INTEREST STATEMENT

None.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/cge.14499.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

13990004, 2024, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/cge.14499 by Arctic University of Norway - UIT Tromso, Wiley Online Library on [06/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

ETHICS STATEMENT

Western Norway, Regional committee for medical and health research ethics—604007, project ID: 2629394.

PATIENT CONSENT FOR PUBLICATION

Written consent was received from the affected individuals or their parents/carers.

ORCID

Sofia Douzgou https://orcid.org/0000-0001-8890-7544

REFERENCES

1. Criswick VG, Schepens CL. Familial exudative vitreoretinopathy. *Am J Ophthalmol*. 1969;68(4):578-594.
2. Gilmour DF. Familial exudative vitreoretinopathy and related retinopathies. *Eye (Lond)*. 2015;29(1):1-14.
3. Ober RR, Bird AC, Hamilton AM, Sehmi K. Autosomal dominant exudative vitreoretinopathy. *Br J Ophthalmol*. 1980;64(2):112-120.
4. Ye X, Wang Y, Cahill H, et al. Norrin, frizzled-4, and Lrp5 signaling in endothelial cells controls a genetic program for retinal vascularization. *Cell*. 2009;139(2):285-298.
5. Xiao H, Tong Y, Zhu Y, Peng M. Familial exudative vitreoretinopathy-related disease-causing genes and Norrin/beta-catenin signal pathway: structure, function, and mutation spectrums. *J Ophthalmol*. 2019;2019:5782536.
6. Wang X, Chen J, Xiong H, Yu X. Genotype-phenotype associations in familial exudative vitreoretinopathy: a systematic review and meta-analysis on more than 3200 individuals. *PloS One*. 2022;17(7):e0271326.
7. Kondo H, Hayashi H, Oshima K, Tahira T, Hayashi K. Frizzled 4 gene (FZD4) mutations in patients with familial exudative vitreoretinopathy with variable expressivity. *Br J Ophthalmol*. 2003;87(10):1291-1295.
8. Khan AO, Lenzner S, Bolz HJ. A family harboring homozygous FZD4 deletion supports the existence of recessive FZD4-related familial exudative vitreoretinopathy. *Ophthalmic Genet*. 2017;38(4):380-382.
9. van der Ende SR, Meyers BS, Capasso JE, et al. Severe familial exudative vitreoretinopathy, congenital hearing loss, and developmental delay in a child with biallelic variants in FZD4. *JAMA Ophthalmol*. 2022;140(9):889-893.
10. Berger W, van de Pol D, Warburg M, et al. Mutations in the candidate gene for Norrie disease. *Hum Mol Genet*. 1992;1(7):461-465.
11. Rentzsch P, Schubach M, Shendure J, Kircher M. CADD-splice-improving genome-wide variant effect prediction using deep learning-derived splice scores. *Genome Med*. 2021;13(1):31.
12. Dan H, Wang D, Huang Z, et al. Whole exome sequencing revealed 14 variants in NDP, FZD4, LRP5, and TSPAN12 genes for 20 families with familial exudative vitreoretinopathy. *BMC Med Genomics*. 2022;15(1):54.
13. Bertalovitz AC, Pau MS, Gao S, Malbon CC, Wang HY. Frizzled-4 C-terminus distal to KTXXXW motif is essential for normal dishevelled recruitment and Norrin-stimulated activation of Lef/Tcf-dependent transcriptional activation. *J Mol Signal*. 2016;11:1.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hoem G, Pastore A, Bratland E, Christoffersen T, Stornaiuolo M, Douzgou S. Severe isolated exudative vitreoretinopathy caused by biallelic *FZD4* variants. *Clinical Genetics*. 2024;105(6):661-665. doi:10.1111/cge.14499

13990004, 2024, 6, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/cge.14499 by Arctic University of Norway - UIT Tromso, Wiley Online Library on [06/08/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License