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Key Words:	FZD4, LRP5, NDP, familial exudative vitreoretinopathy, Norrie disease



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Identification of 21 novel variants in *FZD4*, *LRP5*, *NDP* and an overview of the mutation spectrum in familial exudative vitreoretinopathy and Norrie disease

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Abstract

Wnt signaling is a crucial component of the cell machinery orchestrating a series of physiological processes such as cell survival, proliferation and migration. Amongst the plethora of roles that Wnt signaling plays, its canonical branch regulates eye organogenesis and angiogenesis. Mutations in the genes encoding the low density lipoprotein receptor protein 5 (*LRP5*) and frizzled 4 (*FZD4*), acting as co-receptors for Wnt ligands, cause familial exudative vitreoretinopathy (FEVR). Moreover, mutations in the gene encoding NDP, a ligand for these Wnt receptors, cause Norrie disease and FEVR. Both FEVR and Norrie disease share similar phenotypic characteristics including abnormal vascularisation of the peripheral retina and formation of fibrovascular masses in the eye that can lead to blindness. In this mutation update, we report 21 novel variants for *FZD4*, *LRP5* and *NDP*, and discuss the putative functional consequences of missense mutations. In addition, we provide a comprehensive overview of all previously published variants in the aforementioned genes and summarise the phenotypic characteristics in mouse models carrying mutations in the orthologous genes. The increasing molecular understanding of Wnt signaling, related to ocular development and blood supply, offers more tools for accurate disease diagnosis which may be important in the development of therapeutic interventions.

KEY WORDS: *FZD4*; *LRP5*; *NDP*; familial exudative vitreoretinopathy; FEVR; Norrie disease, ND

Introduction

Familial exudative vitreoretinopathy (FEVR; MIM# 133780) and Norrie disease (ND; MIM# 310600) are two inherited retinal disorders with highly overlapping ocular manifestations that are caused by alterations in the Wnt signaling network. FEVR and ND share an abnormal vascularisation of the peripheral retina with the formation of retinal folds, retinal detachment and in many cases the creation of a fibrovascular membrane located behind the lens [Laqua, 1980; van Nouhuys, 1982; Miyakubo et al., 1984; Shukla et al., 2003]. Genetic analyses have thus far identified three causative genes for the two disorders i.e. frizzled 4 (*FZD4*, located on 11q14.2; MIM# 604579), low density lipoprotein 5 (*LRP5*, located on 11q13.2; MIM# 603506), and Norrin (*NDP*, located on Xp11.3; MIM# 300658). Those genes encode proteins involved in the evolutionary highly conserved Wnt signaling network which plays an important role in eye development and angiogenesis. FEVR is a genetically heterogeneous disease and is inherited in an autosomal dominant (adFEVR; MIM# 133780) [Criswick and Schepens, 1969; Gow and Oliver, 1971; van Nouhuys, 1982; Feldman et al., 1983; van Nouhuys, 1985], autosomal recessive (arFEVR; MIM# 601813) or an X-linked recessive mode (MIM# 305390) [Laqua, 1980; Chen et al., 1993a; de Crecchio et al., 1998], with autosomal dominant being the most prominent mode of inheritance. Mutations in *FZD4* and *LRP5*, which are located on 11q14.2 and 11q13.2 respectively, are associated with autosomal dominant FEVR [Robitaille et al., 2002; Toomes et al., 2004a]. In addition, one family has been found to link to the EVR3 locus on chromosome 11p12-p13 [Downey et al., 2001]. Mutations in *LRP5* have also been associated with arFEVR [Jiao et al., 2004] whereas X-linked recessive FEVR is caused by mutations in the

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3 Norrie disease gene (*NDP*) located on Xp11.3 [Berger et al., 1992b; Chen et al., 1993a]. The
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5 pathologic features of the disease initiate from the abnormal retinal development due to the
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7 incomplete vascularisation of the peripheral retina and/or retinal blood vessel differentiation
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9 [Canny and Oliver, 1976]. The resulting avascular peripheral retinal zone [Miyakubo et al.,
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11 1982; van Nouhuys, 1982; Miyakubo et al., 1984; Shukla et al., 2003] is the hallmark of the
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13 disease and in many mildly affected patients may be FEVR's only manifestation. In more severe
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15 cases, retinal complications such as the formation of holes and tears, neovascularisation,
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17 exudation, vitreous haemorrhage, macular ectopia, falciform folds and retinal detachment, can
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19 result in blindness.
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24 Norrie disease (ND; MIM# 310600) is a severe X-linked recessive form of congenital blindness
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26 which in about one third of the cases is accompanied by mental retardation and deafness
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28 [Warburg, 1966; Bleeker-Wagemakers et al., 1985]. A characteristic intra-ocular mass in the
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30 retina (pseudoglioma) can lead to microphthalmia [Townes and Roca, 1973; Johnston et al.,
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32 1982]. Other ocular findings include the development of cataract, alterations in the composition
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34 of the vitreous body [Warburg, 1975], vitreoretinal haemorrhages, creation and deposition of
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36 retrolental fibrovascular tissue, retinal folding and detachment accompanied by subretinal
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38 exudates [Polomeno et al., 1987; Enyedi et al., 1991] which are signs bearing high phenotypic
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40 overlap with FEVR [van Nouhuys, 1982]. ND is genetically homogeneous and is caused by
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42 mutations in *NDP* [Berger et al., 1992b; Chen et al., 1993b].
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48 In addition to causing FEVR, defects in *LRP5* have also been associated with two groups of bone
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50 abnormalities. The first group includes osteoporosis–pseudoglioma syndrome (OPPG; MIM#
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52 259770), a rare autosomal recessive condition which initiates during early childhood. The major
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54 symptoms of OPPG include visual loss due to several vitreoretinal dysplasias and bone weakness
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3 which results in multiple fractures and deformities as the bone mass reduces. Carriers of OPPG-
4 associated *LRP5* mutations are often found to have reduced bone mass density. The second
5 group of diseases comprises endosteal hyperostosis (MIM# 144750), osteosclerosis (MIM#
6 144750), osteopetrosis (MIM# 607634), van Buchem disease type 2 (VBCH2; MIM# 607636),
7 and high-bone-mass trait (HBM; MIM# 601884). The aforementioned conditions are all
8 characterised by a high-bone-mass phenotype and are inherited in autosomal dominant mode.
9

10 FZD4, LRP5 and NDP are components of the Norrin-induced FZD4/LRP5/ β -catenin signaling
11 pathway. It has been demonstrated that Wnt signaling pathways are one of the main drivers for
12 vascular development in the mammalian eye. Consequently, mutations in these three genes cause
13 FEVR and ND that have an almost identical pathologic impact on intraretinal vasculature.
14

15 Recently a series of dedicated experiments revealed that a new gene, *TSPAN12* (located on
16 7q31.31; MIM# 613138) plays a key-role in Norrin-induced β -catenin signaling transduction and
17 regulation (Fig. 1). *TSPAN12* is a member of the tetraspanin family that share certain specific
18 structural features that distinguishes them from other proteins that pass the membrane four times.
19 The protein was postulated to act via a mechanism that enhances FZD4 multimerisation and
20 clustering by binding to Norrin multimers, a crucial step for induction of physiological levels of
21 signaling [Junge et al., 2009].
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46 **Wnt signaling and eye vasculature: brief overview**

47 The Wnt signaling transduction network influences cell survival, differentiation, proliferation
48 and migration by a highly orchestrated transcriptional regulation of target genes. The network
49 can be roughly categorised in two broad branches; the canonical or Wnt/ β -catenin pathway and
50 the non-canonical pathway which includes the Wnt/calcium signaling and planar cell polarity
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3 pathways. Wnt genes encode a family of soluble, secreted cysteine-rich proteins which are often
4 glycosylated and/or palmitoylated. Wnts can act as ligands for Frizzled receptors which in
5 concert with LRP5/6 co-receptors form a ternary complex [Tamai et al., 2000] and subsequently
6 initiate Wnt canonical signaling. Canonical Wnt signaling regulates the amount of β -catenin that
7 reaches the nucleus via the action of Disheveled proteins that block its phosphorylation by GSK-
8 3 [Li et al., 1999] and proteolytic degradation in the proteasome [Polakis, 2000]. Presence of β -
9 catenin inside the nucleus promotes transcription of several genes implicated in different
10 processes. Wnts are expressed in a wide variety of tissues and the specific type of Wnt-Frizzled
11 induced signaling is determined mostly by the nature of the different interactions and the
12 locations in which these occur. FZD4 has been shown to act also in the non-canonical Wnt
13 signaling pathway [Robitaille et al., 2002] by regulating components belonging to the
14 Wnt/calcium signaling network.

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Wnt signaling plays also an important role in eye organogenesis and angiogenesis. Beta-catenin
regulation is present in critical steps of ophthalmogenesis such as anterior neural tissue definition
from the remaining brain compartments, eye field formation, and fine definition of the neural
retina [Fuhrmann, 2008]. In addition, ocular blood vessel formation during development initiates
with the formation of a provisional blood vessel system in the extraretinal hyaloid region called
the hyaloid vascular system (HVS). This system is responsible for the supply of nutrients to the
early vitreal structure and the retina. When the intraretinal vasculature develops, the HVS
recedes in the maturing eye.

Retinal angiogenesis is dependent on TSPAN12, FZD4 and LRP5 to initiate the β -catenin
signaling cascade upon NDP binding (Fig. 1).

Mutations in *FZD4*

FZD4 is a member of the Frizzled gene family and encodes a protein of 537 amino acids. Similar to the other members of the Frizzled family it is composed of a seven pass transmembrane helix and an extracellular Frizzled (FZ) domain (Supp. Figure S1).

To date, a total of 32 different mutations have been reported for *FZD4* (Supp. Table S1). Ten mutations result in a premature stop codon, 21 are missense changes and one is an in-frame deletion of two amino acids (p.M493_W494del). No splice mutations have been reported for *FZD4* and mutations are not clustering in a specific “hotspot”. All patients with *FZD4* mutations are heterozygous carriers, except for a 5-month-old baby carrying a homozygous change (p.R417Q) for which both parents harbouring the same mutation heterozygously exhibited mild phenotypes [Kondo et al., 2003] suggesting an autosomal dominant mode of inheritance. The effect of a few *FZD4* nucleotide variants has been assayed functionally. Robitaille et al. [2002] measured the *FZD4* dependent induction of β -catenin signaling upon activation of the receptor. They found that p.M493_494del and p.L501SfsX533 mutant proteins did not exert any detectable activity. Two additional studies, using Norrin dependent signaling reporter assays, showed the reduced activity of *FZD4* mutants (p.M105V, p.M157V, p.W319X and p.R417Q) ranging from ~30% to 95% of the wild-type levels [Xu et al., 2004; Qin et al., 2008].

In this study we report five novel variants in *FZD4*, one of which is located in exon 1 (p.E40Q) and the other four in exon 2 (p.C204Y, p.E286X, p.D428SfsX2 and p.L501SfsX33). Because *FZD4* contains only two exons, protein truncating mutations in exon 2 likely do not result in complete nonsense mediated decay (NMD) but rather in a non-functional protein.

The *FZD4* proteins containing the two protein-truncating mutations p.E286X, p.D428SfsX2 (Table 1, Supp. Table S5) are predicted to lack the conserved KTXXXW domain and PDZ

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3 binding motif (Supp. Figure S1). The absence of these elements probably abolishes the ability of
4
5 FZD4 to induce the downstream β -catenin signaling pathway. The KTXXXW domain is located
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7 directly after the seventh transmembrane (TM) helix at the C terminus and is essential for
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9 generation of FZD4-mediated signal transduction [Umbhauer et al., 2000]. Moreover, a truncated
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11 protein (p.L501SfsX33) that contained the KTXXXW domain but lacked the PDZ binding motif
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13 was shown to be defective. The mutated protein was absent from the plasma membrane probably
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15 due to impaired oligomerisation of the wild-type protein with the mutant form which lead to the
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17 retention of FZD4 in the endoplasmic reticulum [Kaykas et al., 2004].
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21 In addition to the two protein-truncating mutations we also identified three novel missense
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23 changes. Variant p.E40Q was found in one sporadic patient (Table 1). The wild-type glutamic
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25 acid residue is located at the N-terminus directly following the putative signal peptide at the FZ
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27 domain (Supp. Figure S1). According to the secondary structure prediction [Bryson et al., 2005],
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29 this residue is located at a coiled domain on the surface of the protein where interactions might
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31 occur. The function of the FZ domain is not fully elucidated, although mutagenesis and crystal
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33 structure studies suggest that it may be important for interaction with other proteins such as Wnts
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35 or NDP [Dann et al., 2001]. Glutamic acid is a negatively charged amino acid and is often
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37 involved in ionic interactions. The mutant glutamine has the same molecular structure except for
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39 the negatively charged oxygen. The pathogenic effect of this change is therefore likely due to the
40
41 loss of ionic interactions of the FZ domain with other proteins or by itself. In addition, the same
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43 patient carries a second nucleotide variant in *LRP5*, the splice site mutation c.4489-1G>A. In
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45 order to better assess the disease causing potential of the two aforementioned changes, the
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47 affected mother was also included in the study. She was found to carry both variants i.e. *FZD4*
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49 p.E40Q and *LRP5* splice site mutation c.4489-1G>A, which does not exclude a digenic mode of
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3 inheritance. Notably, previous studies have also provided some evidence that FEVR may not
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5 exclusively rely on a monogenic model of inheritance. Shastry and Trese [2004] reported that a
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7 factor V change co-segregated with the *FZD4* mutation in a patient with autosomal dominant
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9 FEVR.
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12 The p.C204Y variant (Table 1, Supp. Table S5) is located in a stretch of residues that connects
13
14 the FZ domain with the first TM domain of FZD4. Using the PHDacc-server ([Rost, 1996];
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16 accessible via “Project HOPE”, <http://www.cmbi.ru.nl/hope/>), secondary structure prediction
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18 indicates that the mutated cysteine is placed in a large coiled region, buried in the core of the
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20 protein preceding the first TM helix. Mutation of a cysteine residue, possibly employed to form a
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22 disulfide bond, into a tyrosine, a hydrophilic amino acid with a large bulky side chain, is
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24 expected to lead to destabilisation or even unfolding of the domain. In family W06-237 we
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26 identified the p.G525R variant in FZD4 (Table 1, Supp. Figure S5). This missense change affects
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28 an amino acid located in the intracellular C-terminal tail of FZD4, in a stretch of residues
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30 predicted to form a coil. This residue is partly exposed to the solvent and partly buried. The two
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32 following residues (tryptophan and valine) are predicted to be completely buried in the core of
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34 the protein and by being hydrophobic, they are probably important forming the core of the C-
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36 terminal domain. Glycine residues are known for their flexibility as no other residue can make
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38 the same backbone angles. It seems possible that the residues preceding tryptophan and valine
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40 form a typical structure that is needed to locate the hydrophobic tryptophan and valine in their
41
42 correct position. This is underlined by the fact that the glycine residue at position 525 is preceded
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44 by an asparagine (a residue often present in structural turns) and by another flexible glycine. The
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46 p.G525R variant changes the flexible glycine into a less flexible arginine, thereby disturbing the
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48 putative backbone structure that is needed for the positions of tryptophan and valine. This
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3 potentially could affect the normal function of KTXXXW and PDZ binding in the Wnt/ β catenin
4 pathway, thereby impeding the action of FZD4 receptor complex. All the changed residues are
5 highly conserved among several FZD4 orthologues (Supp. Figure S2). We have summarized the
6 clinical data of five FEVR patients with *FZD4* variants in Supp. Table S5. Their visual acuity
7 ranges from normal to complete blindness. Macular ectopia is frequently observed.
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18 **Mutations in *LRP5***

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20 LRP5 is a composite cell surface receptor protein that consists of 1615 amino acids and belongs
21 to the low density lipoprotein receptor (LDLR) superfamily. Its function is associated with the
22 process of receptor-mediated endocytosis of a wide spectrum of ligands. Upon ligand binding,
23 LRP5 can act synergistically with FZD4 or other members of the Frizzled family by activating
24 the canonical Wnt and/or Norrin/ β -catenin pathway and induce the transcription of target genes
25 [Pinson et al., 2000; Wehrli et al., 2000; He et al., 2004].
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34 LRP5 consists of four extracellular domains, each of which is composed of six segments. Those
35 segments form a β -propeller structure that is followed by an EGF-like domain (Fig. 2A). Five of
36 those segments are YWTD LDL-class B repeats, while the sixth does not contain the required
37 YWTD motif to be recognized as a LDL-class B repeat. The first two propeller domains were
38 suggested to be important for interaction with the Wnt/Frizzled complex [Mao et al., 2001]. The
39 four propeller domains are followed by three LDL-class A repeats (also known as LDL-receptor
40 like ligand binding domains), one TM domain and a cytoplasmic region with one or multiple
41 short signals for receptor internalisation through coated pits. LRP5 is highly expressed in many
42 tissues including bone, liver, heart, retina, skin, pancreas during various stages of development.
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3 Moreover, whilst part of a very potent and diverse signaling network such as the β -catenin
4 pathway, LRP5 is a major player in morphological development sugar and lipid metabolism.
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8 Thus far, 86 different mutations have been reported for LRP5 of which 26 introduce premature
9 stop codons, 56 mutations are missense changes and four changes affect splicing (Supp. Table
10 S2). Whereas *LRP5* mutations that are associated with FEVR are scattered throughout the gene,
11 other mutations in *LRP5* that seem to be clustered locally cause different phenotypes. Individuals
12 suffering from a group of disorders demonstrating high-bone-mass and sclerosing bone
13 dysplasias, show autosomal dominant missense variants clustered in exons 2-5 which encode the
14 first “ β -propeller” domain of LRP5. These changes are hypothesised to act in a dominant
15 negative way. The mutant LRP5 proteins are impaired from binding to Wnt pathway antagonists,
16 e.g. SOST or DKK-1, because of defective receptor action or cell trafficking, thereby promoting
17 abnormal growth in bone forming cells [Boyden et al., 2002; Zhang et al., 2004; Semenov and
18 He, 2006].
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34 *LRP5* variants have also been associated with OPPG, a rare autosomal recessive condition for
35 which the vast majority of loss-of-function mutations identified are located in the second and
36 third “ β -propeller” domain and the following LDL repeats of LRP5. Of the FEVR-causing
37 mutations, four mutations result in premature stop codons, whereas at least six missense changes
38 have been associated with the autosomal dominant form of the disease. In addition, eleven
39 missense variants (three homozygous and two compound heterozygous) have been associated
40 with autosomal recessive or sporadic FEVR cases. All these changes seem to result in a reduced
41 or lost interaction with FZD4 and NDP and thus disturbing the normal Wnt signaling cascade
42 that regulates retinal vessel formation.
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3 Functional characterisation of nucleotide changes in *LRP5* has been performed by various
4 groups. Gong et al. [2001] studied the effect of p.Q853X and p.E1270RfsX169 by transiently
5 transfecting COS-7 cells with mammalian expression constructs containing either the wild-type
6 or mutant *LRP5* sequences. They demonstrated that even if the mutant proteins were synthesised
7 they were not able to be secreted, due to impaired cell trafficking, adding evidence to the
8 assumption that OPPG results from loss-of-function mutations. Boyden et al. [2002] studied the
9 effects of p.G171V by performing *in vitro* and *in vivo* experiments measuring Wnt signaling
10 output. They used a luciferase activity assay, which was responsive to *LRP5* by co-transfection
11 experiments in NIH3T3 cells and by measuring fibronectin levels in affected individuals with the
12 high-bone-mass phenotype. The p.G171V variant did not influence normal Wnt signaling in the
13 absence of Wnt antagonists. However, when such an antagonist (e.g. Dkk-1) was present, the
14 mutant *LRP5* construct was able to reduce its inhibitory action. Zhang et al. [2004] showed that
15 the p.G171V mutation seemed to disrupt the Mesd-mediated recruitment of *LRP5* to the surface
16 of osteoblasts which occurs via the first β -propeller domain. They proposed that paracrine Dkk-
17 1, which is produced by osteoclasts and binds to the third “ β -propeller” domain of *LRP5*, has
18 limited target availability i.e. minimal antagonising potential, boosting autocrine Wnt action in
19 the osteoblasts. Adding to the complexity of events that act synergistically for high-bone-mass
20 phenotype, it was also postulated that p.G171V was able to directly reduce binding ability of a
21 bone specific *LRP5* antagonist, SOST, to the first β -propeller structure, potentially affecting
22 bone mass regulation [Semenov and He, 2006]. Ai et al. [2005] designed a series of expression
23 constructs containing OPPG-associated mutations (p.S356L, p.T390K, p.G404R, p.D434N,
24 p.G520V and p.G610R) and FEVR-associated mutations (p.T173M, p.R570Q, p.Y1168H,
25 p.C1361G and p.E1367K) along with wild-type *LRP5* and transiently transfected HEK293T cells
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3 in an *ex vivo* reporter assay for Wnt and Norrin signal transduction. With the exception of
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5 p.S356L and p.G520V, OPPG-associated *LRP5* mutations, which affected the first or second β -
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7 propeller domain, resulted in impaired subcellular trafficking of mutant LRP5. However, there
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9 was no clear-cut correlation between assay activity and phenotypes. When FEVR-associated
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11 mutations were tested in the same assay they showed variable results. The p.Y1168H missense
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13 variant, that causes autosomal dominant FEVR, almost completely abolished Wnt and Norrin
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15 signal transduction whereas p.T173M had no significant impact. A similar effect was shown for
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17 two other mutations associated with autosomal recessive FEVR (p.R570Q and p.E1367K).
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19 Finally, it was demonstrated in concordance with all the previous experiments, that LRP5
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21 mutants p.R444C and p.A522T exhibited a variable reduction in the Norrin dependent Wnt
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23 signaling (45% and 26% respectively) that correlated with the mild phenotype observed in the
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25 patients who carry these changes [Qin et al., 2008].
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32 In this study, we report two new missense (p.E441K, p.C1253F), one new nonsense variant
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34 (p.W993X), and one novel splice site (c.4489-1G>A) change. The p.E441K variant has been
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36 identified in one sporadic FEVR patient (Table 2, Supp. Table S5). It is located in the second “ β -
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38 propeller” domain of the protein, at an evolutionary highly conserved position (Supp. Figure S3).
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40 As our structure prediction model (Fig. 2B) points out, the negatively charged and hydrophilic
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42 glutamic acid, creates ionic interactions with two positively charged arginine residues in its close
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44 proximity (residues indicated in blue), thereby stabilising and formulating the local structure of
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46 the whole domain. Introduction of an additional positive residue such as lysine is likely to disturb
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48 the ionic interactions, potentially destabilising the whole domain structure and therefore
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50 impeding its normal function. This could have an effect on its potential interaction with NDP,
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52 which is also supported by the aforementioned functional analysis of p.R444C. More
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3 interestingly a different mutation, p.D434N, which was also characterised by decreased Wnt
4 signaling potential, was associated with OPPG. The second variant, p.C1253F, is located in the
5 EGF-like domain following the third “β-propeller” module of LRP5 (Fig. 2C) and also affects a
6 well conserved residue (Supp. Figure S3). The role of this domain is unclear. The third “β-
7 propeller” structure and the three LDL-class A repeats play a role in binding of ligands such as
8 Dkk-1. The EGF-like domains in general are stabilised by three disulfide bridges (Fig. 2C). The
9 introduction of a phenylalanine at the position of cysteine residue is predicted to disturb one of
10 the existing disulfide bridges. Moreover, phenylalanine has a much bulkier side chain when
11 compared with cysteine and might exert steric hindrance effects. Interestingly, the same person
12 carrying the p.C1253F variant was found to carry a second LRP5 missense variant, p.G610R,
13 previously reported to be associated with FEVR (in a compound heterozygous mode) and with
14 OPPG (Supp. Table S2). The p.G610R variant, when present heterozygously, was shown in at
15 least one case to result in low bone mass density. The father of the proband was found to carry
16 the p.C1253F variant while the mother was carrying the p.G610R. Based on the clinical picture
17 of this family (Supp. Table S5) and mutation segregation data an autosomal recessive mode of
18 inheritance can be suggested, although autosomal dominant inheritance cannot be totally
19 excluded.
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43 Furthermore, a nonsense change (p.W993X) was found in one sporadic patient with FEVR
44 which results in a premature stop codon in exon 13 likely causing NMD and thus leading to
45 LRP5 haploinsufficiency (Table 2, Supp. Table S5). Finally, we identified a splice-acceptor
46 mutation, c.4489-1G>A, in the same person that harbours the FZD4 p.E40Q variant. The effect
47 of this mutation is predicted to be skipping of exon 22 and the subsequent in frame deletion of 20
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3 amino acids. No RNA was available to test the effect of the splice site change. We summarised
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5 the clinical data for three patients with *LRP5* variants in Supp. Table S5.
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10 ***LRP5* polymorphisms**

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12 Many groups have tried to find associations between *LRP5* polymorphisms and osteoporosis,
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14 low bone mass index, hypercholesterolemia, obesity and increased body mass index. The non-
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16 synonymous p.A1330V (rs3736228:C>T) SNP in exon 18 has been in the center of attention in a
17
18 large number of studies. This polymorphism was linked with low bone mass determination at the
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20 lumbar spine, femoral neck and total hip [Koay et al., 2004]. Subsequently, at least six more
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22 groups reported putative association of p.A1330V alone or synergistically with other
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24 polymorphisms in *LRP5* or other genes, with bone mass determination and hypercholesterolemia
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26 [Ferrari et al., 2005; van Meurs et al., 2006; Suwazono et al., 2006; Giroux et al., 2007; Saarinen
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28 et al., 2007; Suwazono et al., 2007].
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34 Qin et al. [2008] utilising a Norrin dependent reporter demonstrated an unexpected decrease in
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36 Wnt signaling activity in the case of SNPs p.Q89R (rs41494349:A>G) and p.A1330V. These
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38 variants may play a role in the wide phenotypic heterogeneity observed in people that harbour
39
40 the same pathogenic nucleotide variants in *LRP5*.
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45 ***NDP* mutations**

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47 The Norrie disease protein gene (*NDP*) encodes Norrin, a small secreted and cysteine rich
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49 protein that consists of 133 amino acids with weak homology to the transforming growth factor
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51 (TGF)- β family of ligands [Berger et al., 1992a; Chen et al., 1992; Meindl et al., 1992; Meitinger
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53 et al., 1993]. Norrin consists of two major parts: a signal peptide at the amino-terminus of the
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3 protein that directs its localisation and a region containing a typical motif of six cysteines
4 forming a cysteine-knot that provides the structural conformation required for receptor binding
5 and subsequent signal transduction [Meitinger et al., 1993]. Our 3D model of NDP agrees with
6 structures previously described [Vitt et al., 2001] and contains two antiparallel β -sheets and one
7 α -helical structure (Fig. 3A).

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15 Although Norrin is not structurally related to the Wnt family of proteins, it resembles their
16 function by binding with high affinity to the receptor Frizzled-4, requiring LDL receptor-related
17 protein (LRP) as a co-receptor and by activating the FZD4/LRP5/ β -catenin signaling pathway
18 Wnt signaling pathway [Xu et al., 2004]. One of the multiple routes that this pathway is involved
19 in, is the transcriptional control and regulation of genes that are under the influence of TCF/Lef
20 binding sites. NDP, together with FZD4 and LRP5, effectively plays a role in the development of
21 the cells in the retinal neural epithelium by setting off the retreat of the HVS and in parallel by
22 patterning the external and deep retinal vasculature which is necessary for retinal function
23 [Luhmann et al., 2005].

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Ninety-five nucleotide variants have been reported for NDP (Supp. Table S4). Twenty-four
mutations result in the introduction of a premature stop codon, 58 are missense changes, six
changes affect splice sites and for seven variants their effect is different or unknown. Most of the
mutations result in Norrie disease and a smaller percentage in X-linked FEVR. Five NDP
changes (p.R41S, p.Y44X, p.C96W, p.L108P and p.R121W) have been suggested to be
associated with persistent fetal vasculature syndrome, Coats disease, and retinopathy of
prematurity (ROP). These distinct clinical entities share some common pathological features
such as abnormal retinal blood vessel growth which may result in scarring and retinal
detachment.

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3 Two groups have performed functional assays for *NDP* variants. Xu et al. [2004] using a co-
4 transfection signaling assay in STF cells in parallel with immunoblotting, assessed total protein
5 production, secretion efficiency to the extracellular matrix and potency for signaling
6 transduction. They analysed 18 *NDP* mutations associated with FEVR or ROP. All missense
7 mutants but one (p.L13R), showed no production or secretion efficiency aberrations; all but one
8 (p.K58N) resulted in 20-80% decreased Wnt signaling potential. Moreover, Qin et al. [2008]
9 after testing two groups of *NDP* missense variants associated with either FEVR (p.R41K,
10 p.K54N, p.R115L, p.R121W) or Norrie disease (p.K58N, p.A63D, p.R97P and p.R121W), in a
11 similar way showed 17-96% reduced signaling potential. Only p.K58N, in agreement with the
12 previous study, resulted in increased signaling ability. Interestingly, the authors suggested that
13 mutants p.K54N, p.K58N and p.R115L disturb binding of Norrin with other factors possibly
14 related to Norrin's physiological function. Finally, no mutant was observed to have any impact
15 on the integrity of the protein.
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34 Here, we report 12 novel nucleotide variants in *NDP* associated with Norrie disease or X-linked
35 FEVR. These include six missense variants (p.C55R, p.G67E, p.G67R, p.F89L, p.S92P, p.P98L),
36 one nonsense (p.S111X), three frameshift (p.H4RfsX21, p.S9PfsX4, p.Y44MfsX60) and two
37 splice site mutations (c.-208+2T>G, c.-208+5G>A). The missense change p.C55R influences a
38 cysteine residue located on the “top” of one of the antiparallel β -sheets forming a disulphide
39 bridge with a cysteine on the top of the other β -sheet (Fig. 3B). The exact function of this bond is
40 unclear, but it could be speculated that it might be necessary to stabilise the conformation of the
41 two β -sheet domains which contain hydrophobic residues that can interact with another *NDP*
42 monomer. Without the disulphide bridges the two sheets will have more freedom to move,
43 thereby making it difficult to form the correct structure for dimerisation. Moreover, this
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3 disulphide bridge and amino acid location was shown to be evolutionary conserved (Supp.
4 Figure S4) [Meitinger et al., 1993].
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8 The glycine residue at position 67 is highly conserved amongst many NDP orthologues (Supp.
9 Table S4) and the p.G67E and p.G67R variants change a small and flexible glycine into either a
10 glutamic acid (negatively charged) or into an arginine (positively charged). Glycine is “required”
11 at this position for the protein to adopt a typical backbone conformation crucial for formation of
12 the cysteine-knot (Fig. 3C). No other residue can make the correct backbone angles and
13 therefore, mutation of the glycine will disturb cysteine-knot formation and the structure of the
14 protein.
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18 The missense change p.F89L is located in a highly conserved domain (Supp. Table S4) that
19 adopts an α -helical structure. This amino acid is predicted to be one of the residues that
20 participate in the dimerisation of NDP [Meitinger et al., 1993]. The hydrophobic phenylalanine
21 interacts with other hydrophobic residues on the other monomer. Leucine is also hydrophobic,
22 but has a smaller sidechain (Fig. 3D). It seems possible that this leucine could make the same
23 type of interactions but because of altered stereo-specificity the dimer formation could be less
24 strong and stable.
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28 The missense change p.S92P affects a serine which precedes a cysteine in the cysteine-knot
29 motif and was identified in a simplex Norrie disease patient (Table 3, Supp. Table S5). As a
30 “consensus” it is very critical that the cysteines are placed correctly in NDP as they are crucial
31 for its structure. The missense change introduces proline (Fig. 3E) which is known to be a very
32 rigid residue that often forces the backbone to bend and thereby might disturb the formation of
33 the disulphide bridge and thus the conformation of NDP. Finally, p.P98L is located close to the
34 last cysteine in the knot motif and to the unbound cysteine that is needed to form dimers (Fig.
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3 3F). The local backbone structure that is created by proline, might be necessary for the correct
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5 positioning of cysteines. A leucine will cause more flexibility in the backbone which might result
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7 in difficulties during dimerisation and/or formation of the cysteine-knot. Another possibility is
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9 that the hydrophobic side chain of proline might be needed for interactions with another
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11 monomer.
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15 The four novel protein truncating mutations likely result in NMD of the RNA or a truncated
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17 protein and thereby in a reduction or absence of NDP activity. Moreover, the two novel splice
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19 mutations, c.-208+2T>G and c.-208+5G>A, affect the highly conserved splice donor site of exon
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21 1. The two most likely effects are that either intron 1 is retained in the mature mRNA or that
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23 exon 2 is not spliced into the mRNA. In the latter case, the mRNA would lack the ATG residing
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25 in exon 2, as well as the 58 most N-terminal amino acids. Inclusion of intron 1 may have an
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27 effect on the stability of the mRNA. The c.-208+5G>A variant segregates with the disease
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29 phenotype in the family as it is present in two affected males. The clinical information of one ND
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31 patient is presented in Supp. Table S5.
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39 **Animal models**

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41 Several mouse models have been made that either have no or partially functional *Fzd4*, *Lrp5* and
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43 *Ndph* (Norrie disease pseudoglioma mouse homologue), in order to shed more light into the
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45 biochemical properties of these proteins and understand the pathology of the associated diseases.
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47 Gene disruption in murine *Fzd4* using a *Fzd4-lacZ* knock-in reporter allele assessed by X-gal
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49 staining provided in depth analysis of its expression. During littermate development, *Fzd4*
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51 seemed to be transiently present in a wide variety of tissues. In mature *Fzd4*^{+/-} mice, expression
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53 was observed in the Purkinje cells of the cerebellum, in the retina, in the inner hair cells in the
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3 organ of Corti, and in the sensory epithelium of the otolith organs [Wang et al., 2001]. Moreover,
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5 a prominent *Fzd4* expression throughout development was also demonstrated in the ovary with
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7 high prevalence in the corpus luteum [Hsieh et al., 2002]. *Fzd4*^{-/-} mice are viable although they
8
9 are subject to high mortality rates (~50%) and impaired growth, beginning in the second
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11 postnatal week [Wang et al., 2001]. The *Fzd4*^{-/-} mice also displayed progressive hearing loss and
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13 an inoperative swollen esophagus around P8 [Wang et al., 2001]. Xu et al. [2004] demonstrated
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15 that young adult mice show compromised intraretinal vasculature. Problematic blood supply of
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17 the retina especially in the nerve fiber layer (NFL) and outer plexiform layer (OPL) was noted.
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19 No significant differences in anatomic and/or phenotypic characteristics were noticed between
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21 *Fzd4*^{+/+} and *Fzd4*^{+/-} mice.
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27 Similar to *Fzd4*, *Lrp5* was found to be expressed in many tissues in the mouse. However, its
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29 expression was most abundant in CNS neurons, the islets of Langerhans, liver, osteoblasts and
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31 ocular macrophages [Figuroa et al., 2000; Kato et al., 2002]. In an effort to replicate the
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33 phenotypic manifestation of OPPG, *LRP5*^{-/-} mice were generated by disrupting exon 6 of the
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35 gene with the insertion of an IRES-LacZ-Neomycin cassette and thus resulting to a prematurely
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37 truncated protein [Kato et al., 2002]. These *Lrp5*^{-/-} mice were viable and fertile; however a
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39 fraction of them died prematurely because of bone fractures. The mice displayed low bone mass
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41 phenotype from 2 weeks and decreased bone volume and mineral content of the ECM
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43 surrounding osteoblasts. In 70% of *Lrp5*^{-/-} mice hyaloid vessels regression was delayed at 6
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45 months of age. This was postulated to result from failed macrophage-mediated apoptosis. *Lrp5*^{+/-}
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47 mice displayed the same bone phenotype to a lesser extent and had no eye-related abnormalities.
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49 Moreover, Fujino et al. [2003], generated *Lrp5*^{-/-} mice by disrupting a ligand binding repeat in
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51 exon 18. All mice appeared normal until the sixth month of their life when parietal bone
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3 weakness was noted in 70% of *Lrp5*^{-/-} of the female population. The *Lrp5*^{-/-} mice also displayed
4 impaired chylomicron clearance, hepatic uptake and glucose-induced insulin secretion. The
5 authors did not report on eye-related abnormalities. Two other studies, [Babij et al., 2003; Akhter
6 et al., 2004] described transgenic mice with the p.G171V mutation, which is associated with the
7 human high-bone-mass phenotype. The LRP5_{G171V} mice showed greater bone mass, density,
8 structure and strength. Finally, Xia et al. [2008] identified a ENU-induced recessive mouse
9 mutant line that bears many similarities with the eye pathology that FEVR shows in humans. The
10 mutant *r18* mouse carries a frameshift mutation which causes a premature stop at codon 1596
11 and the replacement of the 39 most C-terminal amino acids by 20 different ones. In *Lrp5*^{r18/r18}
12 mice, the retina appeared hypopigmented and the retinal arteries weak. Moreover, early in their
13 growth, the mice developed retinal haemorrhage. The retinal vasculature was disorganized,
14 immature, and leaky, with less and smaller blood vessels. Meagre retinal blood supply was
15 postulated to be a consequence of defective capillary lumen formation.
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34 *Ndph* mutant mice replicate most of the symptoms associated with Norrie disease and partially
35 that of FEVR, both in terms of eye/retinal symptomatology and auditorial defects. The mutant
36 mice display retrolental structures in the vitreous body and a perturbed ganglion cell layer along
37 with the formation of pseudogliomas in the posterior vitreal chamber [Berger et al., 1996;
38 Richter et al., 1998]. All the different cell layers of the retina were reduced along with the
39 photoreceptors, but specific segments of the retina such as the OPL could completely disappear
40 [Berger et al., 1996; Lenzner et al., 2002]. Moreover, the *Ndph* knockout mice developed severe
41 structural irregularities of the retinal vascular system formation. The hyaloid blood vessels
42 delayed to regress [Richter et al., 1998; Luhmann et al., 2005; Ohlmann et al., 2005] and both
43 superficial and intraretinal vasculature was severely impaired because a large number of blood
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3 vessels or capillaries was defective or even absent. The lack of normal blood supply that
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5 nourishes the different retinal cell layers results in local hypoxic conditions, especially in the
6
7 inner retina. This was associated with increased expression of vasodilatation indicators such as
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9 eNOS and VEGF, and increased permeability of retinal blood vessels [Luhmann et al., 2005;
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11 Kaur et al., 2006]. Defects in the cochlear stria vascularis of *Ndph*^{y/-} animals resulted in
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13 progressive hearing loss, leading to deafness, similar to the human phenotype [Rehm et al.,
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15 2002]. Adding to that, more light was shed on the biochemical role of NDP by overexpressing it
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17 transgenically in the eyes of *Ndph*^{y/-} mice under the control of lens specific promoter [Ohlmann
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19 et al., 2005]. Ectopically expressed Norrin surprisingly restored the normal retinal vasculature,
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21 while respecting the normal local architecture of the blood vessels. Recently, the fact that
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23 vascular changes occur in the cerebellum of hemizygous *Ndph*^{y/-} animals was noted as the first
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25 evidence of a brain phenotype in mice that could be correlated with the mental retardation seen
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27 in one third of human Norrie disease patients [Luhmann et al., 2008]. Finally, Schafer et al.
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29 [2009] provided further evidence about the multi-dimensional role of NDP during retinal
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31 angiogenesis. They postulated that the function of NDP may not only be limited to
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33 transcriptional regulation of β -catenin target genes that for example regulate blood vessel
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35 formation and integrity, but also play a role in the transcription of other targets like *Plvap* [Stan
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37 et al., 1999; Carson-Walter et al., 2005]. The latter would expand Norrin's functional spectrum,
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39 which might provide us with an insight into the phenotypic heterogeneity observed even in
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41 individuals of the same family suffering from FEVR or ND. The parallel contribution of other
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43 genetic determinants could affect the severity of disease symptoms by putatively altering target
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45 gene expression, something that potentially could be explored as a diagnostic or prognostic tool
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47 regarding the phenotypic severity of patients.
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Clinical, diagnostic, biological relevance and future prospects

With the present study on *FZD4*, *LRP5*, and *NDP*, we have attempted to provide a comprehensive summary of mutations that have been reported to affect these genes, with a focus on FEVR and Norrie disease. The three proteins co-operate in the Wnt/ β -catenin and/or Norrin/ β -catenin signaling pathway, by regulating angiogenesis and maintaining blood vessel integrity in the retina. Patients suffering from FEVR and to a lesser degree, Norrie disease, are often difficult to diagnose. Non-penetrance in FEVR can be observed in up to 25% of mutation carriers [Riveiro-Alvarez et al., 2005; Boonstra et al., 2009] and genotype-phenotype correlations are often not accurate enough. Wu et al. [2007] have suggested that *NDP* mutations affecting cysteine residues that play a firm role in the formation of the cysteine knot motif resulted in severe retinal dysgenesis and were diagnosed as Norrie disease. Nucleotide variants that affected non-cysteine residues were associated with FEVR-like phenotypes. Furthermore, it was also postulated that mutations in *LRP5* found in patients with FEVR or OPPG can result both in ocular and bone abnormalities, classifying both diseases under the same phenotypic spectrum [Qin et al., 2005]. The relatively small number of 28 exons and 32 amplicons that need to be analysed for these three genes renders Sanger sequence analysis cost effective. Molecular testing is important for diagnostic and prognostic purposes in individuals suspected to suffer from FEVR or ND, especially when coupled with a detailed family history. At least 40% of patients with FEVR do not carry mutations in *FZD4* or *LRP5* [Toomes et al., 2004a; Qin et al., 2005; Boonstra et al., 2009] so we are pursuing the identification of additional FEVR genes. Recently, in a cohort of eleven Dutch families that were tested negative for mutations in the ORF of *FZD4*, *LRP5* and *NDP* respectively; we identified *TSPAN12* as a gene causative for autosomal

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3 dominant FEVR [Nikopoulos et al., 2010]. The gene was mutated in five out of the eleven Dutch
4 families tested rendering it a relative frequent cause of FEVR. In parallel, Poulter et al. [2010]
5 identified the involvement of *TSPAN12* in FEVR by identifying seven novel mutations in this
6 gene in a cohort of 70 FEVR patients in whom known FEVR had already been excluded.. Our
7 emerging understanding of Wnt signaling offers hope that pharmaceutical agents that selectively
8 target this pathway will be developed to treat some phenotypic characteristics of these diseases in
9 the future.
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23 24 **Patients and Methods**

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27 The tenets of the Declaration of Helsinki were followed and informed consent was obtained from
28 all patients who participated in this study prior to donation of a blood sample. These studies were
29 approved by the local medical ethical commission.
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33 For mutation analysis of the probands, DNA was isolated from blood leukocytes by an automated
34 procedure with the magnetic bead platform from Chemagen AG (Baeswider, Germany). The
35 *FZD4* (2 exons, 6 amplicons), *LRP5* (23 exons, 23 amplicons), and *NDP* (3 exons, 3 amplicons)
36 genes were scanned for mutations by using standard sequencing methodologies on a genetic
37 analyzer (model 3730; ABI) with primers flanking the coding exons and the adjacent splice sites.
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39 Primers and PCR conditions are available on request.
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48 In this study we analysed probands from 16 different FEVR families. From those families, 11
49 were sporadic cases and five had two or more affected individuals. In total those families
50 comprise 20 affected and six not-affected individuals. In the 16 families we identified four
51 probands harboring aberrant nucleotide changes in *FZD4*, three in *LRP5*, and one harboring
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3 changes in both *FZD4* and *LRP5*. In seven families we did not identify any variants in those
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5 genes. We also analysed 29 sporadic cases and 17 families with two or more affected individuals
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7 with ND or ND-like features. Our screening identified 27 probands harboring aberrant nucleotide
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9 changes in the *NDP* ORF and four female index-patients being carriers of such changes.
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11 Probands of 15 families were tested negative for *NDP* changes and seven of those did not contain
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13 changes in the ORF of *FZD4* and *LRP5*.
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17 In families in which a genetic variant in one of the genes was identified, DNA of all available
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19 affected and non affected family members was analyzed for segregation of the specific variant by
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21 analysis of the relevant exons using the same conditions when this applicable. All novel variants
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23 in *FZD4* and *LRP5* were absent among 100 control alleles tested, or 100 X chromosomes in case
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25 of novel variants in *NDP*.
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34 **Acknowledgments**

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Legends to Figures

Figure 1. Schematic representation of the activation of the Norrin/ β -catenin canonical signaling pathway from TSPAN12/FZD4/LRP5 co-receptors upon activation by NDP. Mutations in the corresponding genes have been associated with impaired activation of the pathway and result in FEVR and Norrie disease, two conditions that not only share common molecular aetiologies but to a high extent show phenotypic overlap.

Figure 2. Schematic representation of LRP5 and 3D modelling of LRP5 missense changes. A) Schematic overview of LRP5 and its different domains. B) Overview of the second β -propeller domain of LRP5 and a close-up of the p.E441K change in the second β -propeller domain. The side chain of the wild-type residue (glutamic acid) in position 441 is shown in green, whereas the mutant lysine is shown in red. Two neighbouring arginines are coloured blue. C) Overview of one of the EGF-like domains and the p.C1253F change. Pair of cysteines forming disulfide bridges are shown in yellow. One of these cysteines is mutated into a phenylalanine, depicted in red.

Figure 3. 3D modelling of NDP missense changes: A) Ribbon model of NDP. The protein is coloured in grey, the cysteine side chains that participate in forming disulfide bonds and creating the critical knot-motif are shown in yellow. The wild-type and mutant residues are depicted in green and red respectively. B) Close-up of the p.C55R missense change. The wild-type cysteine creates a bond with the neighboring cysteine. The arginine residue at position 55 disrupts the disulfide bond. C) Close-up of the cysteine knot in NDP and missense change p.G67R. The cysteines forming the typical knot-motif are indicated. NDP contains an additional cysteine-bond

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3 which is shown in grey. Additionally, assumptions on the effect of p.G67E on the cysteine knot
4 motif can be drawn from the same figure as the stereochemical structure of glutamic acid is very
5 similar to arginine. D) Close-up of the p.F89L missense change. The side chains of
6 phenylalanine and leucine are shown in green and red respectively. E) Close-up of the p.S92P
7 missense change. The mutation precedes one of the important bonding cysteines in the protein
8 disturbing the creation of the disulfide bond and possibly affecting the local structure of NDP. F)
9 Close-up of the missense change p.P98L. The change is located close to the last cysteine in the
10 cysteine knot motif and to the unbound cysteine that is needed to form dimers. Conversion of
11 proline to leucine will probably have an impact on the local structure of the protein averting its
12 normal function.
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Table 1. Novel *FZD4* sequence variants that are likely to cause familial exudative vitreoretinopathy

Nucleotide variant	Effect	Exon	Occurrence in patients (probands)	Occurrence in control alleles	Segregation
Truncating changes					
c.856G>T	p.E286X	2	1/16	0/100	Yes
c.1282_1285del	p.D428SfsX2	2	1/16	0/100	Yes
Missense changes					
c.118G>C ^A	p.E40Q	1	1/16	0/100	Simplex
c.611G>A	p.C204Y	2	1/16	0/100	Simplex
c.1573G>C	p.G525R	2	1/16	0/100	Yes

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (GenBank NM_012193.2), according to journal guidelines (www.hgvs.org/mutnomen). The translation initiation codon is codon 1 (GenBank NP_036325.2).

^AThis patient also carries a splice site mutation in *LRP5* (c.4489-1G>A), see Table 2.

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Table 2. Novel *LRP5* sequence variants that are likely to cause familial exudative vitreoretinopathy

Nucleotide variant	Effect	Exon	Occurrence in patients (probands)	Occurrence in control alleles	Segregation
Truncating changes					
c.2978G>A	p.W993X	13	1/16	0/100	Simplex
Missense changes					
c.1321G>A	p.E441K	6	1/16	0/100	Yes
c.3758G>T ^B	p.C1253F	17	1/16	0/100	Simplex
c.4489-1G>A ^A	Splice defect	22	1/16	0/100	Simplex

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (GenBank NM_002335.2), according to journal guidelines (www.hgvs.org/mutnomen). The translation initiation codon is codon 1 (GenBank NP_002326.2).

^AThis patient also carries a second missense variant in *FZD4* p.E40Q (c.118G>C), see Table 1.

^BThis patient also carries a second missense variant in *LRP5* p.G610R (c.1828G>A).

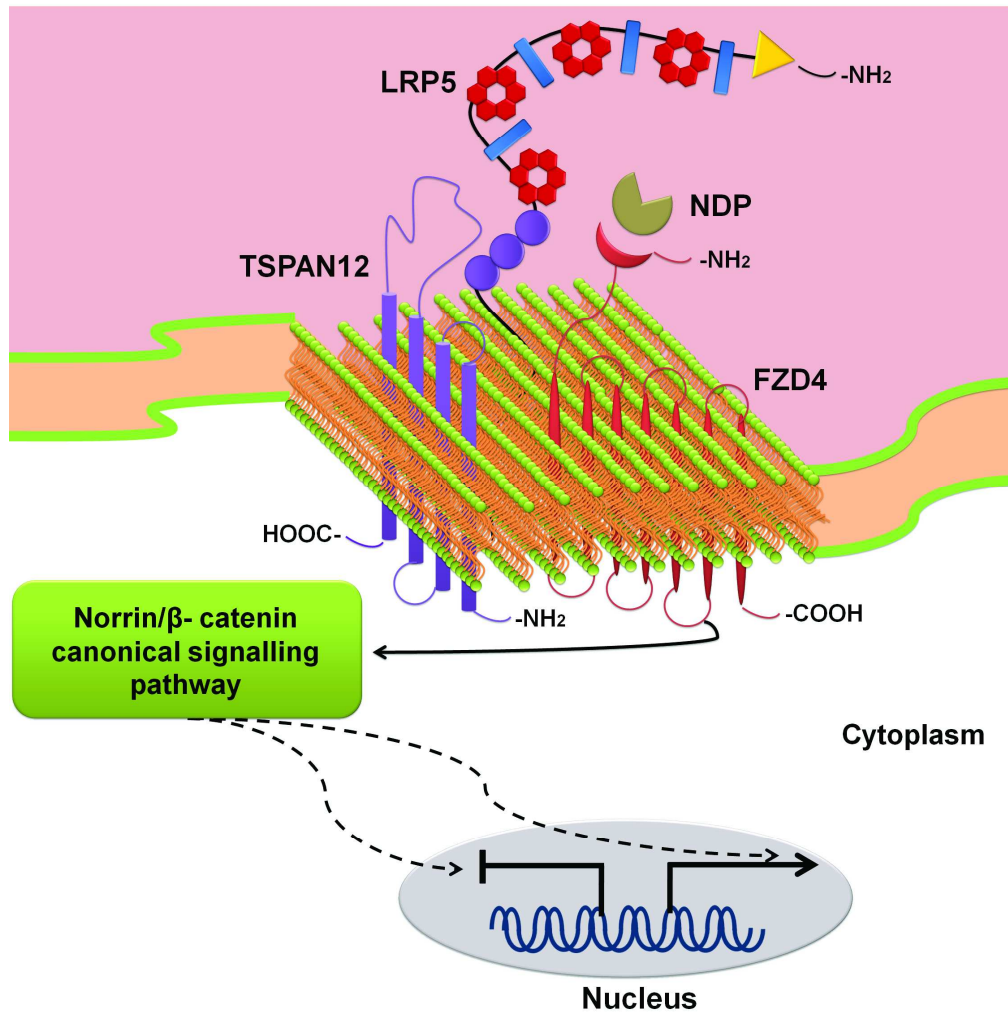
Table 3. Novel *NDP* sequence variants that are likely to cause Norrie Disease

Nucleotide variant	Effect	Exon	Occurrence in patients (probands)	Occurrence in control alleles	Segregation
Truncating changes					
c.11_12del	p.H4RfsX21	2	1/46	0/100	Simplex
c.25_40del	p.S9PfsX4	2	1/46	0/100	Simplex
c.129delC	p.Y44MfsX60	2	1/46	0/100	Simplex
c.332C>A	p.S111X	3	1/46	0/100	Simplex
Missense changes					
c.163T>C	p.C55R	2	1/46	0/100	Simplex
c.199G>A	p.G67R	3	1/46	0/100	Simplex
c.200G>A	p.G67E	3	1/46	0/100	Simplex
c.267C>A	p.F89L	3	1/46	0/100	Simplex
c.274T>C	p.S92P	3	1/46	0/100	Simplex
c.293C>T	p.P98L	3	1/46	0/100	Simplex
Splice-site changes					
c.-208+2T>G	Splice defect	1	1/46	0/100	Simplex
c.-208+5G>A	Splice defect	1	1/46	0/100	Yes

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (GenBank NM_000266.3), according to journal guidelines (www.hgvs.org/mutnomen). The translation initiation codon is codon 1 (GenBank NP_000257.1).

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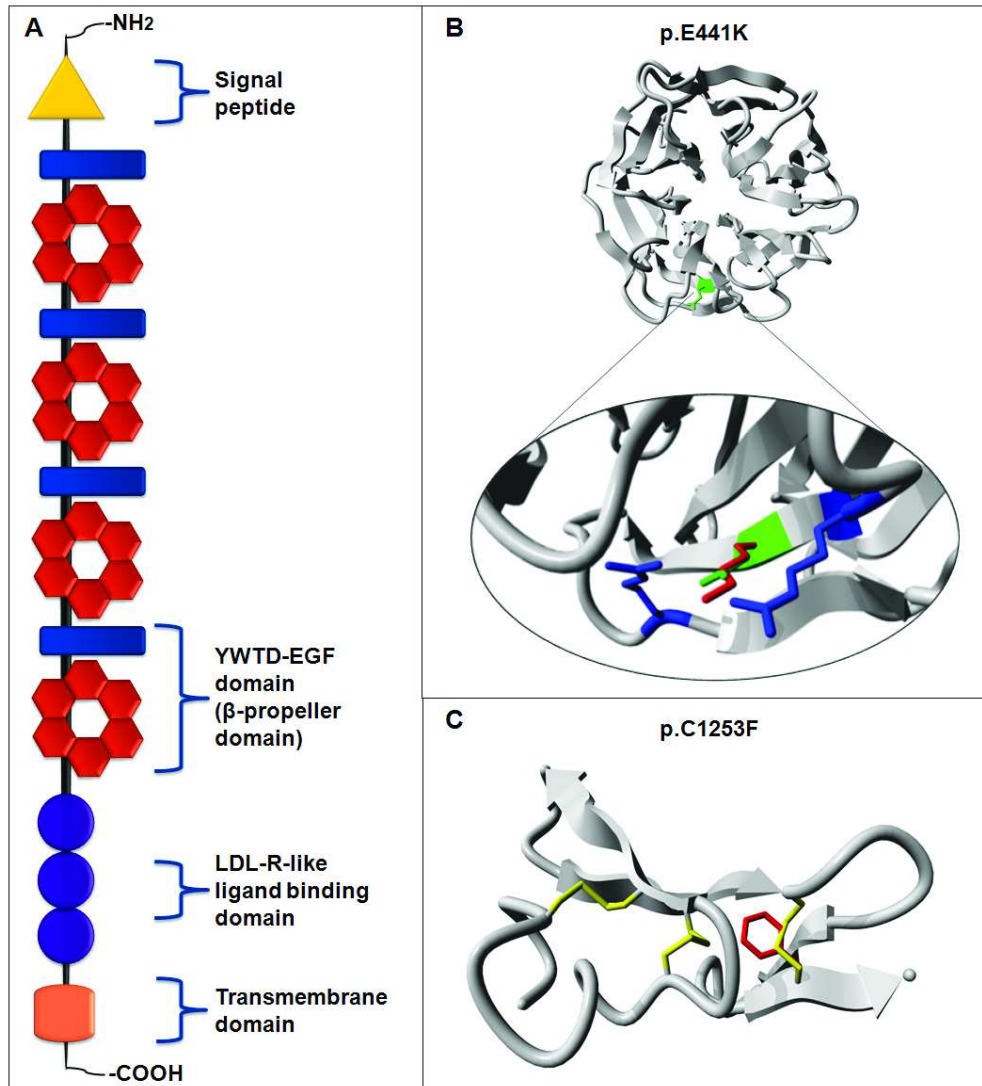
Figure 1



Schematic representation of the activation of the Norrin/β-catenin canonical signaling pathway from TSPAN12/FZD4/LRP5 co-receptors upon activation by NDP. Mutations in the corresponding genes have been associated with impaired activation of the pathway and result in FEVR and Norrie disease, two conditions that not only share common molecular aetiologies but to a high extent show phenotypic overlap.

283x298mm (200 x 200 DPI)

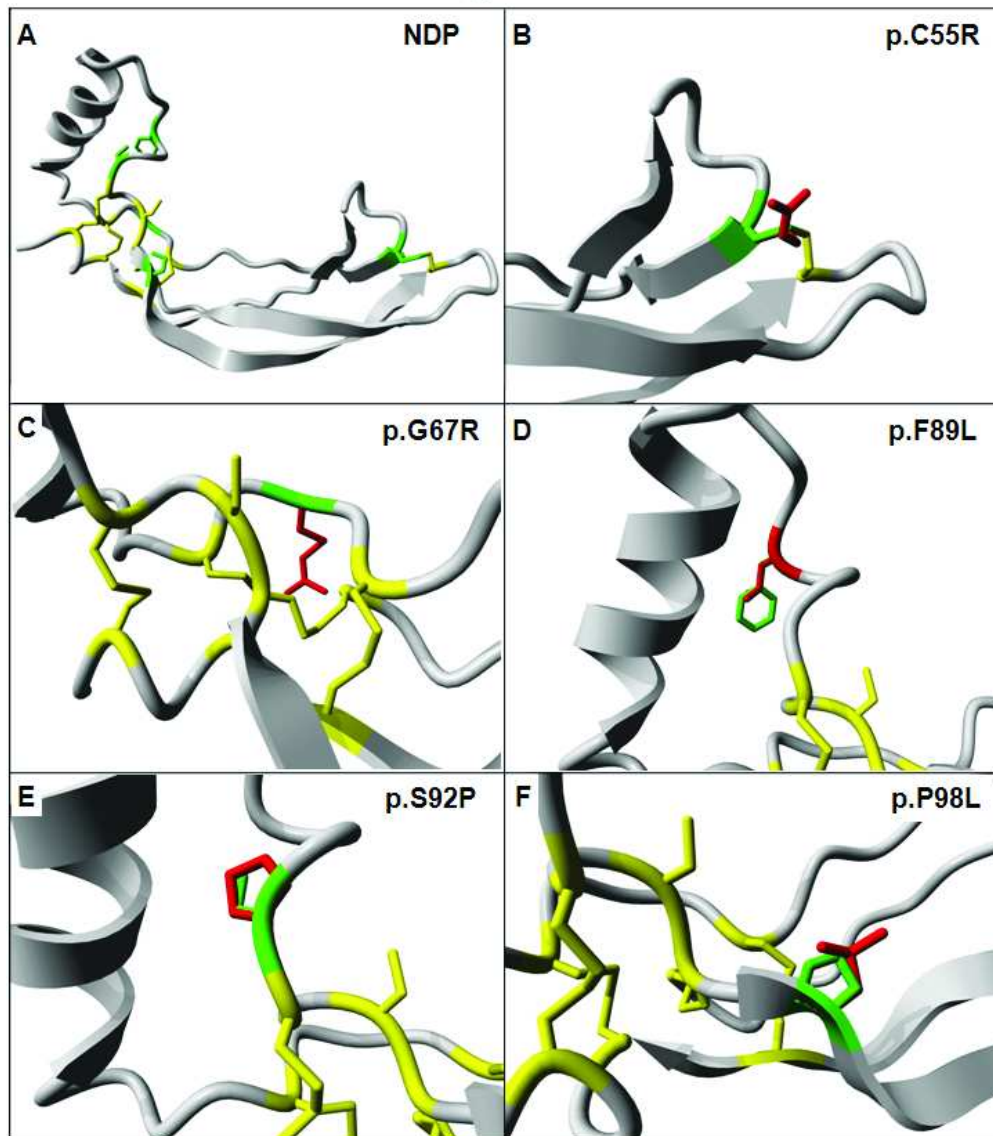
Figure 2



Schematic representation of LRP5 and 3D modelling of LRP5 missense changes. A) Schematic overview of LRP5 and its different domains. B) Overview of the second β-propeller domain of LRP5 and a close-up of the p.E441K change in the second β-propeller domain. The side chain of the wild-type residue (glutamic acid) in position 441 is shown in green, whereas the mutant lysine is shown in red. Two neighbouring arginines are coloured blue. C) Overview of one of the EGF-like domains and the p.C1253F change. Pair of cysteines forming disulfide bridges are shown in yellow. One of these cysteines is mutated into a phenylalanine, depicted in red.

135x156mm (200 x 200 DPI)

Figure 3

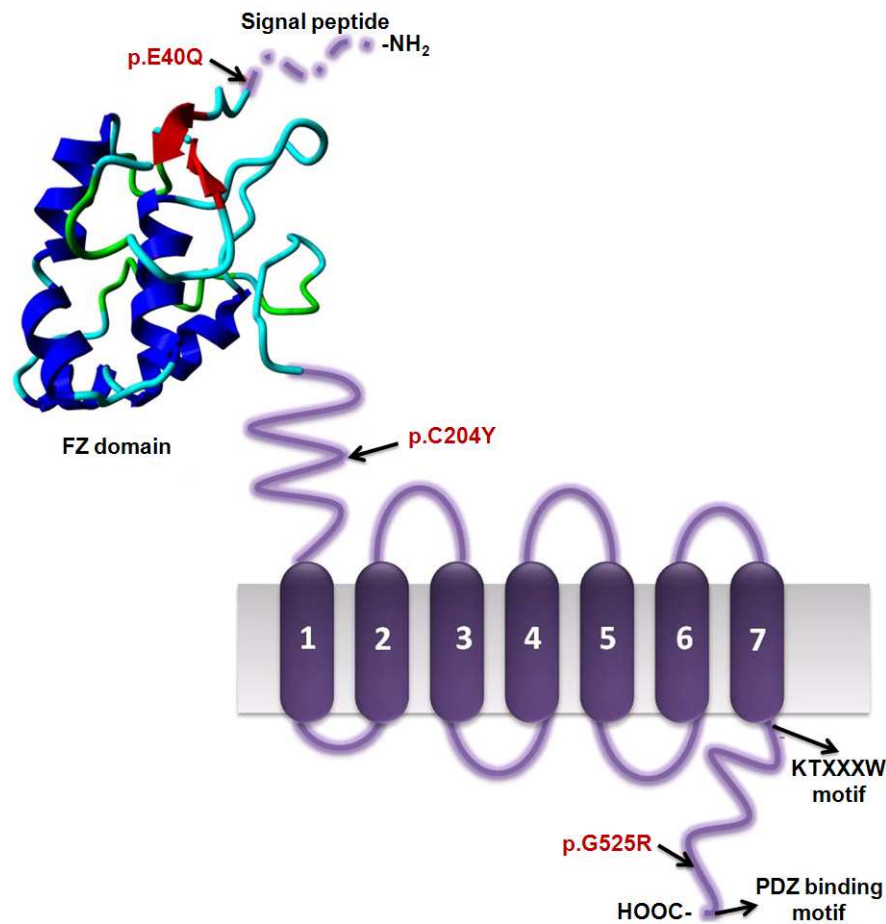


3D modelling of NDP missense changes: A) Ribbon model of NDP. The protein is coloured in grey, the cysteine side chains that participate in forming disulfide bonds and creating the critical knot-motif are shown in yellow. The wild-type and mutant residues are depicted in green and red respectively. B) Close-up of the p.C55R missense change. The wild-type cysteine creates a bond with the neighboring cysteine. The arginine residue at position 55 disrupts the disulfide bond. C) Close-up of the cysteine knot in NDP and missense change p.G67R. The cysteines forming the typical knot-motif are indicated. NDP contains an additional cysteine-bond which is shown in grey. Additionally, assumptions on the effect of p.G67E on the cysteine knot motif can be drawn from the same figure as the stereochemical structure of glutamic acid is very similar to arginine. D) Close-up of the p.F89L missense change. The side chains of phenylalanine and leucine are shown in green and red respectively. E) Close-up of the p.S92P missense change. The mutation precedes one of the important bonding cysteines in the protein disturbing the creation of the disulfide bond and possibly

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2
3 affecting the local structure of NDP. F) Close-up of the missense change p.P98L. The change is
4 located close to the last cysteine in the cysteine knot motif and to the unbound cysteine that is
5 needed to form dimers. Conversion of proline to leucine will probably have an impact on the local
6 structure of the protein averting its normal function.

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Supp. Figure S1. Topology of the Frizzled 4 receptor and the position of novel missense variants. The 7 transmembrane domains are shown as purple cylindrical columns numbered 1 through 7. The FZ-domain was modelled using the mouse Fzd8 homolog, which is 40% identical with human FZD4. Amino acids connecting the transmembrane domains are shown as purple lines; no structure could be predicted for these residues. The putative signal peptide is shown as a dashed line. The KTXXXW and PDZ binding motifs located in the C-terminal tail are indicated with arrows.

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4	A.	Missense variant				p.E40Q		
5		<i>Homo sapiens</i>	30	LLGPARGFGDE	-EERRCDPIR		49	
6		<i>Macaca mulatta</i>	30	LLGPARGFGDE	-EERRCDPIR		49	
7		<i>Mus musculus</i>	30	LLRPTL L FGFDE	-EERRCDPIR		49	
8		<i>Bos taurus</i>	30	LVEPVRGFGDE	-EERRCDPIR		49	
9		<i>Monodelphis domestica</i>	219	LLGQTRAFGDE	-EEMTCDPIR		238	
10		<i>Gallus gallus</i>	18	LLGGARGFGDE	-EERRCDPIR		37	
11		<i>Xenopus laevis</i>	16	VVGLIAGFGDE	-EERSCDPIR		35	
12		<i>Gastrosteus aculeatus</i>	22	ALREVEAFGDE	VEEMTCDPIR		42	
13								
14	B.	Missense variant				p.C204Y		
15		<i>Homo sapiens</i>	194	VKRSLNCVLKCGYDAGLYSRS			214	
16		<i>Macaca mulatta</i>	194	VKRSLNCVLKCGYDAGLYSRS			214	
17		<i>Mus musculus</i>	194	VKRSLNCVLKCGYDAGLYSRS			214	
18		<i>Bos taurus</i>	194	VKRSLNCVLKCGYDAGLYSRS			214	
19		<i>Monodelphis domestica</i>	383	VKRSLNCVLKCGYDAGLYNRS			403	
20		<i>Gallus gallus</i>	182	VKRNLDCVLKCGYDAGLYSRS			202	
21		<i>Xenopus laevis</i>	180	VKRSMNCVLKCGYD	SGLYNRL		200	
22		<i>Gastrosteus aculeatus</i>	187	LKQSESCALQCGYD	SGLYRGA		207	
23								
24	C.	Missense variant				p.G525R		
25		<i>Homo sapiens</i>	518	KREKR---	GNGWVKPGKGET		535	
26		<i>Macaca mulatta</i>	518	KREKR---	GNGWVKPGKGET		535	
27		<i>Mus musculus</i>	518	KREKR---	GNGWVKPGKGET		533	
28		<i>Bos taurus</i>	518	KREKR---	GNGWVKPGRGET		533	
29		<i>Monodelphis domestica</i>	707	KREKR---	GNGWIKPGKGET		724	
30		<i>Gallus gallus</i>	506	KREKR---	ADGWVKPGKGET		523	
31		<i>Xenopus laevis</i>	504	KRKKR---	VDGWVKPGKGET		521	
32		<i>Gastrosteus aculeatus</i>	511	SRGGKPARGE	GWIKPGKGET		521	

Supp. Figure S2. Amino acid sequence alignment of human FZD4 with orthologous proteins from, rhesus monkey, mouse, cow, opossum, chicken, frog, and stickleback. Ten upstream and 10 downstream amino acids of the missense variants p.E40Q (panel A), p.C204Y (panel B) and p.G525R (panel C) are depicted. Residues identical across all sequences are black on a white background whereas similar amino acids are indicated in white on a gray background. Non similar and weakly similar amino acids are indicated in white on a black background. The amino acid residue at the position of the missense change is indicated in bold. GenBank and/or Ensembl protein ID numbers for FZD4 amino acid alignment: *Homo sapiens* NP_036325.2; *Macaca mulatta* XP_001103927.1; *Mus musculus* NP_032081.3; *Bos taurus* XP_874584.2; *Monodelphis domestica* XP_001376661.1; *Gallus gallus* NP_989430.1; *Xenopus laevis* NP_001083922.1; *Gastrosteus aculeatus* ENSGACP00000010666.

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4	A.	Missense variant				p.E441K		
5		Homo sapiens	431	Y	W	T	D	T
6		Pan troglodytes	517	Y	W	T	D	T
7		Mus musculus	430	Y	W	T	D	T
8		Bos taurus	618	Y	W	T	D	T
9		Gallus gallus	430	Y	W	T	D	T
10		Anolis carolinensis	405	Y	W	T	D	T
11		Xenopus laevis	421	Y	W	T	D	T
12		Danio rerio	408	Y	W	T	D	T
13		Drosophila melanogaster	492	Y	W	T	D	T
14								
15	B.	Missense variant				p.C1253F		
16		Homo sapiens	1243	H	L	V	L	L
17		Pan troglodytes	1329	H	L	V	L	L
18		Mus musculus	1242	H	L	V	L	L
19		Bos taurus	1430	H	L	V	L	L
20		Gallus gallus	1244	H	L	V	L	L
21		Anolis carolinensis	1217	H	L	V	L	L
22		Xenopus laevis	1235	H	L	V	L	L
23		Danio rerio	1217	H	L	V	L	L
24		Drosophila melanogaster	1303	H	L	V	L	L
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Supp. Figure S3. Amino acid sequence alignment of human LRP5 with orthologous proteins from chimpanzee, mouse, cow, chicken, lizard, frog, zebra fish and fruit fly. Ten amino acids in the vicinity of missense changes p.E441K (panel A) and p.C1253F (panel B) are depicted. Residues identical across all sequences are black on a white background whereas similar amino acids are indicated in white on a gray background. Non similar and weakly similar amino acids are indicated in white on a black background. The amino acid residue at the position of the missense change is bolded. GenBank and/or Ensembl protein ID numbers for LRP5 amino acid alignment: *Homo sapiens* NP_002326.2; *Pan troglodytes* XP_508605.2; *Mus musculus* NP_032539.1; *Bos taurus* XP_614220.3; *Gallus gallus* NP_001012915.1; *Anolis carolinensis* ENSACAP00000014430; *Xenopus laevis* NP_001079163.1; *Danio rerio* XP_696943.3; *Drosophila arrow* NP_524737.2.

			p. C55R		p. G67E p. G67R		p. S92P		p. F89L		p. P98L		
	Homo sapiens	47	SISHPLYK C SSKMVLLARCEGHCSQASRSEPLVSFSTVLKQ PFRS SCHCC R PQTSK L KALRL	108									
	Pan troglodytes	47	SISHPLYK C SSKMVLLARCEGHCSQASRSEPLVSFSTVLKQ PFRS SCHCC R PQTSK L KALRL	108									
	Mus musculus	45	SISHPLYK C SSKMVLLARCEGHCSQASRSEPLVSFSTVLKQ PFRS SCHCC R PQTSK L KALRL	108									
	Sus scrofa	47	SISHPLYK C SSKMVLLARCEGHCSQASRSEPLVSFSTVLKQ PFRS SCHCC R PQTSK L KALRL	108									
	Bos taurus	47	SISHPLYK C SSKMVLLARCEGHCSQASRSEPLVSFSTVLKQ PFRS SCHCC R PQTSK L KALRL	106									
	Canis familiaris	47	SISHPLYK C SSKMVLLARCEGHCSQASRSEPLVSFSTVLKQ PFRS SCHCC R PQTSK L KALRL	108									
	Echinops telfairi	47	SISHPLYK C SSKMVLLARCEGHCSQASRSEPLVSFSTVLKQ PFRS SCHCC R PQTSK L KALRL	108									
	Gallus gallus	47	SISHPLYK C SSKMVLLARCE G HC S QASRSEPLVSFSTVLKQ PFRS TCHCC R PQTSK L KAMRL	108									
	Anolis carolinensis	47	SISHPLYK C SSKMVLLARCE G HC S QASRSEPLVSFSTVLKQ PFRS TCHCC R PQTSK L KAMRL	108									
	Xenopus laevis	46	SIN H PO H K C TAKMVLLARCE G HC S QASRSEPLVSFSTVLKQ PFRS TCHCC R PQTSK L KAMRL	107									
	Danio rerio	53	TI I TH P LYK N SKMVLLARCE G HC S HTSRSD P L S FS S VLKQ P F K NT C CC R P H TSK L KAMRL	114									

Supp. Figure S4. Protein sequence alignment of human NDP with orthologues from chimpanzee, mouse, pig, cow, dog, tenrec, chicken, lizard, frog and zebrafish. Residues identical across all sequences are black on a white background whereas similar amino acids are indicated in white on a gray background. Non similar and weakly similar amino acids are indicated in white on a black background. The amino acid residue at the position of the missense change is bolded. GenBank and/or Ensembl protein ID numbers for NDP amino acid alignment: *Homo sapiens* NP_000257.1; *Pan troglodytes* XP_528948.1; *Mus musculus* NP_035013.1; *Sus scrofa* NP_001106528; *Bos taurus* NP_001039555.1; *Canis familiaris* XP_855261.1; *Echinops telfairi* ENSP00000367301; *Gallus gallus* XP_416765.1; *Anolis carolinensis* ENSACAP00000017627; *Xenopus laevis* NP_000257.1; *Danio rerio* XP_001338820.1.

Supp Table S1. *FZD4* sequence variants that are likely to be pathogenic

Nucleotide variant	Effect	Exon	Occurrence in patients (proband)	Occurrence in control alleles	Segregation	Disease	Reference
Truncating changes							
c.244_251delins27	p.F82AfsX53	1	1/53	0/200	Yes	FEVR	[Nallathambi et al., 2006]
c.856G>T	p.E286X	2	1/8	0/100	Yes	FEVR	This study
c.957delG	p.W319CfsX5	2	1/40	0/200	Simplex	FEVR	[Toomes et al., 2004b]
c.957G>A	p.W319X	2	5/20, 1/24	0/80, 0/300	Yes, <i>De novo</i>	FEVR, FEVR	[Kondo et al., 2003; Boonstra et al., 2009]
c.1282_1285del	p.D428SfsX2	2	1/8	0/100	Yes	FEVR	This study
c.1286_1290del	p.K429RfsX28	2	1/21	N/A	Yes	FEVR	[Muller et al., 2008]
c.1488G>A	p.W496X	2	1/20	0/80	Yes	FEVR	[Boonstra et al., 2009]
c.1498delA	p.T500LfsX13	2	1/40	0/200	Simplex	FEVR	[Toomes et al., 2004b]
c.1501_1502del	p.L501SfsX33	2	1/2, 1/40	0/306, 0/200	Yes, Yes	FEVR, FEVR	[Robitaille et al., 2002; Toomes et al., 2004b]
c.1513C>T	p.Q505X	2	1/40	0/200	Yes	FEVR	[Toomes et al., 2004b]
Missense changes							
c.107G>A	p.G36D	1	1/40	0/400	Simplex	FEVR	[Toomes et al., 2004b]
c.118G>C	p.E40Q	1	1/8	0/100	Simplex	FEVR	This study ^A
c.313A>G	p.M105V	2	1/24	0/300	Yes	FEVR	[Kondo et al., 2003]
c.314T>C	p.M105T	2	1/40	0/400	Simplex	FEVR	[Toomes et al., 2004b]
c.341T>C	p.I114T	2	1/2	N/A	Yes	FEVR	[Robitaille et al., 2009]
c.469A>G	p.M157V	2	1/40	0/400	Yes	FEVR	[Toomes et al., 2004b]
c.541T>C	p.C181R	2	1/2	0/240	Yes	FEVR	[Omoto et al., 2004]
c.609G>C	p.K203N	2	1/104	0/346	Yes	ROP	[Ells et al., 2010]
c.611G>A	p.C204Y	2	1/8	0/100	Simplex	FEVR	This study [Nallathambi et al., 2006]
c.612T>C	p.C204R	2	1/53	0/200	Yes	FEVR	
c.668T>A	p.M223K	2	1/20	0/80	Yes	FEVR	[Boonstra et al., 2009]

								[MacDonald et al., 2005]
c.766A>G	p.I256V	2	1/20	0/200	Simplex	FEVR		
c.1005G>C	p.W335C	2	1/56	0/300	Yes	FEVR		[Qin et al., 2005]
c.1024A>G	p.M342V	2	1/1,	0/120,	Yes,	FEVR,		[Yoshida et al., 2004;
			1/56	0/300	Simplex	FEVR		Qin et al., 2005]
c.1109C>G	p.A370G	2	1/104	0/346	Simplex	ROP		[Ells et al., 2010]
c.1250G>A	p.R417Q	2	2/24	0/300	Yes	FEVR		[Kondo et al., 2003]
c.1333A>C	p.T445P	2	1/20	0/80	Yes	FEVR		[Boonstra et al., 2009]
c.1463G>A	p.G488D	2	1/24	0/300	Yes	FEVR		[Kondo et al., 2003]
c.1474G>C	p.G492R	2	1/2	N/A	Yes	FEVR		[Muller et al., 2008]
c.1490C>T	p.S497F	2	1/40	0/400	Simplex	FEVR		[Toomes et al., 2004b]
c.1573G>C	p.G525R	2	1/8	0/100	Yes	FEVR		This study
Miscellaneous changes								
c.1479_1484del	p.M493_W494del	2	1/2	0/306	Yes	FEVR		[Robitaille et al., 2002]

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (GenBank NM_012193.2), according to journal guidelines (www.hgvs.org/mutnomen). The translation initiation codon is codon 1 (GenBank NP_036325.2).

^AThis patient also carries a splice site mutation in *LRP5* (c.4489-1G>A), see Supp. Table S2.

FEVR, familial exudative vitreoretinopathy (autosomal dominant); ROP, retinopathy of prematurity; N/A, information not available or screening was not performed.

Supp. Table S2. *LRP5* sequence variants that are likely to be pathogenic

Nucleotide variant	Effect	Exon	Occurrence in patients (proband)	Occurrence in control alleles	Segregation	Disease	Reference
Truncating changes							
c.29G>A	p.W10X	1	1/28, 1/37	0/50, N/A	Yes, Yes	OPPG, OPPG	[Gong et al., 2001; Ai et al., 2005]
c.209_210TC>AA	p.F70X	2	1/37	N/A	Yes	OPPG	[Ai et al., 2005]
c.765G>A	p.W255X	4	1/37	N/A	Yes	OPPG	[Ai et al., 2005]
c.789C>A	p.C263X	4	1/37	N/A	Yes	OPPG	[Ai et al., 2005]
c.803_812del	p.G269RfsX4	4	1/56	0/362	Simplex	arFEVR	[Qin et al., 2005]
c.1000_1004dup	p.C336GfsX50	5	1/37	N/A	Yes	OPPG	[Ai et al., 2005]
c.1275G>A	p.R425X		3/3	N/A	Yes	OPPG	[Streeten et al., 2008]
c.1282C>T	p.R428X	6	1/28, 1/37	0/50, N/A	Yes, Yes	OPPG, OPPG	[Gong et al., 2001; Ai et al., 2005]
c.1453G>T	p.E485X	7	1/28, 1/37	0/50, N/A	Yes, Yes	OPPG, OPPG	[Gong et al., 2001; Ai et al., 2005]
c.1468delG	p.D490MfsX40	7	1/28, 1/37	0/50, N/A	Yes, Yes	OPPG, OPPG	[Gong et al., 2001; Ai et al., 2005]
c.1750C>T	p.Q584X	8	1/37	N/A	Yes	OPPG	[Ai et al., 2005]
c.2151dupT	p.D718X	10	1/28, 1/37	0/50, N/A	Yes, Yes	OPPG, OPPG	[Gong et al., 2001; Ai et al., 2005]
c.2202G>A	p.W734X	10	1/28, 1/37	0/50, N/A	Yes, Yes	OPPG, OPPG	[Gong et al., 2001; Ai et al., 2005]

								2005]
c.2247delG	p.Q750SfsX48	10	2/37	N/A	Yes	OPPG	[Ai et al., 2005]	
c.2305delG	p.D769IfsX29	10	1/27	0/50	Yes	OPPG	[Gong et al., 2001]	
c.2409_2503+79del174	p.G804_G835delfs49X	11	1/37	N/A	Yes	OPPG	[Ai et al., 2005]	
c.2557C>T	p.Q853X	12	1/28, 1/37	0/50, N/A	Yes, Yes	OPPG, OPPG	[Gong et al., 2001; Ai et al., 2005]	
c.2718_2721del	p.M907TfsX52	12	1/37	N/A	Yes	OPPG	[Ai et al., 2005]	
c.2737dupT	p.C913LfsX73	12	1/37, 1/20	N/A, 0/246	Yes, Yes	Primary osteoporosis, OPPG	[Ai et al., 2005; Hartikka et al., 2005]	
c.2978G>A	p.W993X	13	1/8	0/100	Simplex	FEVR	This study	
c.3232C>T	p.R1078X	14	1/37	N/A	Yes	OPPG	[Ai et al., 2005]	
c.3804delA	p.E1270RfsX169	18	1/28, 1/32, 2/37	0/50, 0/400, N/A	Yes, Simplex, Yes	OPPG, FEVR, OPPG	[Gong et al., 2001; Toomes et al., 2004a; Ai et al., 2005]	
c.4105_4106del	p.M1369VfsX2	19	1/37	N/A	Yes	OPPG	[Ai et al., 2005]	
c.4119dupC	p.L1374QfsX176	20	1/32	0/400	Yes	FEVR	[Toomes et al., 2004a]	
c.4512_4517delinsTGT ACAACAT	p.A1505VfsX46	22	1/37	N/A	Yes	OPPG	[Ai et al., 2005]	
c.4600C>T	p.R1534X	23	1/37	N/A	Yes	OPPG	[Ai et al., 2005]	
Missense changes								
c.85G>A	p.A29T	1	1/20	0/246	Yes	Primary osteoporosis	[Hartikka et al., 2005]	
c.331G>T	p.D111Y	2	1/10	0/100	Yes	HBM	[van Wesenbeeck et al., 2003]	
c.433C>T	p.L145F	2	1/56	0/362	Yes	FEVR	[Qin et al., 2005]	
c.461G>T	p.R154M	2	1/1	1/544	Yes	HBM	[Rickels et al.,	

								2005]
c.511G>C	p.G171R	3	1/10	0/100	Yes	HBM		[van
								Wesenbeeck et
								al., 2003]
c.511G>T	p.G171V	3	1/1, 1/1	0/420, 0/2000	Yes, Yes	HBM, HBM		[Boyden et al.,
								2002; Little et
								al., 2002]
								[Toomes et al.,
c.518C>T	p.T173M	3	1/32	0/400	Simplex	FEVR		2004a]
c.607G>A	p.D203N	3	1/32	N/A	Yes	OPPG		[Ai et al., 2005]
c.640G>A	p.A214T	3	1/10	0/100	Yes	HBM		[van
								Wesenbeeck et
								al., 2003]
c.641C>T	p.A214V	3	1/10	0/100	Simplex	HBM		[van
								Wesenbeeck et
								al., 2003]
c.724G>A	p.A242T	4	4/10	0/100	Yes	HBM		[van
								Wesenbeeck et
								al., 2003]
c.731C>T	p.T244M	4	1/37	N/A	Yes	OPPG		[Ai et al., 2005]
c.758C>T	p.T253I	4	2/10	0/100	Yes	HBM		[van
								Wesenbeeck et
								al., 2003]
								[Balemans et
c.844A>G	p.M282V	4	1/1	0/100	Simplex	HBM		al., 2007]
c.920C>T	p.S307F	5	1/37	N/A	Yes	OPPG		[Ai et al., 2005]
c.1042C>T	p.R348W	6	1/37	N/A	Yes	OPPG		[Ai et al., 2005]
c.1058G>A	p.R353Q	6	1/37	N/A	Yes	OPPG		[Ai et al., 2005]
c.1067C>T	p.S356L	6	1/66	0/390	Yes	Idiopathic osteoporosis		[Crabbe et al.,
								2005]
c.1169C>A	p.T390K	6	1/37	N/A	Yes	OPPG		[Ai et al., 2005]
c.1199C>A	p.A400E	6	1/37	N/A	Yes	OPPG		[Ai et al., 2005]
c.1210G>A	p.G404R	6	1/37	N/A	Yes	OPPG		[Ai et al., 2005]

								[Streeten et al., 2008]
c.1225A>G	p.T409A	6	1/3	N/A	Yes	OPPG		[Ai et al., 2005]
c.1300G>A	p.D434N	6	1/37	N/A	Yes	OPPG		This study
c.1321G>A	p.E441K	6	1/8	0/100	Yes	FEVR		[Qin et al., 2005]
c.1330C>T	p.R444C	6	1/56	0/362	Yes	FEVR		[Crabbe et al., 2005]
c.1364C>T	p.S455L	6	1/66	0/390	Yes	Idiopathic osteoporosis		[Crabbe et al., 2005]
c.1378G>A	p.E460K	6	1/32	N/A	Yes	OPPG		[Ai et al., 2005]
c.1432T>A	p.W478R	7	1/1	0/200	Yes	OPPG		[Cheung et al., 2006]
c.1481G>A	p.R494Q	7	1/28, 1/37	0/50, N/A	Yes, Yes	OPPG, OPPG		[Gong et al., 2001; Ai et al., 2005]
c.1513G>T	p.W504C	7	1/1	0/200	Yes	OPPG		[Cheung et al., 2006]
c.1532A>C	p.D511A	7	1/20	0/80	Yes	FEVR		[Boonstra et al., 2009]
c.1559G>T	p.G520V	7	1/37	N/A	Yes	OPPG		[Ai et al., 2005]
c.1564G>A	p.A522T	7	1/56	0/362	Yes	FEVR		[Qin et al., 2005]
c.1592A>T	p.N531I	8	1/1	N/A	Yes	OPPG		[Barros et al., 2007]
c.1604C>T	p.T535M	8	1/56	0/362	Simplex	arFEVR		[Qin et al., 2005]
c.1648G>A	p.G550R	8	1/1	0/120	Yes	arFEVR		[Downey et al., 2006]
c.1708C>T	p.R570W	8	1/28, 2/37	0/50, N/A	Yes, Yes	OPPG, OPPG		[Gong et al., 2001; Ai et al., 2005]
c.1709G>A	p.R570Q	8	1/3	0/200	Yes	arFEVR		[Jiao et al., 2004]
c.1828G>A	p.G610R	9	1/37,	N/A,	Yes,	OPPG,		[Ai et al., 2005;

				1/56	0/362	Simplex	arFEVR	Qin et al., 2005]
								[Qin et al., 2005]
								[Gong et al., 2001]
7	c.1850T>G	p.F617C	9	1/56	0/362	Simplex	arFEVR	[Ai et al., 2005]
9	c.1999G>A	p.V667M	9	1/28	0/50	Yes	OPPG	[Ai et al., 2005]
10	c.2047G>A	p.D683N	9	1/37	N/A	Yes	OPPG	[Ai et al., 2005]
11	c.2197T>C	p.Y733H	10	1/37	N/A	Yes	OPPG	[Ai et al., 2005]
12								[Jiao et al., 2004]
14	c.2302C>G	p.R752G	19	1/3	0/200	Yes	arFEVR	[Qin et al., 2005]
15								[Boonstra et al., 2009]
16	c.2392A>G	p.T798A	11	1/56	0/362	Yes	FEVR	[Hartikka et al., 2005]
17								[Ai et al., 2005]
18	c.2413C>T	p.R805W	11	1/20	0/80	Yes	FEVR	[Ai et al., 2005]
19	c.3107G>A	p.R1036Q	14	1/20	0/246	Yes	Primary osteoporosis	[Qin et al., 2005]
20								[Toomes et al., 2004a]
22	c.3295G>T	p.D1099Y	15	1/37	N/A	Yes	OPPG	This study ^B
23	c.3337C>T	p.R1113C	15	1/37	N/A	Yes	OPPG	[Toomes et al., 2004a]
24								[Jiao et al., 2004]
26	c.3361A>G	p.N1121D	15	1/56	0/362	Yes	FEVR	[Ai et al., 2005]
27								[Crabbe et al., 2005]
28	c.3502T>C	p.Y1168H	16	1/32	0/400	Yes	FEVR	
29	c.3758G>T	p.C1253F	17	1/8	0/100	Simplex	FEVR	
30								
31	c.4081T>G	p.C1361G	19	1/32	0/400	Simplex	FEVR	
32								
33								
34	c.4147G>A	p.E1367K	20	1/3	0/200	Yes	arFEVR	
35	c.4202G>A	p.G1401D	20	1/37	N/A	Yes	OPPG	
36	c.4609G>A	p.A1537T	23	1/66	0/390	Yes	Idiopathic osteoporosis	
37								
38								
39	Splice-site changes							
40								
41	c.3763+2T>C	Splice defect	17	1/37	N/A	Yes	OPPG	[Ai et al., 2005]
42								
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							[Toomes et al., 2004a]
c.4488+2T>G	Splice defect	21	1/32	0/400	Yes	FEVR	
c.4489-1G>A	Splice defect	22	1/8	0/100	Simplex	FEVR	This study ^A
c.4588+2T>C	Splice defect	22	1/37	N/A	Yes	OPPG	[Ai et al., 2005]

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (GenBank NM_002335.2), according to journal guidelines (www.hgvs.org/mutnomen). The translation initiation codon is codon 1 (GenBank NP_002326.2).

^AThis patient also carries a second missense variant in *FZD4* p.E40Q (c.118G>C), see Supp. Table S1.

^BThis patient also carries a second missense variant in *LRP5* p.G610R (c.1828G>A).

arFEVR, autosomal recessive familial exudative vitreoretinopathy; HBM, high-bone-mass; OPPG, osteoporosis-pseudoglioma syndrome; N/A, information not available or screening was not performed.

Supp. Table S3. *LRP5* sequence variants in patients with osteoporosis–pseudoglioma syndrome and ar familial exudative vitreoretinopathy

Disease	Allele 1		Allele 2		Reference
OPPG	c.29G>A	p.W10X	c.29G>A	p.W10X	[Gong et al., 2001; Ai et al., 2005]
OPPG	c.209_210TC>AA	p.F70X	c.1378G>A	p.E460K	[Ai et al., 2005]
OPPG	c.607G>A	p.D203N	c.1199C>A	p.A400E	[Ai et al., 2005]
OPPG	c.731C>T	p.T244M	c.3763+2T>C	Splice defect	[Ai et al., 2005]
OPPG	c.765G>A	p.W255X	c.1067C>T	p.S356L	[Ai et al., 2005]
OPPG	c.789C>A	p.C263X	c.2202G>A	p.W734X	[Ai et al., 2005]
OPPG	c.920C>T	p.S307F	c.920C>T	p.S307F	[Ai et al., 2005]
OPPG	c.1000_1004dup	p.C336Gfsx50	c.1000_1004dup	p.C336GfsX50	[Ai et al., 2005]
OPPG	c.[1042C>T; 2047G>A; 3337C>T]	p.[R348W; D683N; R1113C]	+	+	[Ai et al., 2005]
OPPG	c.1058G>A	p.R353Q	c.1058G>A	p.R353Q	[Ai et al., 2005]
OPPG	c.1169C>A	p.T390K	+	+	[Ai et al., 2005]
OPPG	c.1210G>A	p.G404E	c.2718_2721del	p.M907TfsX52	[Ai et al., 2005]
OPPG	c.1225A>G	p.T409A	c.1275G>A	p.W425X	[Streeten et al., 2008]
OPPG	c.1275G>A	p.W425X	c.1275G>A	p.W425X	[Streeten et al., 2008]
OPPG	c.1282C>T	p.R428X	c.1282C>T	p.R428X	[Gong et al., 2001; Ai et al., 2005]
OPPG	c.1300G>A	p.D434N	c.1559G>T	p.G520V	[Ai et al., 2005]
OPPG	c.1432T>A	p.W478R	c.1513G>T	p.W504C	[Cheung et al., 2006]
OPPG	c.1453G>T	p.E485X	c.4600C>T	p.R1534X	[Gong et al., 2001; Ai et al., 2005]
OPPG	c.1468delG	p.D490MfsX40	c.1468delG	p.D490MfsX40	[Gong et al., 2001; Ai et al., 2005]

Disease	Allele 1		Allele 2		Reference
					2005]
OPPG	c.1481G>A	p.R494Q	c.1481G>A	p.R494Q	[Gong et al., 2001; Ai et al., 2005]
					[Barros et al., 2007]
OPPG	c.1592A>T	p.N531I	c.1592A>T	p.N531I	[Gong et al., 2001; Ai et al., 2005]
OPPG	c.1708C>T	p.R570W	c.1708C>T	p.R570W	[Jiao et al., 2004]
arFEVR	c.1709G>A	p.R570Q	c.1709G>A	p.R570Q	[Ai et al., 2005]
OPPG	c.1750C>T	p.Q584X	c.4512_4517delinsTGTACAACAT	p.A1505VfsX46	[Ai et al., 2005]
OPPG	c.1828G>C	p.G610R	+	+	[Ai et al., 2005]
arFEVR	c.1828G>C	p.G610R	c.3758G>T	p.C1253F	This study
					[Gong et al., 2001]
OPPG	c.1999G>A	p.V667M	c.1999G>A	p.V667M	[Gong et al., 2001; Ai et al., 2005]
OPPG	c.2151dupT	p.D718X	c.2151dupT	p.D718X	[Ai et al., 2005]
					[Gong et al., 2001]
OPPG	c.2197T>C	p.Y733H	c.2247delG	p.Q749fsX797	[Ai et al., 2005]
					[Gong et al., 2001]
OPPG	c.2202G>A	p.W734X	+	+	[Ai et al., 2005]
OPPG	c.2247delG	p.Q750SfsX48	c.2738dupT	p.C913LfsX73	[Jiao et al., 2004]
					[Gong et al., 2001]
arFEVR	c.2302C>G	p.R752G	c.2302C>G	p.R752G	[Ai et al., 2005]
					[Gong et al., 2001]
OPPG	c.2305del	p.D769IfsX29	+	+	[Ai et al., 2005]
OPPG	c.2409_2503+79del174	p.G804_G835delfsX49	c.3232C>T	p.R1078X	[Ai et al., 2005]
OPPG	c.2557C>T	p.Q853X	c.2557C>T	p.Q853X	[Gong et al., 2001; Ai et al., 2005]
OPPG	c.3295G>T	p.D1099Y	+	+	[Gong et al., 2001; Ai et al., 2005]

Disease	Allele 1		Allele 2		Reference
OPPG	c.3804delA	p.E1270RfsX169	c.3804delA	p.E1270RfsX169	[Gong et al., 2001; Ai et al., 2005]
OPPG	c.4105_4106del	p.M1369VfsX2	+	+	[Ai et al., 2005] [Jiao et al., 2004]
arFEVR	c.4147G>A	p.E1367K	c.4147G>A	p.E1367K	[Jiao et al., 2004]
OPPG	c.4202G>A	p.G1401D	c.4588+2T	Splice defect	[Ai et al., 2005]

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (GenBank NM_002335.2), according to journal guidelines (www.hgvs.org/mutnomen). The translation initiation codon is codon 1 (GenBank NP_002326.2).

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Supp. Table S4. *NDP* sequence variants that are likely to be pathogenic

Nucleotide variant	Effect	Exon	Occurrence in patients (proband)	Occurrence in control chromosomes	Segregation	Disease	Reference
Truncating changes							
c.11_12del	p.H4RfsX21	2	1/13	0/100	Simplex	ND	This study
c.24_27dup	p.F10IfsX17	2	1/17	0/22 ^A	Simplex	ND	[Berger et al., 1992b]
c.25_40del	p.S9PfsX4	2	1/13	0/100	Simplex	ND	This study
c.50dupT	p.I18DfsX8	2	1/3	N/A	Yes	ND	[Gal et al., 1996]
c.65delC	p.T22KfsX10	2	1/26	0/100	Yes	ND	[Schuback et al., 1995]
c.86C>G	p.S29X	2	1/6	0/20	Simplex	ND	[Meindl et al., 1992]
c.109C>T	p.R37X	2	1/1	N/A	Yes	ND	[Ott et al., 2000]
c.128dupA	p.H43QfsX14	2	N/A	N/A	N/A	ND	[Caballero et al., 1996]
c.129delC	p.Y44MfsX60	2	1/13	0/100	Simplex	ND	This study
c.131dupA	p.Y44X	2	1/1	0/150	Yes	ROP	[Hatsukawa et al., 2002]
c.136delG	p.D46IfsX58	2	1/26	0/100	Yes	ND	[Schuback et al., 1995]
c.170C>G	p.S57X		1/17	0/22 ^A	Simplex	ND	[Berger et al., 1992b]
c.205delT	p.C69AfsX35	3	1/26	0/100	Yes	ND	[Schuback et al., 1995]
c.218C>A	p.S73X	3	1/2	0/60	Yes	ND	[Walker et al., 1997]
c.236_240del	p.S80QfsX67	3	1/5	0/75	<i>De novo</i>	ND	[Riveiro-Alvarez et al., 2005]
c.285C>A	p.C95X	3	2/5	0/81 ^A	Yes	ND	[Wu et al., 2007]
c.291delG	p.Q99RfsX5	3	1/17	0/22 ^A	Simplex	ND	[Berger et al., 1992b]
c.325C>T	p.R109X	3	1/2, 2/26	0/200, 0/100	Yes, Yes	ND, ND	[Schuback et al., 1995; Mintz-Hittner et al., 1996]

c.330C>A	p.C110X	3	2/17	0/22 ^A	Simplex	ND	[Berger et al., 1992b]
c.332C>A	p.S111X	3	1/13	0/100	Simplex	ND	This study
c.360C>A	p.Y120X	3	1/5	0/75	Yes	ND	[Riveiro-Alvarez et al., 2005]
c.378T>A	p.C126X	3	1/5, 1/2	0/60, N/A	Simplex, Yes	ND, Ocular phenotypes	[Fuchs et al., 1996; Kellner et al., 1996]
c.383_384delinsAA	p.C128X	3	1/1	0/4	Yes	ND	[Wong et al., 1993]
c.384C>A	p.C128X	3	1/26	0/100	Yes	ND	[Schuback et al., 1995]
Missense changes							
c.38T>G	p.L13R	2	1/1	0/78	Yes	ND	[Fuchs et al., 1996]
c.47T>C	p.L16P	2	1/2	0/50	Yes	ND	[Yamada et al., 2001]
c.53T>A	p.I18K	2	1/65	0/360	Simplex	xIFEVR	[Kondo et al., 2007]
c.112C>T	p.R38C	2	1/21, 2/5	0/75, 0/75	Simplex, Yes	xIFEVR, ND	[Royer et al., 2003; Riveiro-Alvarez et al., 2005]
c.115T>C	p.C39R	2	1/1, 1/52	0/100, 0/81 ^A	Yes, Simplex	ND, ND	[Joos et al., 1994; Wu et al., 2007]
c.122G>A	p.R41K	2	1/5	0/54 ^A	Simplex	xIFEVR	[Shastry et al., 1997a]
c.123G>C	p.R41S	2	1/15	0/81 ^A	Simplex	PFVS	[Wu et al., 2007]
c.125A>G	p.H42R	2	2/5, 1/13, 1/52	0/54 ^A , 0/130, 0/81 ^A	Yes, Simplex, Simplex	xIFEVR, ND, xIFEVR	[Shastry et al., 1997a; Dickinson et al., 2006; Wu et al., 2007]
c.129C>G	p.H43Q	2	1/21	0/75	Yes	ND	[Royer et al., 2003]
c.131A>G	p.Y44C	2	1/6, 1/21	0/20, 0/75	Simplex, Simplex	ND, ND	[Meindl et al., 1992; Royer et al., 2003]
c.133G>A	p.V45M	2	1/21	0/75	Simplex	ND	[Royer et al., 2003]
c.134T>A	p.V45E	2	1/1	N/A	Simplex	ND	[Lev et al., 2007]
c.162G>C	p.K54N	2	2/65, 1/20	0/360, 0/60 ^A	Yes, Simplex	xIFEVR, ND	[Kondo et al., 2007; Boonstra et al., 2009]
c.163T>C	p.C55R	2	1/13	0/100	Simplex	ND	This study
c.174G>T	p.K58N	2	1/2,	0/45 ^A ,	Yes,	ND, xIFEVR,	[Fuentes et al., 1993;

1				1/5	0/54 ^A	Simplex		Shastri et al., 1997a]
2								
3								
4	c.179T>A	p.V60E	3	1/6	0/20	Simplex	ND	[Meindl et al., 1992]
5	c.181C>A	p.L61I	3	1/52	0/81 ^A	Simplex	xIFEVR	[Wu et al., 2007]
6	c.181C>T	p.L61F	3	1/17,	0/22 ^A ,	Simplex,	ND,	[Berger et al., 1992b;
7				1/1	0/115	Yes	ND	Rehm et al., 1997]
8	c.182T>C	p.L61P	3	1/26	0/100	Yes	ND	[Schuback et al.,
9								1995]
10	c.188C>A	p.A63D	3	1/26,	0/100,	Yes,	ND,	[Schuback et al., 1995;
11				3	1/1	Yes	ND	Zaremba et al., 1998]
12	c.194C>G	p.C65W	3	1/26	0/100	Yes	ND	[Schuback et al.,
13								1995]
14	c.194G>A	p.C65Y	3	1/1,	0/45,	Yes,	ND,	[Strasberg et al., 1995;
15				1/5	0/81 ^A	Simplex	ND	Wu et al., 2007]
16	c.199G>A	p.G67R	3	1/13	0/100	Simplex	ND	This study
17	c.200G>A	p.G67E	3	1/13	0/100	Simplex	ND	This study
18	c.206G>C	p.C69S	3	1/1	0/60	Yes	ND	[Chen et al., 1993b]
19	c.220C>T	p.R74C	3	1/17,	0/22 ^A ,	Simplex,	ND	[Berger et al., 1992b;
20				1/2,	0/60,	<i>De novo</i> ,	ND	Fuchs et al., 1996;
21				1/5	0/144 ^A	Yes	ND	Allen et al., 2006]
22	c.223T>C	p.S75P	3	1/2	0/50	Yes	ND	[Yamada et al., 2001]
23	c.224C>G	p.S75C	3	1/17	0/22 ^A	Simplex	ND	[Berger et al., 1992b]
24	c.267C>A	p.F89L	3	1/13	0/100	Simplex	ND	This study
25	c.268C>T	p.R90C	3	2/21	0/75	Yes, <i>De novo</i>	ND	[Royer et al., 2003]
26	c.269G>C	p.R90P	3	1/17	0/22 ^A	Simplex	ND	[Berger et al., 1992b]
27	c.274T>C	p.S92P	3	1/13	0/100	Simplex	ND	This study
28	c.283T>C	p.C95R	3	1/1	0/60	Yes	ND	[Isashiki et al., 1995]
29	c.284G>T	p.C95F	3	1/1	N/A	Yes	ND	[Khan et al., 2004]
30	c.287G>A	p.C96Y	3	1/17,	0/22 ^A ,	Simplex,	ND,	[Berger et al., 1992b;
31				1/6,	0/20,	Simplex,	ND, xIFEVR	Meindl et al., 1992;
32				1/2	0/174	<i>De novo</i>		Shastri et al., 1999]
33	c.288C>G	p.C96W	3	1/1	0/60	Yes	Coats' disease	[Black et al., 1999]
34								
35	c.289C>G	p.R97P	3	1/2	0/98	Yes	ND	[Rivera-Vega et al.,
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4	c.290G>C	p.R97P	3	1/65	0/360	Simplex	ND		[Kondo et al., 2007]
5	c.293C>T	p.P98L	3	1/13	0/100	Simplex	ND		This study
6	c.302C>T	p.S101F	3	1/2	0/60	Yes	ND		[Walker et al., 1997]
7	c.307C>G	p.L103V	3	1/13	0/130	Simplex	xIFEVR		[Dickinson et al.,
8									2006]
9									
10	c.310A>C	p.K104Q	3	1/4	0/75	Yes	ND		[Meindl et al., 1995]
11	c.312G>T	p.K104N	3	1/1	0/100	Yes	ND		[Riveiro-Alvarez et
12									al., 2006]
13									
14	c.313G>A	p.A105T	3	1/2	0/52 ^A	Yes	ND		[Torrente et al., 1997]
15	c.323T>C	p.L108P	3	1/13	0/75 ^A	Simplex	ROP		[Shastry et al., 1997b]
16	c.328T>G	p.C110G	3	1/2	0/45 ^A	Yes	xIFEVR		[Torrente et al., 1997]
17	c.328T>C	p.C110R	3	1/5	0/60	<i>De novo</i>	ND		[Fuchs et al., 1996]
18	c.335G>A	p.G112E	3	1/2	0/144 ^A	Yes	ND		[Allen et al., 2006]
19	c.344G>T	p.R115L	3	1/65	0/360	Simplex	xIFEVR		[Kondo et al., 2007]
20	c.353C>A	p.A118D	3	1/2	0/174	Yes	ND		[Shastry et al., 1999]
21	c.359A>G	p.Y120C	3	1/5	0/54 ^A	Simplex	xIFEVR		[Shastry et al., 1997a]
22	c.361C>T	p.R121W	3	1/4,	0/75,	Yes,	ND,		[Meindl et al., 1995;
23				1/2,	N/A,	Yes,	ND,		Kellner et al., 1996;
24				2/13,	0/75 ^A ,	Yes,	ROP,		Shastry et al., 1997b;
25				1/52	0/81 ^A	Simplex	xIFEVR		Wu et al., 2007]
26	c.362G>T	p.R121L	3	1/2	0/100	Yes	xIFEVR		[Mintz-Hittner et al.,
27									1996]
28	c.362G>A	p.R121Q	3	1/2,	0/75 ^A	Yes,	ND,		[Fuentes et al., 1993;
29				1/4,	0/75,	Yes,	xIFEVR, ND,		Meindl et al., 1995;
30				1/15,	0/75,	Yes,	ND		Riveiro-Alvarez et al.,
31				1/20	0/60	Simplex			2005; Boonstra et al.,
32									2009]
33	c.368T>A	p.I123N	3	2/26,	0/100,	Yes,	ND,		[Schuback et al., 1995;
34				1/1	N/A	Yes	ND		Sims et al., 1997]
35	c.370C>T	p.L124F	3	1/1	0/60	Yes	xIFEVR		[Chen et al., 1993a]
36	c.377G>C	p.C126S	3	1/2	N/A	Yes	ND		[Gal et al., 1996]
37	c.382T>C	p.C128R	3	1/21	0/75	Simplex	ND		[Royer et al., 2003]
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Splice-site changes							
c.-208+1G>A	Splice mutation		1/5	0/60	Simplex	ND	[Fuchs et al., 1996]
c.-208+2T>G	Splice mutation		1/13	0/100	Simplex	ND	This study
c.-208+5G>A	Splice mutation		2/13	0/100	Simplex,	ND	This study
c.62+1G>C	Splice mutation		1/5	0/60	Simplex	ND	[Fuchs et al., 1996]
c.62+1G>A	Splice mutation		1/65	0/360	Simplex	ND	[Kondo et al., 2007]
c.62-1G>C	Splice mutation		1/21	0/75	Yes	ND	[Royer et al., 2003]
Miscellaneous changes							
c.1A>G	p.M1?	2	2/2, 1/3, 1/21	0/60 ^A N/A, 0/75	Yes, Yes, Simplex	ND, ND, ND	[Isashiki et al., 1995; Gal et al., 1996; Royer et al., 2003]
c.2T>G	p.M1?	2	1/26	0/100	Yes	ND	[Schuback et al., 1995]
c.2_3del	p.M1?	2	N/A	N/A	N/A	ND	[Caballero et al., 1996]
[c.282C>A; ins285CTC; 286T>G]	p.H94delinsQC	3	1/26	0/100	Yes	ND	[Schuback et al., 1995]
c.360_368del	p.R121_I123del	3	1/26	0/100	Yes	ND	[Schuback et al., 1995]
c.397delT	p.S133PfsX129	3	1/17	0/22 ^A	Simplex	ND	[Berger et al., 1992b]
c.399delC	p.X134EextX127	3	1/1	N/A	Simplex	ND	[Riveiro-Alvarez et al., 2008]

Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (GenBank NM_000266.3), according to journal guidelines (www.hgvs.org/mutnomen). The initiation is codon 1 (GenBank NP_000257.1)

^ANumber of alleles is estimated on the basis of equal distribution of control individuals between males and females as this is not specified in the original paper.

ND, Norrie disease; xLFEVR, X-linked familial exudative vitreoretinopathy; ROP, retinopathy of prematurity; N/A, information not available or screening was not performed.

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Supp. Table S5. Summary of clinical data of several FEVR or Norrie disease patients harboring novel mutations in *FZD4*, *LRP5* or *NDP*

Family	Patient	Gene	Mutation	Gender	Age at investigation	Visual acuity		Miscellaneous ocular features	Non-ocular features
						LE	RE		
W05-214	II:1	<i>FZD4</i>	p.E286X	M	6 years	?	6/6	Equatorial zone with avascular peripheral fundus. LE: RD surgery (8 years), no vision.	Not present
	I:1	<i>FZD4</i>	p.E286X	F	53 years	6/6	6/6	RE: Few abnormal temporal retinal branches.	Not present
W06-241	II:1	<i>FZD4</i>	p.D428SfsX2	F	5 years	N/A	N/A	Macular ectopia. Haemorrhagic and exudative areas present in the retina.	Premature delivery
W05-213	IV:2	<i>FZD4</i>	p.C204Y	M	3 years	6/60	No LP	RE: Vitreous haemorrhage and formation of fibrovascular membrane.	Juvenile osteochondrosis
					15 years	20/200	No LP	Total RD LE: Deformation of posterior retina, ectopia of the macula, stretched retinal vessels, preretinal membranes	
					17 years	2/60	No LP	LE: Scleral buckling procedure with cryocoagulation.	
					19 years	1/300	No LP	LE: Lensectomy and vitrectomy. LE: Vitreous haemorrhage, vitreoretinal traction and secondary uveitis.	
W06-237	II:2	<i>FZD4</i>	p.G525R	M	At birth	N/A	N/A	Congenital falciform retinal fold from ciliary body to posterior papilla.	Not present
W05-204	II:1	<i>LRP5</i>	p.W993X	M	26 years	LP	20/100	Macular ectopia and peripheral RD.	
					6 months	N/A	N/A	LE/RE: Leucocoria. Persistent hyperplastic primary vitreous.	Severe developmental delay and learning difficulties; plagiocephaly; autistic features and

									seizures (8 years).
W06-920	II:2	<i>LRP5</i>	p.E441K	F	6 years	0	6/12	RE: Falciform retinal fold.LE:RD	Not present
					18 years	0	CF	RE: Cataract, lens aspiration, secondary glaucoma.	
					26 years	0	CF	LE: pupillary block, glaucoma, iridectomy	
					53 years	0	0	Enucleation of both eyes because of recurrent pain	
W05-205	II:2	<i>LRP5</i>	p.G610R/ p.C1253F	M	1 year	N/A	N/A	LE: RD. LE/RE: Ectopic macula and fibrovascular ridge in the temporal periphery.	Reduced BMI (0.62gr/cm ²),
03-2645		<i>NDP</i>	p.S92P	M	8 years	0	N/A	LE: Total RD.	
					3 weeks	N/A	N/A	Conjunctival inflammation. Irregular pupils due to presence of synechiae in the anterior chamber.	Moderate bilateral hearing loss (2 years); skeletal abnormality affecting cervical spine and forearms (24 years).
					7 months	N/A	N/A	Inflammatory bilateral pseudoglioma.	

For FZD4 the translation initiation codon is codon 1 (GenBank NP_036325.2). For LRP5 the translation initiation codon is codon 1 (GenBank NP_002326.2). For NDP, the translation initiation codon is codon 1 (GenBank NP_000257.1). BMI: Bone mass index, LE: Left eye, LP: light perception, N/A: Not available, RD: Retinal detachment, RE: Right eye.

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