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Identification of 2 Potentially Relevant Gene Mutations Involved in Strabismus Using Whole-Exome Sequencing

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Background: The etiology of strabismus has a genetic component. Our study aimed to localize the candidate causative gene mutant in a Chinese family with strabismus and to describe its underlying etiology.





Material/Methods: Genomic DNA was extracted from the affected individual and his parents in a Chinese pedigree with strabismus. The resulting exomes were sequenced by whole-exome sequencing. After variant calling and filtering, the candidate causative gene mutations were selected for the rarity and predicted damaging effect, which complied with the model of recessive disease transmission.

Results: We examined a Chinese strabismus pedigree with the parents unaffected and 2 offspring affected. Whole-exome sequencing and bioinformatics filtering identified 2 variants including Abelson helper integration site 1 (AHI1) gene and nebulin (NEB) gene. The variant in the AHI1 gene, c.A3257G (p.E1086G), and the altered amino acid had a damaging effect on the encoded protein predicted by Polyphen2. Moreover, this change was located in the conserved SH3 domain of AHI1. Biallelic pathogenic variant in AHI1 gene can cause Joubert syndrome-related disorders with oculomotor apraxia characteristics. Additionally, c.A914G mutation was found in nebulin (NEB) gene. Therefore, we concluded that AHI1 c.3257A>G and NEB c.914 A>G were potential causal variants in this strabismus pedigree.

Conclusions: We detected an AHI1 homozygous mutation in the affected individual. Whole-exome sequencing is a powerful way to identify causally relevant genes, improving the understanding of this disorder.

MeSH Keywords: **DNA Mutational Analysis • Sequence Analysis, DNA • Strabismus**

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Background

Strabismus, also referred to as squint, presents as a misalignment of the eyes. It affects 1% to 4% of the population and affects males and females equally [1]. Strabismus is also a feature of several syndromes, such as congenital fibrosis of extraocular muscles, Duane retraction syndrome, and chronic progressive external ophthalmoplegia. Exotropia is a common subtype of strabismus that is most prevalent in children [2]. Population-based studies show that the familial clustering of strabismus has been observed, and the incidence of specific types of strabismus differs, in different racial groups, which suggests that the etiology of strabismus has genetic factors [3–8]. The prevalence of intermittent exotropia was 3.24% in studies in China [2,9,10].

Michaelides et al. studied the genetics of strabismus and found that it was related to mitochondrial cytopathies and cranial nerve misrouting, which indicated that strabismus has a significant genetic component [11]. However, significant genetic heterogeneity makes it difficult to determine the mode of inheritance and the relevant causative genes. In a large family with nonsyndromic strabismus, Parikh et al. found a presumptive strabismus susceptibility locus to chromosome 7p22.1 with a multipoint LOD score of 4.51 under a model of recessive inheritance. In 6 other multiplex families, linkage to 7p was not observed [1].

Several studies have identified the causative gene mutations associated with strabismus-related disorders, such as congenital stationary night blindness [12], intellectual disability [13], and Kaufman oculocerebrofacial syndrome [14]. However, the identity of the strabismus susceptibility genes has been enigmatic and the genetic mechanisms involved in the pathogenesis of strabismus remain poorly understood [15]. Mutation screening of candidate genes may be necessary to localize genes that predispose humans to strabismus.

Exome sequencing is a powerful and cost-effective tool for dissecting the genetic basis of diseases [16–18]. In the present study, we investigated a Chinese strabismus pedigree with the parents unaffected and 2 offspring affected. By whole-exome sequencing, we identified 2 mutations c.A3257G (p.E1086G) in *AHI1* and c.A914G in *NEB* as the most likely causative mutations of the disorder. Of interest, biallelic pathogenic variant in *AHI1* gene can cause Joubert syndrome-related disorders with oculomotor apraxia characteristics.

Material and Methods

Study subjects

We examined a typical 2-generation strabismus pedigree residing in the Shandong province of China. The proband was a

15-year-old boy who was diagnosed with exotropia. His sister also had exotropia, and both shared common characteristics. The boy was affected with comitant exotropia (II1) (Figure 1A). The amount of tropia was -40Δ (near) and -40Δ (far). Eye examinations focused on the measurement of visual acuity (unaided and best-corrected) and refractive status. Refractive error status was emmetropia. His unaided visual acuity was 1.0 in the right eye and 1.0 in the left eye. The proband's sister was also diagnosed with comitant exotropia (II2) (Figure 1A). The amount of tropia was -35Δ (near) and -35Δ (far). The refractive status was myopia. Her best-corrected visual acuity was VOD1.0 and VOS1.0. Moreover, neither of them had any other symptoms such as nystagmus or Joubert-like symptoms or any sign of hypotonia or vision/retinal. Additionally, their parents, grandparents, and maternal grandparents were unaffected. Their refractive state was emmetropia and the eye position was normal. Their unaided visual acuity was VOD1.0 and VOS1.0. None of them suffered from other congenital disorders. Based on these findings, we considered that the etiology of strabismus in this family had a genetic factor. Therefore, the proband and his parents were enrolled for exome sequencing screening (Figure 1A).

For the purpose of biological investigations, consent forms were signed by all enrolled study subjects. The study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University (2015-012) and complied with the Declaration of Helsinki principles.

Exome sequencing and variant calling

Venous blood was drawn from the affected individual (II-1) and 2 unaffected individuals (I1 and I2) within the examined strabismus pedigree, then stored in tubes containing EDTA. All DNA samples were prepared from the venous blood of the available patients by standard procedures. The qualified genomic DNA was used to perform exome target enrichment using the Agilent SureSelect Human All Exon 50Mb Exon kit (Agilent Technologies, Santa Clara, CA, USA). The captured whole exomes were sequenced on the platform of Illumina HiSeq 2500 Sequencer (Illumina, San Diego, CA, USA). For each sample, sequencing reads with paired-end 125-bp long and mean coverage of 100X was generated.

After filtering the adapter, contaminating reads and low-quality reads, the clean paired reads were then mapped to reference human genome sequence (hg19) using the Burrows-Wheeler Alignment tool (BWA) [19], generating the sequence alignment/map (SAM) file. PCR duplicate reads were further marked and removed using the Picard software program (version 1.07). Genome Analysis Toolkit (GATK) [20] and MuTect software [21] were used to detect the single-nucleotide variants (SNVs), insertions, and deletions within genome-wide regions.

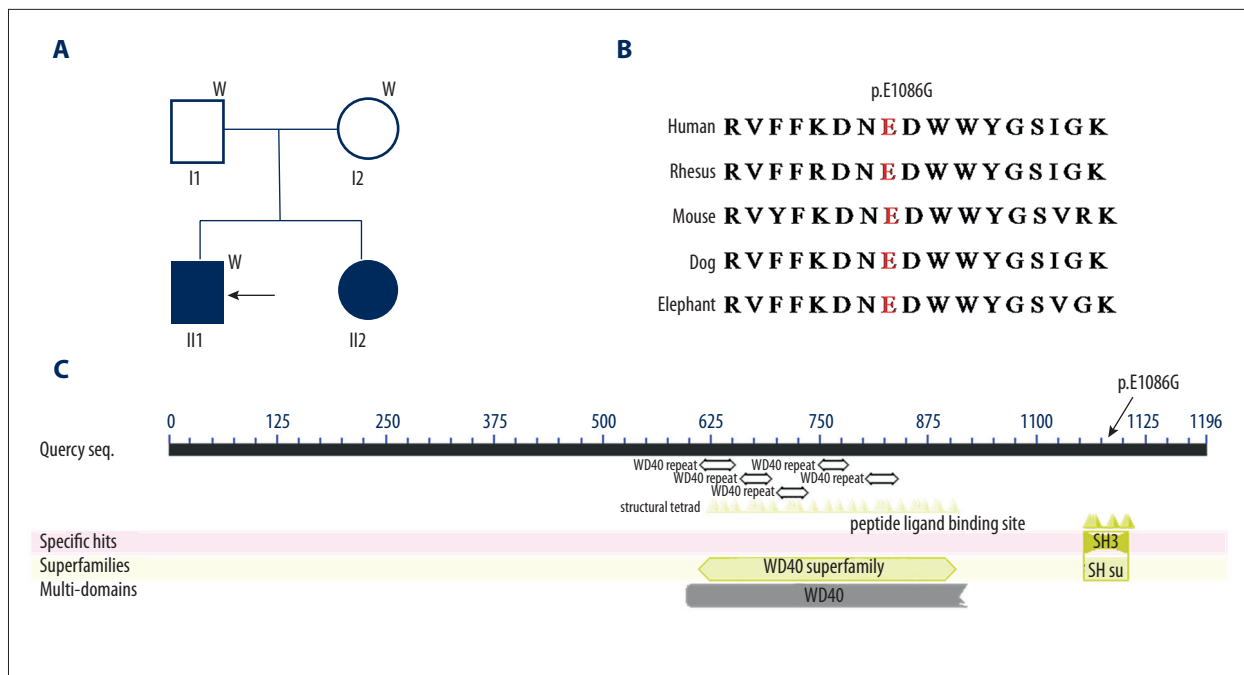


Figure 1. (A) Pedigree for the Chinese family with strabismus. Individuals I: 1, I: 2 and II: 1 underwent exome sequencing. The arrow indicates the proband. W: whole-exome sequencing. (B) The affected amino acid residue is highly conserved across species. (C) Conserved domains in AHI1. The mutation c.A3257G (p.E1086G) was located in the SH3 domain of AHI1.

Variants filtering

To obtain the important candidate genes, the resulting variants were annotated and filtered systematically. The program ANNOVAR (<http://www.openbioinformatics.org/annovar/>) was used to annotate the variants of the information from various genetic variation databases [22]. Based on the reported variant frequencies, the common variants were first excluded with the minor allele frequency (MAF) greater than 0.01 represented in the 1000 Genomes Project. According to the variant location within genes, the variants in the coding region were given higher priority, and the variants that altered the coding sequence (nonsynonymous) were selected. The deleteriousness of selected variants was subsequently predicted by various bioinformatics programs (SIFT, Polyphen2, LRT, MutationTaster, MutationAssessor, FATHMM, and RadialSVM, LR), and the variants were retained whose changes to the protein were damaging. According to the autosomal recessive inheritance pattern, only the variants which were homozygous in the affected individual and heterozygous in the unaffected members were screened. Moreover, we required at least 5X sequence coverage at any given position for genotype calling.

Sanger sequencing for candidate variants

After the systematic filtering, the remaining variants were confirmed by Sanger sequencing. Genomic DNA was prepared from peripheral blood samples of the original 3 individuals

who underwent exome sequencing and 5 additional sporadically affected individuals. Primer-Premier 5.0 (PREMIER Biosoft International, Palo Alto, CA, USA) was used to design oligonucleotide primer sets for the variants from whole-exome sequencing. PCR reactions and Sanger sequencing were performed by Majorbio Company (Shanghai, China).

In silico analysis

Conservation of affected residue was analyzed using the UCSC Genome Browser (<http://genome-asia.ucsc.edu/index.html>) and visualized using MEGA7.0. The protein conserved domains were further identified using the Conserved Domain Search Service (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>).

Results

Genetic analysis

Genomic DNA from I: 1, I: 2 and II: 2 were analyzed by whole-exome sequencing (WES). For each sample, an average of 9.67 Gb raw data were generated with paired-end 125-bp length. After raw-data filtering, we obtained 9.51 Gb clean data. Above 99% clean reads were then aligned to the human reference sequence (hg19) (Table 1). A total of 567 705 single-nucleotide variations (SNVs) were identified by variant detection with the Genome Analysis Toolkit (GATK) and MuTect software.

Table 1. Summary of exome sequencing data.

Sample	Raw data (Gb)	Clean data (Gb)	Map bases rate (%)	Target region map bases (Gb)	Target region map bases rate (%)	Coverage (%)	Mean depth
Father	9.44	9.29	99.16	4.98	53.61	81.31	88.32
Mother	10.57	10.40	99.09	5.81	55.87	81.43	102.75
Proband	9.00	8.85	99.15	4.80	54.24	81.55	84.78
Average	9.67	9.51	99.13	5.20	54.57	81.43	91.95

Among the variants identified by WES, we first removed variants with minor allele frequency >0.01 in the 1000 Genomes Project, resulting in 118 542 variants, and 3191 variants were located in the exonic region. Further filtering of synonymous variants led to 1851 loss-of-function variants, which altered the coding sequence and were more likely associated with the disease. Based on the recessive-inherited mode of the strabismus pedigree, only 2 SNVs fulfilled our filtering criteria: c. 914A-G mutation in NEB (Chr2: 151485334..151734487) and c. 3257A-G mutation in AHI1 (Chr6: 135283972..135497775). The proband of II: 1 was homozygous for these mutations, and his clinically unaffected parents were heterozygous for these variants. Variant frequencies were 0.0228 for mutation in NEB and 0.0357 for mutation in AHI1 in the population of East Asian ancestry from the 1000 Genomes Project.

The c.3257A-G mutation in AHI1 resulted in p.1086E-G change, which was predicted to be damaging by Polyphen2. We searched the literature and found that biallelic pathogenic variants in AHI1 gene were identified in individuals with Joubert syndrome-related disorders, which are characterized by cerebellar ataxia, oculomotor apraxia, hypotonia, neonatal breathing abnormalities, and psychomotor delay [23]. It is reported that the expression of NEB is remarkably decreased in strabismic extraocular muscles compared to normal muscles [24]. Therefore, we considered c.3257A-G mutation in AHI1 and c.914A-G mutation in NEB as the most likely causal variants in this strabismus pedigree.

Sanger sequencing of the candidate causative variants

To further confirm the variants of c.3257A-G mutation in AHI1 in strabismus, Sanger sequencing was performed in 10 individuals with the basic type of strabismus and in 3 healthy individuals. The sister of the proband was also enrolled. The results showed that the mutation was not observed in other individuals with strabismus, indicating the genetic heterogeneity of strabismus. However, the mutation was found to be a homozygous mutation in his sister, which was consistent with the proband.

Mutation of AHI1 gene

The protein in 1086 locus of AHI1 protein sequence is highly conserved across species (human, rhesus monkey, mouse, dog, and elephant). However, we found the rare mutation of c.3257A-G in AHI1 (p.E1086G) (Figure 1B) in this study. Searching for conserved domains within the AHI1 protein indicated that the altered amino acid residue (p.E1086G) was located in the conserved Src Homology 3 (SH3) domain of AHI1 (Figure 1C). SH3 domains are protein interaction domains that bind to proline-rich ligands with moderate affinity and selectivity, and bind preferentially to PxxP motifs. They play versatile and diverse roles in the cell, including the regulation of enzymes, changing the subcellular localization of signaling pathway components, and mediating the formation of a multiprotein complex assembly.

Discussion

Strabismus is characterized as a misalignment of the eyes, which is also a feature of several syndromes such as congenital fibrosis of extraocular muscles [1]. Family studies suggest that there is a strong genetic component to the etiology of strabismus [1,6–8]. Genetic mechanisms of strabismus pathogenesis remain poorly understood [15]. The advent of whole-exome sequencing technologies is a well-justified strategy for discovering candidate causative genes underlying genetic disease phenotypes. In this study, we aimed our efforts in this direction, and whole-exome sequencing was performed on the affected individual and his parents in a Chinese strabismus pedigree, identifying 2 mutations in the genes of AHI1 and NEB, which may be associated with strabismus. Additionally, we detected the AHI1 homozygous mutation in the affected individual in this study.

The inheritance patterns of strabismus are complex and are associated with complex sensory and motor pathways in the retina, thalamus, visual cortex, and brainstem [25–27]. Herein, genetic analysis was conducted on a Chinese strabismus pedigree, and a mutation in AHI1 was identified. AHI1 encodes

Abelson helper integration site 1. The structure of the AHI1 gene contains at least 33 exons and spans 213.7 kb [28]. This gene mutation causes specific forms of Joubert syndrome-related disorders [29]. Genomewide screening of 5 pedigrees with autosomal recessive Joubert syndrome-3 identified 3 independent mutations in the AHI1 gene [30]. Using next-generation sequencing, Najmabadi also identified homozygosity for a nonsense and a missense mutation in the AHI1 gene in affected members of 2 families with Joubert syndrome-3 [31]. Pathogenic variants in AHI1 were identified in 7.3% of 137 persons with Joubert syndrome [32]. Moreover, about 80% of them had retinal dystrophy [33]. AHI1 expression is required for the development of cerebellar and cortical structure, and its missense and frameshift mutations were identified in families with Joubert syndrome plus cortical polymicrogyria [34]. Additionally, Joubert syndrome has been reported to be genetically heterogeneous with mutations in AHI1, suggesting that this genotypic variant of Joubert syndrome may be related to functional abnormalities of the cerebellum and brain stem [35,36]. Our AHI1 variant may play important roles in the pathology of strabismus.

The AHI1 protein includes a SH3 domain, a coiled-coil domain, and 6 WD40 repeats. The AHI1 mutations occurred in a phenotype-specific group of Joubert plus retinal abnormalities, and most of the mutations abolished all of the SH3 domain [32]. Consistent with that, we also found that the altered amino acid residue (p.E1086G) was located in the conserved SH3 domain of AHI1, suggesting its potential roles in strabismus.

Previous studies reported that approximately 15–20% of strabismus was associated with global central nervous system (CNS) defects rather than with ocular disorders [27,37,38]. Additionally, Doherty et al. found that the strabismic patients had anomalous retinal correspondence [39]. It has been suggested that the ipsilateral non-decussating projection from the temporal retina is suppressed in strabismus [40], indicating that the CNS and retina play an important role in the pathology of strabismus. Functional studies on mice showed

that *ahi1*-null mice had defects of retinal outer segment morphogenesis and mislocalization of opsin in photoreceptors, which contributed to the loss of photoreceptors [41]. The protein encoded by AHI1 (also termed *jouberin*) is involved in canonical Wnt signaling. Lancaster found that *ahi1*-mutant mice showed cerebellar hypoplasia and the Wnt reporter activity in the developing cerebellum was decreased [42]. Wnt signaling may participate in the early development of extraocular muscles [43]. These findings suggest that our variant in AHI1 may contribute to strabismus phenotype in this Chinese pedigree.

In this study, we also found another mutation in NEB gene. NEB encodes nebulin, which accounts for 3% to 4% of the total myofibrilla protein and regulates the length of actin filaments and contraction strength [44–46]. Mutations in this gene are associated with recessive nemaline myopathy [47]. It has been reported that NEB has significantly down-regulated expression in strabismic extraocular muscles compared to normal muscles [24], which further demonstrates the relationship between NEB and strabismus.

Conclusions

Our results defined 2 relevant genes, AHI1 and NEB, that may predispose to strabismus, possibly through an additive effect. Our finding revealed the high degree of genetic heterogeneity inherent in the strabismus phenotype, as demonstrated by Parikh et al. [1], who discovered that the presumptive strabismus susceptibility locus to chromosome 7p22.1 was not presented in 6 other multiplex families. We conclude that this Chinese strabismus pedigree best fits a model of autosomal recessive disease transmission. Further research is needed to better understand this disorder.

Conflict of interest

None.

References:

1. Parikh V, Shugart YY, Doheny KF et al. A strabismus susceptibility locus on chromosome 7p. *Proc Natl Acad Sci USA*, 2003; 100: 12283–88
2. Govindan M, Mohny BG, Diehl NN, Burke JP: Incidence and types of childhood exotropia: A population-based study. *Ophthalmology*, 2005; 112: 104–8
3. Holm S: Le strabisme concomitant chez les palénoïdes au Gabon, Afrique équatoriale française. *Acta Ophthalmologica*, 1939; 17: 367–87 [in French]
4. Gover M, Yaukey JB: Physical impairments of members of low-income farm families-11,490 persons in 2,477 Farm Security Administration borrower families, 1940. I. Characteristics of the examined population. II. Defective vision as determined by the Snellen test and other chronic eye conditions. *Public Health Reports*, 1944; 59: 1163–84
5. Sandall GS: Strabismus-Proceedings of the Third Meeting of the International Strabismological Association. *J Neurophysiol*, 1979; 16(8): 1553
6. Cantolino SJ, von Noorden GK: Heredity in microtropia. *Arch Ophthalmol*, 1969; 81: 753–57
7. Paul TO, Hardage LK: The heritability of strabismus. *Ophthalmic Genet*, 1994; 15(1): 1–18
8. Mash AJ, Spivey BE: Genetic aspects of strabismus. *Doc Ophthalmol*, 1973; 34(1): 285–91
9. Yu CB, Fan DS, Wong VW et al: Changing patterns of strabismus: A decade of experience in Hong Kong. *Br J Ophthalmol*, 2002; 86: 854–56
10. Pan CW, Zhu H, Yu JJ et al: Epidemiology of intermittent exotropia in preschool children in China. *Optometry and Vision Science*, 2016; 93: 57–62
11. Michaelides M, Moore AT: The genetics of strabismus. *J Med Genet*, 2004; 41: 641–46

12. Zeitz C, Jacobson SG, Hamel CP et al: Whole-exome sequencing identifies LRIT3 mutations as a cause of autosomal-recessive complete congenital stationary night blindness. *Am J Hum Genet*, 2013; 92: 67–75
13. Alazami AM, Hijazi H, Al-Dosari MS et al: Mutation in ADAT3, encoding adenosine deaminase acting on transfer RNA, causes intellectual disability and strabismus. *J Med Genet*, 2013; 50: 425–30
14. Flex E, Cioffi A, Caputo V et al: Loss of function of the E3 ubiquitin-protein ligase UBE3B causes Kaufman oculocerebrofacial syndrome. *J Med Genet*, 2013; 50: 493–99
15. Traboulsi EI: The genetics of strabismus. *Am Orthop J*, 2001; 51: 67–74
16. Bamshad MJ, Ng SB, Bigham AW et al: Exome sequencing as a tool for Mendelian disease gene discovery. *Nat Rev Genet*, 2011; 12: 745–55
17. Ng SB, Turner EH, Robertson PD et al: Targeted capture and massively parallel sequencing of 12 human exomes. *Nature*, 2009; 461: 272–76
18. Wang JL, Yang X, Xia K et al: TGM6 identified as a novel causative gene of spinocerebellar ataxias using exome sequencing. *Brain*, 2010; 133: 3510–18
19. Li H, Durbin R: Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, 2009; 25: 1754–60
20. McKenna A, Hanna M, Banks E et al: The genome analysis toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*, 2010; 20: 1297–303
21. Cibulskis K, Lawrence MS, Carter SL et al: Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol*, 2013; 31: 213–19
22. Wang K, Li M, Hakonarson H: ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*, 2010; 38: e164
23. Valente EM, Dallapiccola B, Bertini E: Joubert syndrome and related disorders. *Handb Clin Neurol*, 2013; 113: 1879–88
24. Altick AL, Feng CY, Schlauch K et al: Differences in gene expression between strabismic and normal human extraocular muscles. *Invest Ophthalmol Vis Sci*, 2012; 53: 5168–77
25. Hegmann JP, Mash AJ, Spivey BE: Genetic analysis of human visual parameters in populations with varying incidences of strabismus. *Am J Hum Genet*, 1974; 26: 549–62
26. Schlossman A, Priestley BS: Role of heredity in etiology and treatment of strabismus. *Arch Ophthalmol*, 1952; 47: 1–20
27. Maumenee IH, Alston A, Mets M et al: Inheritance of congenital esotropia. *Trans Am Ophthalmol Soc*, 1986; 84: 85–93
28. Jiang X, Hanna Z, Kaouass M et al: Ahi-1, a novel gene encoding a modular protein with WD40-repeat and SH3 domains, is targeted by the Ahi-1 and Mis-2 provirus integrations. *J Virol*, 2002; 76: 9046–59
29. Parisi M, Glass I: Joubert syndrome and related disorders. In: *GeneReviews(R)*. Pagon RA, Adam MP, Ardinger HH et al. (eds.), Seattle (WA), 1993
30. Ferland RJ, Eyaid W, Collura RV et al: Abnormal cerebellar development and axonal decussation due to mutations in AHI1 in Joubert syndrome. *Nat Genet*, 2004; 36: 1008–13
31. Najmabadi H, Hu H, Garshasbi M et al: Deep sequencing reveals 50 novel genes for recessive cognitive disorders. *Nature*, 2011; 478: 57–63
32. Valente EM, Brancati F, Silhavy JL et al: AHI1 gene mutations cause specific forms of Joubert syndrome-related disorders. *Ann Neurol*, 2006; 59: 527–34
33. Valente EM, Brancati F, Dallapiccola B: Genotypes and phenotypes of Joubert syndrome and related disorders. *Eur J Med Genet*, 2008; 51: 1–23
34. Dixon-Salazar T, Silhavy JL, Marsh SE et al: Mutations in the AHI1 gene, encoding joubertin, cause Joubert syndrome with cortical polymicrogyria. *Am J Hum Genet*, 2004; 75: 979–87
35. Parisi MA, Doherty D, Eckert ML et al: AHI1 mutations cause both retinal dystrophy and renal cystic disease in Joubert syndrome. *J Med Genet*, 2006; 43: 334–39
36. Weiss AH, Doherty D, Parisi M et al: Eye movement abnormalities in Joubert syndrome. *Invest Ophthalmol Vis Sci*, 2009; 50: 4669–77
37. Paul TO, Hardage LK: The heritability of strabismus. *Ophthalmic Genet*, 1994; 15: 1–18
38. Nordlöw W: Squint – the frequency of onset at different ages, and the incidence of some associated defects in a Swedish population. *Acta Ophthalmol (Copenh)*, 1964; 42: 1015–37
39. Doherty AL, Bowers AR, Luo G, Peli E: The effect of strabismus on object detection in the ring scotoma of a monocular bioptic telescope. *Ophthalmic Physiol Opt*, 2013; 33(4): 550–60
40. ten Tusscher MP: Does dominance of crossing retinal ganglion cells make the eyes cross? The temporal retina in the origin of infantile esotropia – a neuroanatomical and evolutionary analysis. *Acta Ophthalmol*, 2014; 92: e419–23
41. Louie CM, Caridi G, Lopes VS et al: AHI1 is required for photoreceptor outer segment development and is a modifier for retinal degeneration in nephronophthisis. *Nat Genet*, 2010; 42: 175–80
42. Lancaster MA, Gopal DJ, Kim J et al: Defective Wnt-dependent cerebellar midline fusion in a mouse model of Joubert syndrome. *Nat Med*, 2011; 17: 726–31
43. Noden DM, Trainor PA: Relations and interactions between cranial mesoderm and neural crest populations. *J Anat*, 2005; 207: 575–601
44. Witt CC, Burkart C, Labeit D et al: Nebulin regulates thin filament length, contractility, and Z-disk structure *in vivo*. *Embo J*, 2006; 25: 3843–55
45. Bang ML, Caremani M, Brunello E et al: Nebulin plays a direct role in promoting strong actin-myosin interactions. *FASEB J*, 2009; 23: 4117–25
46. Gokhin DS, Bang ML, Zhang J et al: Reduced thin filament length in nebulin-knockout skeletal muscle alters isometric contractile properties. *Am J Physiol Cell Physiol*, 2009; 296: C1123–32
47. Pelin K, Hilpela P, Donner K et al: Mutations in the nebulin gene associated with autosomal recessive nemaline myopathy. *Proc Natl Acad Sci USA*, 1999; 96: 2305–10