Original Article

Next-generation sequencing reveals a novel NDP gene mutation in a Chinese family with Norrie disease

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Purpose: Norrie disease (ND) is a rare X-linked genetic disorder, the main symptoms of which are congenital blindness and white pupils. It has been reported that ND is caused by mutations in the *NDP* gene. Although many mutations in *NDP* have been reported, the genetic cause for many patients remains unknown. In this study, the aim is to investigate the genetic defect in a five-generation family with typical symptoms of ND. **Methods:** To identify the causative gene, next-generation sequencing based target capture sequencing was performed. Segregation analysis of the candidate variant was performed in additional family members using Sanger sequencing. **Results:** We identified a novel missense variant (c.314C>A) located within the *NDP* gene. The mutation cosegregated within all affected individuals in the family and was not found in unaffected members. By happenstance, in this family, we also detected a known pathogenic variant of retinitis pigmentosa in a healthy individual. **Conclusion:** c.314C>A mutation of *NDP* gene is a novel mutation and broadens the genetic spectrum of ND.

Key words: BGISEQ-500, mutation, *NDP* gene, Norrie disease

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Norrie disease (ND; OMIM 310600) is a rare X-linked genetic disorder, the main symptoms of which are congenital blindness and white pupils.[1,2] It can cause abnormal development of the retina, while some infants will have a yellow-white massive microstructure with well vasculature in the posterior vitreous at birth or soon after birth. In addition, as the disorder progresses, shrinking of the eyeballs may occur, then the lens appears cloudy and eventually becomes covered by cataracts. About one-third of individuals with ND may develop progressive hearing loss due to vascular and cochlear abnormalities, and more than half experience developmental delays with cognitive impairment and/or behavioral disorders.[3] The exact, annual incidence and prevalence of ND are unknown, but more than 400 cases have been detailed in the literature. It is not associated with any specific racial or ethnic group, but affected patients are almost always male, while females are typically carriers.

ND is caused by mutations in the *NDP* (Xp11.3) gene, which encodes a secreted protein with a cystine-knot motif, which activates the Wnt/beta-catenin pathway. A diagnosis of ND is based on a combination of characteristic, clinical ocular findings, and molecular genetic testing of the *NDP* gene. No biochemical or functional assays are available as disease markers. Thus, identifying new mutations for clinical, genetic diagnosis of ND is crucial.

The purpose of this study was to identify the underlying genetic defect in a Chinese family with ND, and to provide clinical guidance for birth defect prevention. Targeted sequencing enables us to identify a novel *NDP* gene mutation (p.Ala105Glu) in two affected patients. Surprisingly, we observed a false-positive mutation in *SLC7A14* gene related to retinitis pigmentosa (RP) when analyzing data generated from an unaffected family member.

Methods

Patients

A five-generation Chinese family, consisting of five affected males, was recruited. Complete clinical diagnosis was carried out on each of them. Diagnosis of ND, with typical symptoms, was established based on ophthalmic examination. Family pedigrees are shown in Fig. 1, wherein is depicted the two affected and six unaffected family members participating in the study. All the participants had given written and informed consent and were also involved in publication. All procedures were approved by the ethics committee of the hospital and adhered to the tenets of the Declaration of Helsinki.

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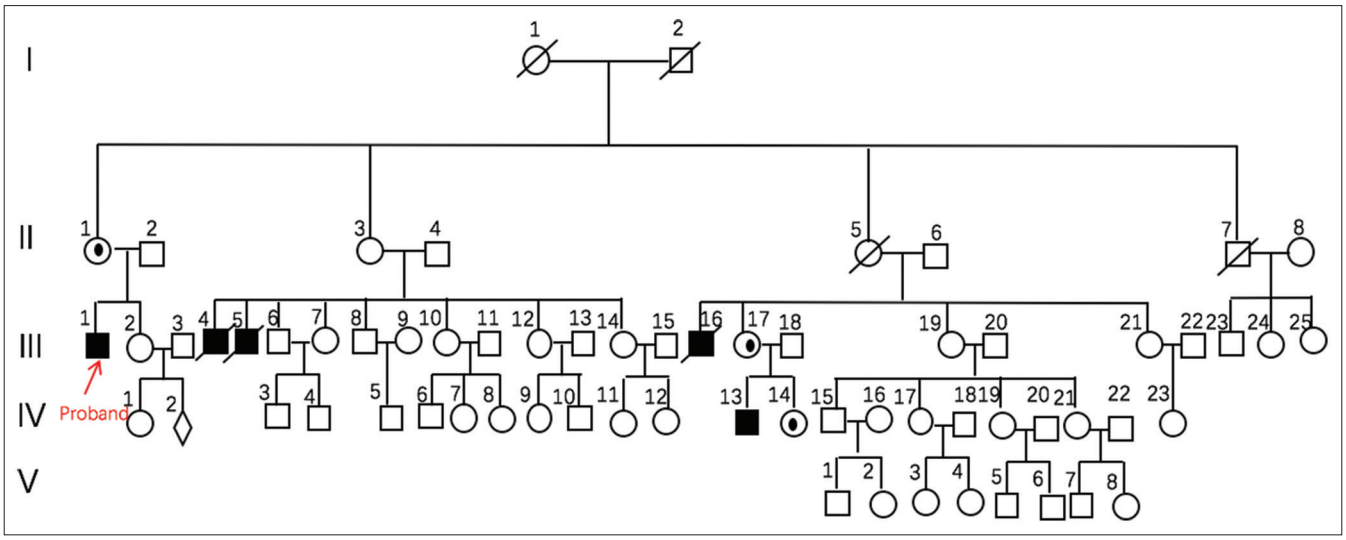

Figure 1: Pedigree of the Norrie disease family, as reported in this study

Capture panel

A gene capture panel was designed to encompass the exons and UTR region of 366 eye disease-related genes, with a target region size of 1.7 M. The capture probes were custom designed and produced by BGI. Before the study, we tested the sensitivity of our method using four samples, by sequence capture performed on two platforms (Illumina's HiSeq sequencer, and BGI's BGISEQ-500 sequencer, respectively). Briefly, all samples had an average depth of more than ×200, and the coverage of target region was around 96% using BGISEQ-500.

Next-generation sequencing on BGISEQ-500

Genomic DNA of two affected (III-1, IV-13) and one unaffected (II-1) family member was extracted from peripheral blood, using a standard protocol. Paired-end DNA libraries were quantified and sequenced on BGISEQ-500. Using DNA nanoballs and combinational probe-anchor synthesis developed from Complete Genomics sequencing technology, it generates short reads on a large scale which can help fulfill the growing demands for sequencing.[4]

Bioinformatics analysis

WES data were mapped to the human reference genome (UCSC hg19) using Burrows-Wheeler aligner (BWA-MEM, version 0.7.10). [5] Variants calling were performed using Genome Analysis Tool Kit (GATK, version 3.3).[6] All variants were annotated by Annovar[7] and SnpEff.[8] Then, the variants identified through the above pipeline were further filtered to eliminate benign variants with minor allele frequency (MAF) >0.1% in 1000 Genomes,[9] dbSNP,[10] EXAC,[11] ESP6500[12] database, and internal data. Finally, variant prioritization and selection were performed combining total depth, quality score, MAF, potential deleterious effect, and the existence of mutation reports in common databases such as the Human Gene Mutation Database,[13] the Retinal Information Network,[14] ClinVar,[15] or Online Mendelian Inheritance in Man[16] to evaluate variant calling confidence.

Sanger validation

Primer3 was used to design all polymerase chain reaction primers for validation. Sanger sequencing was used to validate the identified mutations. Segregation studies were undertaken using eight family members (affected individuals III-1 and IV-13; unaffected individuals II-1, III-2, III-18, IV-1, IV-2, and IV-14).

Results

Clinical characteristics

Two males from this family–the proband and his uncle–were affected by the disease. Patient III-1 is a 44-year-old man with congenital blindness, who has been taking medication since the age of 16 after presenting with epilepsy. The examination revealed that he suffers from microphthalmia and atrophy. Besides this, previous clinical reports had shown that he had a shallow anterior chamber and iris atrophy.

Patient IV-13 was born with the ability to sense light; however, as he grew older, the binocular pupils of his eyes began to turn white, and yellow-white lump tissues were found in the posterior vitreous tissue. When he was 1-year-old, both of his eyes had become entirely blind.

Mutation identification

To identify the causative variants in the ND family cohort, we performed targeted capture sequencing of 366 retina disease genes, using our capture panel described in the methods. High-quality results were obtained [Table 1], with mean coverage within the target region in excess of 94%.

Following bioinformatics analysis, a hemizygous missense mutation c.314C>A in Exon3/CDS2 of *NDP* completely segregated in both of the two affected family members (III-1, IV-13) as well as in one unaffected family member (II-1). This mutation causes an amino acid change from alanine to glutamic acid at position 105 (p.Ala105Glu). Both SIFT and PolyPhen predicted p.Ala105Glu to be damaging, with high hazard scores. Following this result, we applied a further mutation validation strategy, by Sanger sequencing, with additional family members [Fig. 2]. The results confirmed that the disease in this family was due to this mutation (III-1 and IV-13 were hemizygous patients; III-2, IV-1, and IV-2 were unaffected; II-1, III-17, and IV-14

Table 1: Quality of datasets

Sample	Q20	Q30	Average depth	Depth ≥30 (%)	Coverage (%)	Capture efficiency (%)
II-1	95.168	86.161	144.15	92.69	94.15	53.27
III-1	94.475	84.762	220.74	91.91	94.72	42.85
IV-7	95.315	86.583	182.07	93.29	94.48	39.35

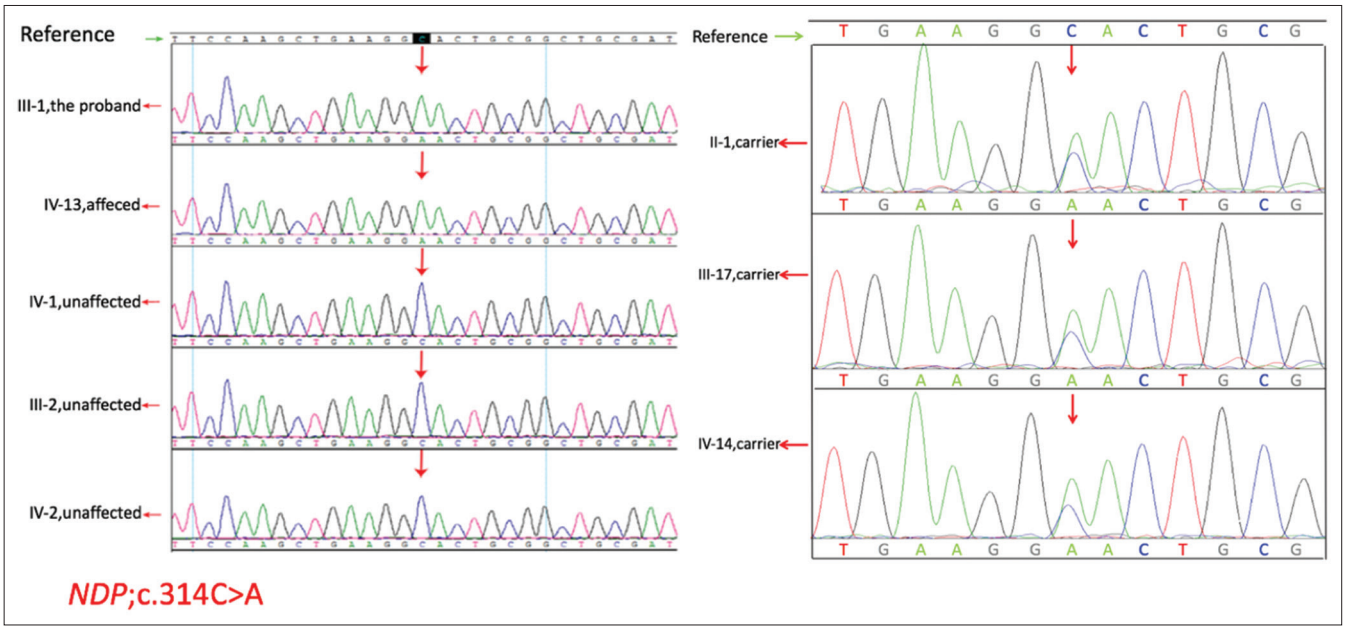

Figure 2: Detected mutations in the *NDP* genes in patients with Norrie disease. Partial sequences of *NDP* from the Norrie disease patients (III-1, IV-13: Hemizygous for the c.314C>A genotype), controls (IV-1, III-2, IV-2), and carriers (II-1, III-17, IV-14: Heterozygous for the genotype)

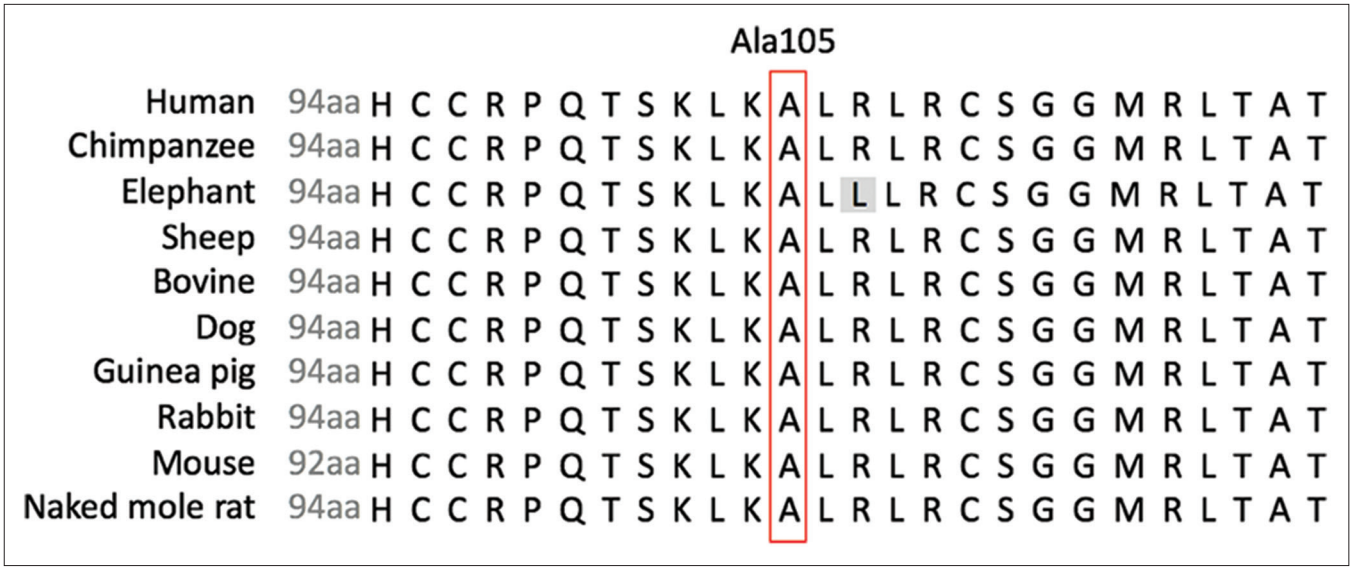

Figure 3: Protein sequence alignment of human NDP with its orthologs. Protein alignment showing conservation of residues *NDP* Ala105 across ten species. The mutation occurred at evolutionarily conserved amino acids (in the red box)

were heterozygous carriers). Multiple orthologous sequence alignment revealed that 105 codon alanine of *NDP* was highly conserved amino acids across different species [Fig. 3]. It suggests that any mutation at those codons may have a deleterious effect.

Interestingly, in an unaffected individual (II-1) without any retina disease symptoms present at the clinical examination, we found an *SLC7A14* mutation[17] c.1391G>T (p.Cys464Phe) by sequencing, which has been reported to be a RP pathogenic variant in four cases. In

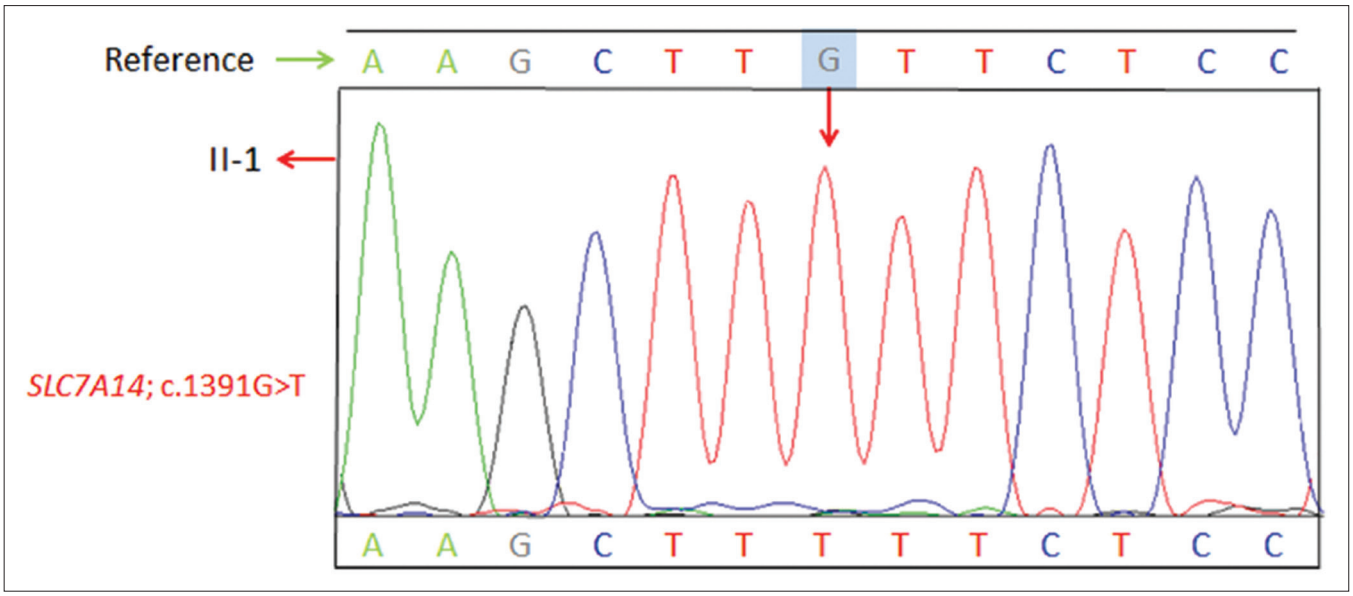

Figure 4: Detected a mutation in the SLC7A14 genes in II-1

addition, Sanger sequencing was used to further identify the variation [Fig. 4].

Discussion

BGISEQ-500 achieves the international standard of excellence for sequencing, and in particular, meets the demands of clinical application. It integrates automatic sample preparation, sequencing process, and data analysis, performing several applications with a one-touch operation. It has proven that it can produce data of the highest standard, with performance concordant to other, highest standard platforms, such as Sanger sequencing.

Besides this evaluation of the BGISEQ-500, we have developed a targeted sequencing-based diagnostic gene panel for ophthalmic diseases, which can offer a precise and cost-effective detection of disease-causing gene variants for clinical use, encompassing both known and novel mutations.

ND is a rare X-linked genetic disorder that leads to blindness in males. According to the statistics, of more than 60% of ND patients with point mutations (>60%), about 20% of patients have deletions of the *NDP* gene.[18] *NDP* encodes Norrin protein which contains the cystine-knot domain.[19] Most mutations of *NDP* gene associated with ND are related to this domain, such as P98 L (c.293C>T) and S111X (c.332C>A).[20] In this study, we report upon a family with a history of typical ND. A novel mutation (c.314C>A, p.Ala105Glu) in the *NDP* was found in two hemizygous, affected male individuals (III-1, IV-13) and in one heterozygous, unaffected individual (II-1). This mutation in exon 3 at codon 105 involves in the cystine knot. Previously, the same missense mutation was reported in one patient with X-linked familial exudative vitreoretinopathy (FEVR).[21] This result suggests that X-linked FEVR and ND may be caused by the same mutation in the *NDP* gene.

Indeed, FEVR is characterized by abnormal vascularization of the peripheral retina. In contrast, for this family cohort, the affected individuals were congenitally blind, which is the typical symptom of ND. Thus, genetic testing is necessary to ensure correct diagnosis, and our work may provide new data and clues for additional research helping with the diagnosis of this disease.

According to pedigree analysis and determination of the mutation, ND can be localized to be an X-linked, recessive genetic disease. According to the fetal karyotype analysis, the proband's sister (III-2) was pregnant with a boy, who might also suffer from congenital blindness. To determine whether the male fetus (IV-2) inherited the pathogenic mutation, we used amniotic fluid to detect the mutation (c.314C>A) in *NDP*. The test result showed that IV-2 did not carry this mutation. So far, no symptoms of ND have been detected in this boy after his birth, providing some corroboration with our findings. From this case, it may be inferred that our work should be of great significance in the clinical prevention of birth defects for affected families such as this one.

Notably, when we analyzed the proband mother's data (II-1), we found her not only to be carrying a heterozygous mutation of the *NDP* gene (c.314C>A), but also to be carrying a known, pathogenic homozygous mutation of the *SLC7A14* gene (c.1391G>T), which was previously reported to be present in four patients with RP. However, clinical examination of the individual showed that she did not have any detectable symptoms of RP. Therefore, we suggest that the clinical significance, or at least the penetrance of this genotype in causing the RP phenotype, requires further research.

Conclusion

We identified a novel mutation c.314C>A in *NDP* gene caused Norrie disease in a Chinese family. Our finding broadens the genetic spectrum of ND and indicates the feasibility of panel-based next generation sequencing for inherited disease.

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Conflicts of interest

There are no conflicts of interest.

References

1. Norrie G. Causes of blindness in children. Acta Ophthal 1927;5:357-86.
2. Krill AE. Norrie's disease. A congenital progressive oculo-acoustico-cerebral degeneration. Am J Ophthal 1967;64 Suppl 89:1.
3. Sims KB. NDP-Related Retinopathies - GeneReviews® - NCBI Bookshelf[J]. Gene, 2009.
4. Fehlmann T, Reinheimer S, Geng C, Su X, Drmanac S, Alexeev A, *et al.* CPAS-based sequencing on the BGISEQ-500 to explore small non-coding RNAs. Clin Epigenetics 2016;8:123.
5. Li H, Durbin R. Fast and accurate long-read alignment with burrows-wheeler transform. Bioinformatics 2010;26:589-95.
6. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, *et al.* The genome analysis toolkit: A mapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 2010;20:1297-303.
7. Wang K, Li M, Hakonarson H. ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 2010;38:e164.
8. Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, *et al.* A program for annotating and predicting the effects of single nucleotide polymorphisms, snpEff: SNPs in the genome of drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) 2012;6:80-92.
9. Siva N. 1000 genomes project. Nat Biotechnol 2008;26:256.
10. Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, *et al.* DbSNP: The NCBI database of genetic variation. Nucleic Acids Res 2001;29:308-11.
11. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, *et al.* Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536:285-91.
12. Tennessen JA, Bigham AW, O'Connor TD, Fu W, Kenny EE, Gravel S, *et al.* Evolution and functional impact of rare coding variation from deep sequencing of human exomes. Science 2012;337:64-9.
13. Stenson PD, Mort M, Ball EV, Howells K, Phillips AD, Thomas NS, *et al.* The human gene mutation database: 2008 update. Genome Med 2009;1:13.
14. Daiger S, Sullivan L, Rossiter B. RetNet: Retinal Information Network(https://sph.uth.edu/retnet/). [Last accessed on 2013 Feb 26].
15. Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, *et al.* ClinVar: Public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res 2014;42:D980-5.
16. Amberger JS, Bocchini CA, Schiettecatte F, Scott AF, Hamosh A. OMIM.org: Online Mendelian inheritance in man (OMIM®), an online catalog of human genes and genetic disorders. Nucleic Acids Res 2015;43:D789-98.
17. Jin ZB, Huang XF, Lv JN, Xiang L, Li DQ, Chen J, *et al.* SLC7A14 linked to autosomal recessive retinitis pigmentosa. Nat Commun 2014;5:3517.
18. Rivera-Vega MR, Chiñas-Lopez S, Vaca AL, Arenas-Sordo ML, Kofman-Alfaro S, Messina-Baas O, *et al.* Molecular analysis of the NDP gene in two families with Norrie disease. Acta Ophthalmol Scand 2005;83:210-4.
19. Meitinger T, Meindl A, Bork P, Rost B, Sander C, Haasemann M, *et al.* Molecular modelling of the Norrie disease protein predicts a cystine knot growth factor tertiary structure. Nat Genet 1993;5:376-80.
20. Nikopoulos K, Venselaar H, Collin RW, Riveiro-Alvarez R, Boonstra FN, Hooymans JM, *et al.* Overview of the mutation spectrum in familial exudative vitreoretinopathy and Norrie disease with identification of 21 novel variants in FZD4, LRP5, and NDP. Hum Mutat 2010;31:656-66.
21. Salvo J, Lyubasyuk V, Xu M, Wang H, Wang F, Nguyen D, *et al.* Next-generation sequencing and novel variant determination in a cohort of 92 familial exudative vitreoretinopathy patients. Invest Ophthalmol Vis Sci 2015;56:1937-46.