BMC Medical Genomics

RESEARCH Open Access

Whole-exome sequencing uncovers the genetic basis of hereditary concomitant exotropia in ten Chinese pedigrees

Wenhua Duan[1*], Taicheng Zhou[2], Xiaoru Huang[3], Dongqiong He[1] and Min Hu[2]

Abstract

Purpose To explore possible pathogenic genes for concomitant exotropia using whole-exome sequencing.

Methods In this study, 47 individuals from 10 concomitant exotropia (including intermittent exotropia and constant exotropia) pedigrees were enrolled. Whole-exome sequencing was used to screen mutational profiles in 25 affected individuals and 10 unaffected individuals. Sanger sequencing and in silico analysis were performed for all participants. Two target genes were used to capture the sequences of 220 sporadic samples.

Results All 10 concomitant exotropia pedigrees presented autosomal dominant inheritance with childhood onset (3.35 ± 1.51 years old). Eleven different missense variants were identified among seven potential pathogenic genes (*COL4A2*, *SYNE1*, *LOXHD1*, *AUTS2*, *GTDC2*, *HERC2* and *CDH3*) that cosegregated with pedigree members. All variants were predicted to be deleterious and had low frequencies in the general population. Distinct variants of *COL4A2* were present in three pedigrees, and distinct variants of *SYNE1* were present in two pedigrees. Fifteen variants in *AUTS2* and four variants in *GTDC2* were identified in 220 patients with sporadic concomitant exotropia using a target-capture sequencing approach.

Conclusion This is the first study to explore the genetic mechanism of concomitant exotropia and identify seven associated genes (*COL4A2*, *SYNE1*, *LOXHD1*, *AUTS2*, *GTDC2*, *HERC2* and *CDH3*) that may be candidate genes causing concomitant exotropia. More samples and in-depth studies are needed to verify these findings.

Keywords Genetics, Concomitant exotropia, Strabismus

Introduction

Strabismus is one of the most common eye diseases, affecting approximately 2.4–4.6% of children of different races and regions [1, 2]. Strabismus can affect a patient's vision, binocular vision function, appearance, balance control, job selection, and quality of life [3–5].

Concomitant exotropia is a common type of concomitant strabismus. In Asians, concomitant exotropia is more common than esotropia is, with an incidence of 1–2% in children [6]. Patients with concomitant exotropia are likely to be undiagnosed because they usually have unaffected corrected visual acuity and eye movement.

*Correspondence:
Wenhua Duan
drduanwh@163.com
[1]The First People's Hospital of Yunnan Province (The Affiliated Hospital of Kunming University of Science and Technology), Kunming, Yunnan Province, China
[2]The Affiliated Hospital of Yunnan University (The Second People's Hospital of Yunnan Province), Kunming, Yunnan Province, China
[3]Yunnan University, Kunming, Yunnan Province, China

Families with a history of hereditary concomitant exotropia often have multiple affected members; if the optimal intervention period is missed, the stereoscopic vision of affected patients cannot be effectively improved and restored [7]. The pathogenesis of concomitant exotropia is attributed to genetic, environmental (external) and microenvironmental (internal) factors [8]. Risk factors for concomitant exotropia in childhood include heredity, ametropia, astigmatism, ethnic origin, low birth weight (<1500 g), the presence of hypoxia at birth, and smoking by pregnant women [9, 10].

In recent years, an increasing number of clinical observations have shown that concomitant strabismus has a clear familial tendency [11–13], suggesting that hereditary factors play a very important role in unexplained concomitant exotropia. Research on familial exotropia continues to grow, with some genetic susceptibility genes already identified. For example, Min et al. used whole-exome sequencing to examine a two-generation family with concomitant exotropia, identifying AHI1 c.3257 A>G and NEB c.914 A>G as potential causal variants [14]. Similarly, Gong et al. studied a three-generation Chinese family in which both a 7-year-old girl and her mother had intermittent exotropia, identifying PAX3 c.434G>T (p.R145L) as a potential causative variant [15]. These results expand our understanding of the relevant genetic etiology of concomitant exotropia. However, there have been few studies on concomitant exotropia, which include small genetic pedigrees and insufficient sample sizes, and its specific pathogenic genes and mechanisms are still unclear. In addition, some genetic research on concomitant strabismus has not distinguished between esotropia and exotropia [16, 17], which may increase the difficulty of identifying pathogenic genes. To date, we have conducted genetic studies on specific concomitant exotropia populations to explore possible pathogenic genes.

The aim of this study was to identify potential pathogenic genes associated with concomitant exotropia through whole-exome sequencing and analysis of variant profiles in ten Chinese families.

Patients and methods

Sample collection and diagnostic procedures

All patients were from Yunnan, China. The patients we identified were limited to those with concomitant exotropia, including both intermittent and constant exotropia. The patients were defined as individuals from pedigrees with at least two affected members. All patients underwent a careful slit-lamp examination by an experienced ophthalmologist to rule out any ocular diseases, including eye movement-related disorders such as nystagmus. Patients with any of these ocular diseases or with nervous system or other systemic diseases were excluded from the study. The proband and available pedigree members were examined for best corrected visual acuity, Hirschberg corneal reflex test, angle of deviation at near (33 cm) and far (5 m) distances using the prism and alternate cover test, stereopsis, ocular appearance and fundus photography. The prism test was performed after 1 h of monocular coverage (full break of binocular fusion). Affected members with concomitant exotropia were defined as having a horizontal misalignment of 10.00 or more diopters in the prism test at either near or far distances from the primary position. Unaffected individuals were defined as having unaffected corneal reflection and stereopsis. Finally, 47 participants (32 affected and 15 unaffected) from 10 pedigrees were included in the study.

Whole-exome sequencing and data analysis

Peripheral blood was obtained from all 47 participants and collected for DNA extraction (AP-MN-BL-GDNA-250, Axygen, USA). Whole-exome sequencing was used to screen the mutational profiles of 25 affected individuals and 10 unaffected individuals. Exome capture was performed using the Agilent SureSelect Human All Exon V6 capture kit (Santa Clara, CA, USA). Sequencing was conducted on the DNBSEQ high-throughput sequencing platform developed by BGI Technology Services Co., Ltd. (BGI-Shenzhen, China).

We used 1 μg of genomic DNA to construct whole-genome libraries. All the clean data from each sample were subsequently mapped to the human reference genome (HG19) [18]. We strictly followed the recommended best practices for variant analysis with the Genome Analysis Toolkit (GATK) to ensure accurate variant calling. Base quality score recalibration and duplicate read marking were performed using GATK [19]. The sequencing depth and coverage for each individual were calculated based on the alignments. The GATK HaplotypeCaller tool was used to detect single-nucleotide polymorphisms (SNPs) and insertion-deletions (InDels) simultaneously.

To narrow down candidate variants, the main filtering criteria included the following: (1) population frequency in databases, selected from databases with less than 0.005 variant frequencies; (2) prediction of pathogenicity, using programs such as SIFT, PolyPhen2, MutationTaster, MutationAssessor, FATHMM, PROVEAN and CADD [20], to determine whether the variant was harmful (a variant was considered harmful if more than half of the prediction tools indicated harmful effects); and (3) segregation analysis, for which the corresponding genetic model (autosomal dominant, AD/autosomal recessive, AR) of the pedigree was selected, with clear typing, and the number of sequencing case/control samples was fully consistent.

The online software Wayne (VENNY2.1, https://bioinfogp.cnb.csic.es/tools/venny/index.html) was used to explore the candidate genes that were consistent between different pedigrees and the genes in each pedigree that intersected with the Human Phenotype Ontology (HPO, https://hpo.jax.org/app/) for strabismus (HP:0000486, strabismus).

Sanger sequencing

Sanger sequencing was performed on all 47 individuals for segregation analysis. Primers were designed (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) for the candidate genes of each pedigree, PCR was performed (ABI, American), and then first-generation Sanger sequencing was used to validate the candidate variants (genes and primer information are shown in Table 2).

Variant validation

Based on information provided for family members and appropriate gene size, a special full-exon panel for *AUTS2* and *GTDC2* was designed (by BGI Technology Services Co., Ltd.) for capture sequencing to search for other possible variants.

Results

Figure 1 shows the workflow of exome sequencing analysis from sample collection, validation of variants in concomitant exotropia pedigrees, exon capture and sequencing of the target gene, and variant validation.

Pedigree information

Forty-seven individuals from 10 pedigrees were included in this study. All patients were from Yunnan, China. Among the 32 affected patients, 43.75% were male, and 56.25% were female.

Figure 2 shows the pedigrees of the ten studied Chinese families

Among the 10 concomitant exotropia pedigrees, there were 5 intermittent exotropia pedigrees (F1, F2, F4, F5, and F10), 2 constant exotropia pedigrees (F3 and F6), and 3 pedigrees with mixed intermitted and constant exotropia (F7, F8, and F9). Among the 47 individuals, 11 were diagnosed with constant exotropia, 21 with intermittent exotropia, and 15 were unaffected. All pedigrees showed autosomal dominant inheritance (Fig. 2).

The available clinical findings of the studied members of the pedigrees are summarized in Table 1.

Except for participants who were unsure of the exact age of onset, the mean onset age of concomitant exotropia in pedigrees was 3.35±1.51 years (26 patients). All the participants had unaffected corrected visual and eye movements. Stereopsis (unaffected: 40 s of arc)

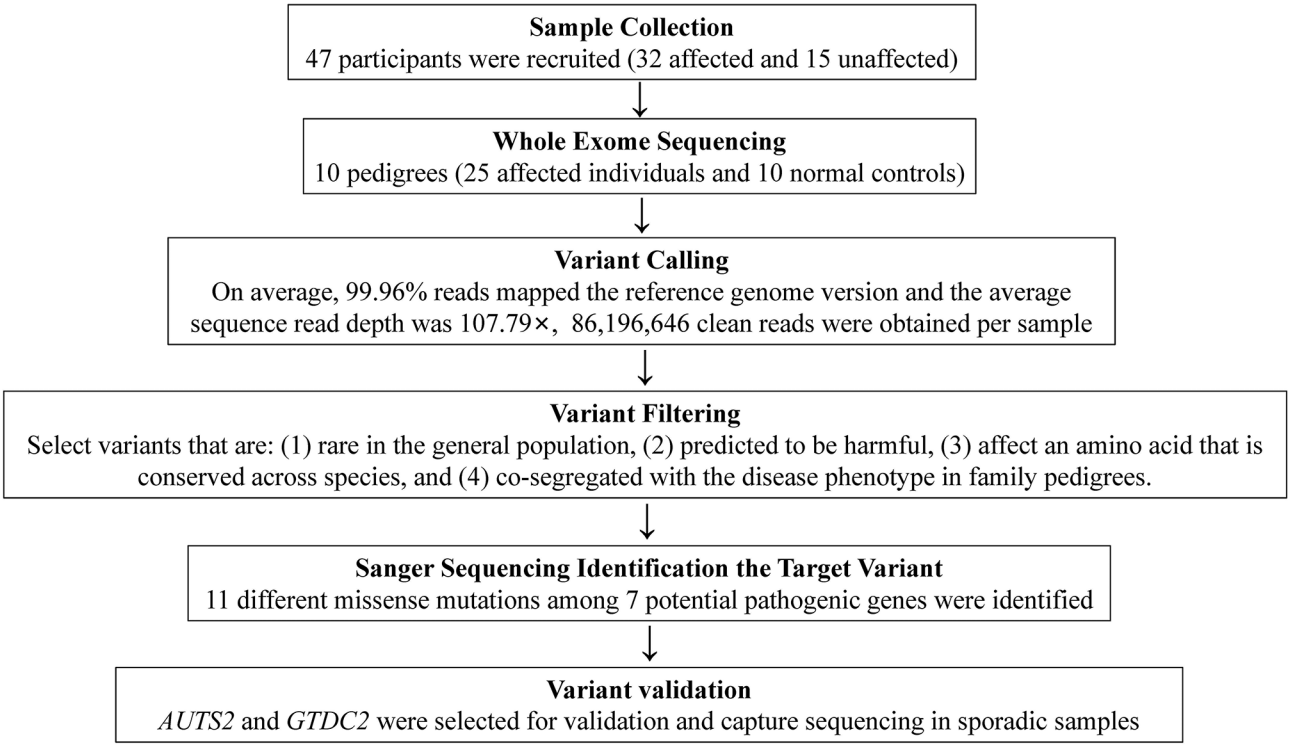

Fig. 1 Workflow for genetic variant detection and validation in concomitant exotropia pedigrees

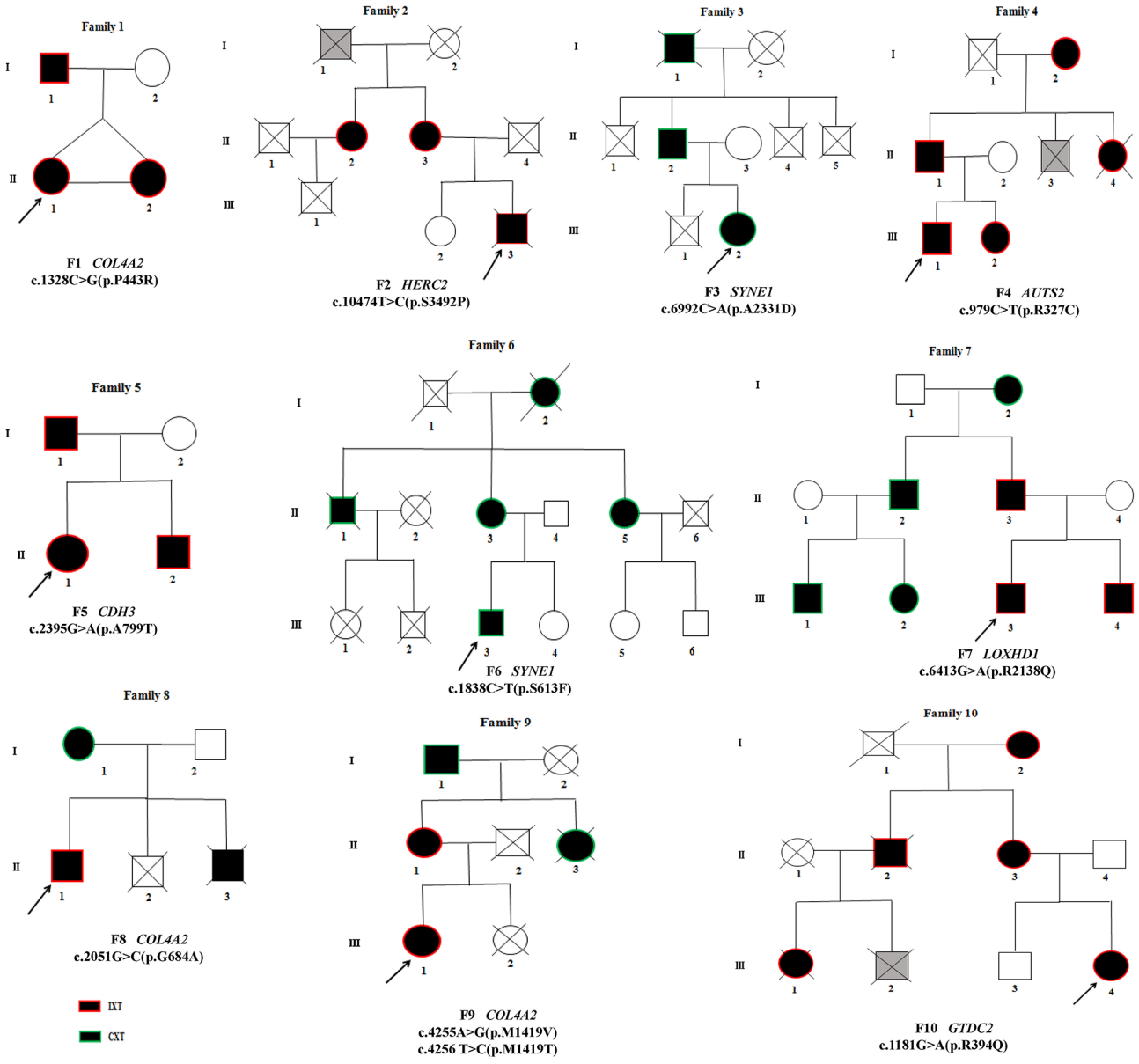

Fig. 2 Pedigrees of 10 families with exotropia. Excluded participants are marked with an "X". Affected individuals are represented by symbols with a black fill, with border colors indicating the type of exotropia: intermittent exotropia (IXT) is marked by a red border, and constant exotropia (CXT) is marked by a green border. Unaffected individuals are shown as unfilled symbols. Individuals with unknown diagnostic status are shaded in gray. Males are depicted as squares, and females are depicted as circles. Arrows indicate the probands

was impaired to varying degrees in 25 (78.1%) affected patients.

Genetic analysis in concomitant exotropia pedigrees

Exome sequencing was performed on 47 individuals from 10 concomitant exotropia pedigrees. After removing low-quality reads, we obtained an average of 86,196,646 clean reads (12,929,496,900 bps). The clean reads of each sample had high Q20 and Q30 values, indicating high sequencing quality. The average GC content was 50.78%. Duplicate reads accounted for approximately 14.85% of the total mapped reads, and the average sequence read depth was 107.79×. On average, per sequenced individual, the whole genome, excluding gap regions, was covered by at least 1 read. We identified an average of 131,079 SNPs, 99.18% of which were represented in dbSNP and 92.7% of which were annotated in the 1000 Genomes Project database. The number of novel InDels was 2,991.

In total, we identified 11 different missense variants among 7 potential pathogenic genes, which cosegregated with pedigree members (Table 3: Candidate genes and variants). Among these genes, 5 genes (*COL4A2*,

Table 1 Clinical data (10 concomitant exotropia pedigrees, 32 affected and 15 unaffected)

Family	Number	Sex	Age	Onset age	Diagnosis	Corrected visual	Corneal reflection (°)	Prism test (N-△)	Prism test (D-△)	Stereopsis-Titmus	Eye movement
F1	F1-I1	M	43	unclear	IXT	OU:1.0	-10	-20	-10	40″	Unaffected
	F1-I2	F	42	—	unaffected	OU:1.0	0	0	0	40″	Unaffected
	F1-II1	F	11	5	IXT	OU:1.0	-15	-40	-30	40″	Unaffected
	F1-II2	F	11	5	IXT	OU:1.0	-15	-40	-30	40″	Unaffected
F2	F2-II2	F	46	1	IXT	OU:1.0	-30	-95	-90	>3000″	Unaffected
	F2-II3	F	44	1	IXT	OD:0.9, OS:1.0	-45	-140	-140	>3000″	Unaffected
	F2-III2	F	28	—	unaffected	OU:1.0	0	0	0	40″	Unaffected
F3	F3-II2	M	41	unclear	CXT	OU:1.0	-25	-70	-60	>3000″	Unaffected
	F3-II3	F	39	—	unaffected	OU:1.0	0	0	0	40″	Unaffected
	F3-III2	F	17	5	CXT	OU:1.0	-20	-60	-50	>3000″	Unaffected
F4	F4-I2	F	67	unclear	IXT	OU:1.0	-15	-30	-20	100″	Unaffected
	F4-II1	M	39	6	IXT	OU:1.0	-10	-20	-15	100″	Unaffected
	F4-II2	F	38	—	unaffected	OU:1.0	0	0	0	40″	Unaffected
	F4-III1	M	20	5	IXT	OU:1.0	-35	-95	-85	>3000″	Unaffected
	F4-III2	F	10	1	IXT	OU:1.0	-20	-90	-85	>3000″	Unaffected
F5	F5-I1	M	38	unclear	IXT	OU:1.0	-10	-20	-15	80″	Unaffected
	F5-I2	F	37	—	unaffected	OU:1.0	0	0	0	40″	Unaffected
	F5-II1	F	15	5	IXT	OU:1.0	-10	-20	-10	40″	Unaffected
	F5-II2	M	11	4	IXT	OU:1.0	-10	-25	-15	40″	Unaffected
F6	F6-II3	F	45	1	CXT	OU:0.9	-30	-105	-100	>3000″	Unaffected
	F6-II4	M	44	—	unaffected	OU:1.0	0	0	0	40″	Unaffected
	F6-II5	F	44	2	CXT	OU:1.0	-25	-90	-90	>3000″	Unaffected
	F6-III3	M	21	3	CXT	OU:0.8	-20	-50	-60	200″	Unaffected
	F6-III4	F	18	—	unaffected	OU:1.0	0	0	0	40″	Unaffected
	F6-III5	F	16	—	unaffected	OU:1.0	0	0	0	40″	Unaffected
	F6-III6	M	9	—	unaffected	OU:1.0	0	0	0	40″	Unaffected
F7	F7-I1	M	68	—	unaffected	OU: 1.0	0	0	0	40″	Unaffected
	F7-I2	F	71	unclear	CXT	OD:0.8, OS:0.5	-15	-40	-30	200″	Unaffected
	F7-II1	F	46	—	unaffected	OU: 1.0	0	0	0	40″	Unaffected
	F7-II2	M	47	5	CXT	OU: 1.0	-15	-30	-20	80″	Unaffected
	F7-II3	M	45	4	IXT	OU: 1.0	-15	-30	-15	40″	Unaffected
	F7-II4	F	43	—	unaffected	OU: 1.0	0	0	0	40″	Unaffected
	F7-III1	M	20	4	CXT	OU: 1.0	-20	-60	-50	200″	Unaffected
	F7-III2	F	14	3	CXT	OU: 1.0	-20	-60	-50	200″	Unaffected
	F7-III3	M	13	3	IXT	OU: 1.0	-15	-30	-25	40″	Unaffected
	F7-III4	M	8	3	IXT	OU: 1.0	-20	-60	-40	100″	Unaffected
F8	F8-I1	F	29	4	CXT	OU:1.0	-35	-80	-70	>3000″	Unaffected
	F8-I2	M	34	—	unaffected	OU:1.0	0	0	0	40″	Unaffected
	F8-II1	M	8	3	IXT	OU:1.0	-20	-50	-40	100″	Unaffected
F9	F9-I1	M	48	4	CXT	OU:1.0	-45	-130	-120	>3000″	Unaffected
	F9-II1	F	25	3	IXT	OU:1.0	-30	-90	-80	200″	Unaffected
	F9-III1	F	4	1	IXT	OU:0.8	-30	-80	-60	400″	Unaffected
F10	F10-I2	F	64	unclear	IXT	OD:0.7, OS:0.8	-10	-20	-15	100″	Unaffected
	F10-II3	F	39	4	IXT	OU:1.0	-30	-100	-90	>3000″	Unaffected
	F10-II4	M	40	—	unaffected	OU:1.0	0	0	0	40″	Unaffected
	F10-III3	M	12	—	unaffected	OU:1.0	0	0	0	40″	Unaffected
	F10-III4	F	5	2	IXT	OU:0.8	-25	-65	-40	200″	Unaffected

Acronyms: M: male; F: female; IXT: intermittent exotropia; CXT: constant exotropia

Table 2 Lists the Sanger sequencing-verified genes that achieved coisolation in each pedigree (including primers, product size, and annealing temperature)

Family ID	Gene	Primer sequence	Product size (bp)	Tm (°C)
F1	*COL4A2*	F: TGTGGGTTGGGAAGAGAACG	725	56.39
		R: GCAGCTCAGATGTTTCGCTG		59.87
F2	*HERC2*	F: TGGGGGAAAGAACCAACTCG	638	56.32
		R: ACACGTGGAAGCAGTAAGCA		57.51
F3	*SYNE1*	F: CACGGCTCAAAGTACACAAGT	374	55.61
		R: CATAGACTCTGAGCAGGCACT		57.57
F4	*AUTS2*	F: TATGCCACACTCGCATGTCA	543	55.4
		R: GAGCAGGCCACTCACCTTAAA		57.57
F5	*CDH3*	F: GAGTGGTTAAGGGACTCGCC	639	56.91
		R: GACTCATAGCCTGTCTCCGC		58.66
F6	*SYNE1*	F: TGAATGAAACCACCGCTCAGT	466	55.61
		R: CAGGAAATCCATGAAGGCTGG		57.57
F7	*LOXHD1*	F: ATCACCTTGGGAAGGGATCA	757	55.4
		R: GGACCCCATGAAGTTCTCAGT		57.57
F8	*COL4A2*	F: ACACTGACTTGCAGGGTAGC	616	57.18
		R: CTAGCCTGGCCCCAACTAAG		58.36
F9	*COL4A2*	F: CCTCTCTGGCATGGGTCAC	739	56.75
		R: CAGAGCACTAGGACCTGGGAA		58.93
F10	*GTDC2*	F: CCCCTAGGCGAGGAGTACAT	586	59.5
		R: CTGTCCACTTCTGCTTCCGT		57.45

Acronyms: F: forward; R: reverse

SYNE1, *AUTS2*, *HERC2*, and *CDH3*) intersected with the Human Phenotype Ontology (HPO) strabismus gene list (HP:0000486).

Figure 3 shows the overlap of genes between some family-specific genes and the Human Phenotype Ontology (HPO) strabismus gene list.

In a multipedigree study of the same hereditary disease, Venn diagram comparison was able to help us quickly identify possible cosegregated genes. All candidate SNPs identified by whole-exome sequencing were verified by Sanger sequencing (the results are shown in Fig. 4).

Figure 4 shows the nucleotide sequences of the cosegregated genes for each pedigree.

Verification of candidate genes in sporadic cases

In accordance with the results of Sanger sequencing and previous data analysis, *AUTS2* and *GTDC2* were selected for validation in sporadic concomitant exotropia samples and an unaffected population. In the validation of the expanded sample, the c.979 C>T (p.R327C) variant in *AUTS2* was not detected in 496 sporadic concomitant exotropia samples or 239 unaffected samples, and the c.1181G>A (p.R394Q) variant in *GTDC2* was not detected in 422 sporadic concomitant exotropia samples or 239 unaffected samples (Table 4). The capture sequencing results revealed that in 220 sporadic

Table 3 Shows chromosome and variant details of the target genes

Family	Country	Gene	CHR	ID	KG	Transcript	Variant	Variant type	Inheritance	Zygosity	Coisolation	Gene-Tag
F1	China	*COL4A2*	13	rs192250572	0.0022	NM_001846.2	c.1328C>G(p.P443R)	Missense	AD	Heterozygote	Yes	Novel
F2	China	*HERC2*	15	rs185865505	0.002	NM_004667.5	c.10474T>C(p.S3492P)	Missense	AD	Heterozygote	Yes	Novel
F3	China	*SYNE1*	6	.	-1	XM_005266877.1	c.6992C>A(p.A2331D)	Missense	AD	Heterozygote	Yes	Novel
F4	China	*AUTS2*	7	rs141599415	0.0008	NM_015570.2	c.979C>T(p.R327C)	Missense	AD	Heterozygote	Yes	Novel
F5	China	*CDH3*	16	rs144117679	-1	NM_001793.4	c.2395G>A(p.A799T)	Missense	AD	Heterozygote	Yes	Novel
F6	China	*SYNE1*	6	rs140135976	0.0028	XM_005266877.1	c.1838C>T(p.S613F)	Missense	AD	Heterozygote	Yes	Novel
F7	China	*LOXHD1*	18	rs148468627	0.0048	NM_144612.6	c.6413G>A(p.R2138Q)	Missense	AD	Heterozygote	Yes	Novel
F8	China	*COL4A2*	13	rs201214647	0.0002	NM_001846.2	c.2051G>C(p.G684A)	Missense	AD	Heterozygote	Yes	Novel
F9	China	*COL4A2*	13	rs531809013, rs191708663	0.002	NM_001846.2	c.4255A>G(p.M1419V), c.4256T>C(p.M1419T)	Missense	AD	Heterozygote	Yes	Novel
F10	China	*GTDC2*	3	rs199612856	0.001	NM_032806.5	c.1181G>A(p.R394Q)	Missense	AD	Heterozygote	Yes	Novel

Acronyms: CHR: chromosome; KG: Public database, approximately 2500 WES/WGS, includes multiple groups; AD: autosomal dominant

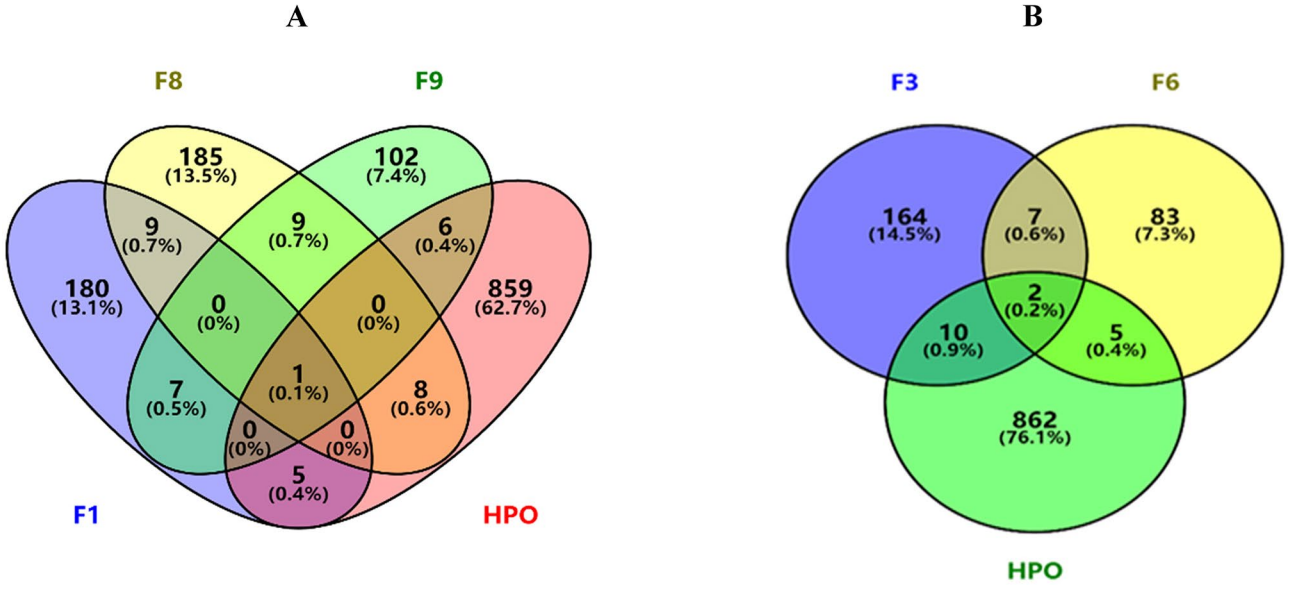

Fig. 3 Venn diagrams showing the overlap of genes between family-specific genes and the Human Phenotype Ontology (HPO) strabismus gene list. (**A**) Venn diagram depicting the overlap of identified genes among Families 1 (F1), 8 (F8), and 9 (F9) with the HPO strabismus gene list (HP:0000486). (**B**) Venn diagram showing the overlap of genes among Families 3 (F3) and 6 (F6) with the HPO strabismus gene list (HP:0000486)

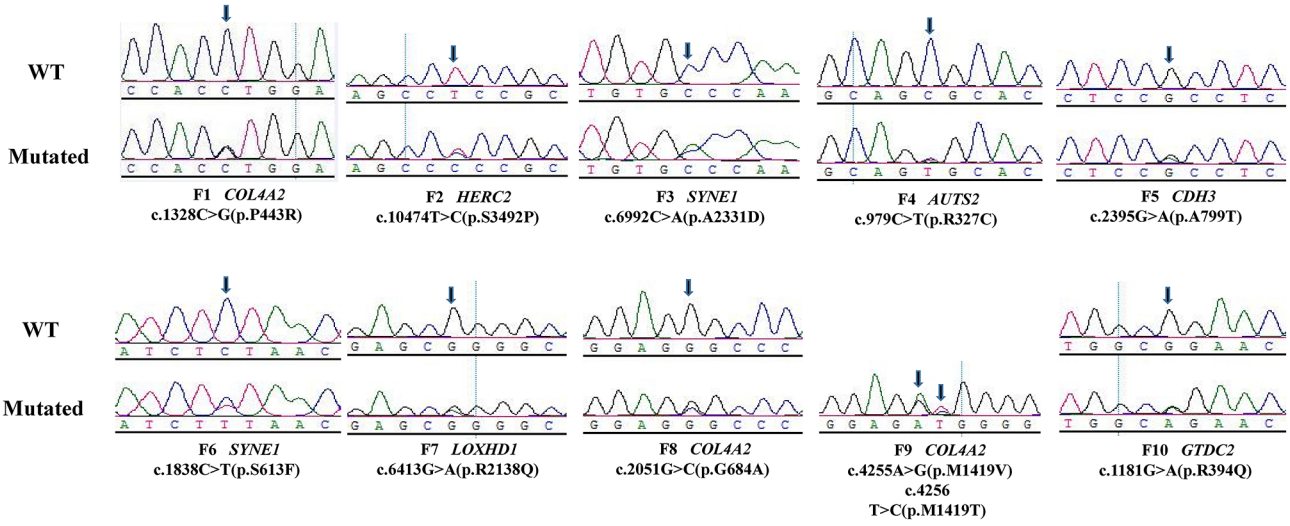

Fig. 4 Chromatograms showing nucleotide sequences for wild-type (WT) and mutated (variant) alleles for each gene. Each panel compares the WT sequence (top) from an unaffected individual with the mutated sequence (bottom) from an affected pedigree member. The arrows indicate the nucleotide position of each variant

Table 4 Shows that *AUTS2* and *GTDC2* were validated in sporadic concomitant exotropia samples and an unaffected population. The *AUTS2* c.979C > T (p.R327C) variant was not detected in 496 sporadic samples or 239 unaffected samples. The *GTDC2* c.1181G > A (p.R394Q) variant was not detected in 422 sporadic samples or 239 unaffected samples.

Gene	Sporadic samples	Unaffected population	Variant	Family
AUTS2	496/0	239/0	c.979C > T (p.R327C)	F4
GTDC2	422/0	239/0	c.1181G > A (p.R394Q)	F10

concomitant exotropia samples, 15 variants in *AUTS2* were captured (Table 5), and 4 variants were captured in *GTDC2* (Table 6).

Discussion

This is the first study to explore the genetic mechanism of concomitant exotropia. Eleven different missense variants were identified among seven potential pathogenic genes (*COL4A2*, *SYNE1*, *LOXHD1*, *AUTS2*, *GTDC2*, *HERC2* and *CDH3*) that cosegregated with pedigree members.

Table 5 A full-exon panel of *AUTS2* was designed for capture sequencing in 220 sporadic concomitant exotropia samples to search for other possible variants. Table 5 shows that in 220 sporadic concomitant exotropia samples, 15 variants were captured (including 12 SNP variant sites and 3 Indels) in *AUTS2*

Sample	Mut_type	Chr	Ref	Alt	Vcf_mut	GT	Func.refGene	cytoband
1	SNP	chr7	C	T	chr7:70256360:C/T	0/1	UTR3	7q11.22
2	SNP	chr7	A	T	chr7:69064357:A/T	0/1	UTR5	7q11.22
3	SNP	chr7	T	G	chr7:69756846:T/G	0/1	UTR3	7q11.22
4	SNP	chr7	C	T	chr7:70254740:C/T	0/1	exonic	7q11.22
5	SNP	chr7	G	A	chr7:70256619:G/A	0/1	UTR3	7q11.22
6	SNP	chr7	T	C	chr7:70256133:T/C	0/1	UTR3	7q11.22
7	SNP	chr7	C	T	chr7:70258170:C/T	0/1	UTR3	7q11.22
8	InDel	chr7	GG	-	chr7:69063488:CGG/C	0/1	UTR5	7q11.22
9	SNP	chr7	A	G	chr7:70254874:A/G	0/1	exonic	7q11.22
10	SNP	chr7	A	G	chr7:70254874:A/G	0/1	exonic	7q11.22
11	SNP	chr7	A	G	chr7:70254874:A/G	0/1	exonic	7q11.22
12	InDel	chr7	-	TATG	chr7:70256153:A/ATATG	0/1	UTR3	7q11.22
13	InDel	chr7	T	-	chr7:69064295:AT/A	0/1	UTR5	7q11.22
14	SNP	chr7	G	A	chr7:70257803:G/A	0/1	UTR3	7q11.22
15	SNP	chr7	A	G	chr7:69757852:A/G	0/1	UTR3	7q11.22

Table 6 A full exon panel of *GTDC2* was designed for capture sequencing in 220 sporadic concomitant exotropia samples to search for other possible variants. Table 6 shows that in 220 sporadic concomitant exotropia samples, 4 SNP variant site variants in *GTDC2* were captured.

Sample	Mut_type	Chr	Ref	Alt	Vcf_mut	GT	Func.refGene	cytoBand
1	SNP	chr3	G	A	chr3:43147568:G/A	0/1	UTR5	3p22.1
2	SNP	chr3	C	T	chr3:43122440:C/T	0/1	exonic	3p22.1
3	SNP	chr3	C	T	chr3:43121729:C/T	0/1	exonic	3p22.1
4	SNP	chr3	C	T	chr3:43147481:C/T	0/1	UTR5	3p22.1

Concomitant strabismus is characterized by an angle of deviation (magnitude of ocular misalignment) that remains the same in all directions of gaze, whichever eye is fixed [21]. Concomitant exotropia includes intermittent exotropia and constant exotropia [22]. Intermittent exotropia in infancy tends to rapidly progress to the constant phase [23]. Intermittent exotropia is more likely to develop into constant exotropia when there are decreases in binocular function and the stability of the eye movement control system [24]. In our study, there were 5 intermittent exotropia pedigrees, 2 constant exotropia pedigrees and 3 mixed pedigrees (both intermittent and constant strabismus members in one pedigree). Intermittent exotropia mostly occurs in children and has a higher incidence in patients with concomitant exotropia and a more pronounced inheritance tendency [22], which is consistent with our research.

The familial nature of isolated or nonsyndromic strabismus has been recognized in the medical literature since Hippocrates [25]. Cantolino and Von Noorden reported that there may be a hereditary component to microtropia, the minor form of strabismus [26]. Pedigree studies suggest that there is a strong genetic component to the etiology of concomitant strabismus, with approximately 30% of probands with strabismus having a pedigree member or close relative with strabismus [11]. To date, most progress in understanding the genetics of strabismus has been made in cases of incomitant strabismus [27–31]. Only a few studies have been performed on concomitant exotropia, and no distinction has been made between concomitant exotropia and esotropia [17, 32].

In this study, we limited the subjects to pedigrees with concomitant exotropia to exclude the interference of other types of strabismus, such as esotropia. Our results revealed that the onset age of individuals in pedigrees was earlier (3.35±1.51 years, 26 cases) than that of individuals with sporadic (6.19±5.45 years, 377 cases) concomitant exotropia. The results revealed that genetic factors play important roles in the early onset of concomitant exotropia. The main refraction of individuals in these pedigrees was myopia, with no significant differences in morbidity or diopter measurements compared with those with sporadic concomitant exotropia.

Based on whole-exome sequencing performed in a subset of members and Sanger sequencing performed in all participants, 47 individuals from 10 concomitant exotropia pedigrees were subjected to in silico analysis. We identified 11 different missense variants among 7 potential pathogenic genes that cosegregated with pedigree members. All variants had a low frequency (<5%) in the general population and could affect the secondary and tertiary structures of proteins.

Four variants of the *COL4A2* gene cosegregated in three pedigree members. *COL4A2* (collagen type IV alpha 2 chain) is a protein-coding gene associated with brain small vessel disease and intracerebral hemorrhage [33]. Among its related pathways are the integrin pathway and nervous system development [34]. Interestingly, ophthalmic diseases associated with *COL4A2* variants include keratoconus, ocular anterior segment dysgenesis, and nonarteritic anterior ischemic optic neuropathy [35]. Neri et al. described a novel *COL4A2* variant that presented with epilepsy and cortical development malformations, and the phenotype included strabismus [36]. In our study, this gene was screened in all three pedigrees with different variant sites.

SYNE1 was the pedigree coisolation gene in F3 (c.6992 C>A(p. A2331D)) and F6 (c.1838 C>T(p. S613F)). *SYNE1* (spectrin repeat-containing nuclear envelope protein 1) encodes a spectrin repeat-containing protein that is expressed mainly in skeletal and smooth muscle [37]. Current studies have revealed that diseases associated with *SYNE1* include spinocerebellar ataxia [38], autosomal recessive 8 [39] and arthrogryposis multiplex congenita [40]. The *SYNE1* gene is highly expressed in the skeletal muscle and nervous systems. Research has also suggested that the Klarsicht/ANC-1/Syne homolog (KASH)-domain-containing protein *SYNE1* plays crucial roles in anchoring both synaptic and nonsynaptic myonuclei, which are important for proper motor neuron innervation [41]. In our study, pedigrees F3 and F6 presented different variants in the *SYNE1* gene that caused different amino acid changes.

AUTS2 was the pedigree coisolation gene in F4, and the variant (c.979 C>T (p.R327C)) was not detected in 496 sporadic concomitant exotropia samples or 239 unaffected samples (Table 4). A total of 220 sporadic samples were subsequently used for capture sequencing to search for other possible variants. The results revealed 15 variants (including 12 SNPs and 3 Indels) at multiple sites in *AUTS2* (Table 5). Among them, three samples had the same SNP site variant (c.2600 A>G (p.K867R)). Therefore, *AUTS2* may be a pathogenic gene that causes hereditary concomitant exotropia. *AUTS2* (activator of transcription and developmental regulator) is a protein-coding gene; it has been implicated in neurodevelopment and is a candidate gene for numerous neurological disorders [42], including intellectual disability, developmental delay, and autism spectrum disorders [43]. This finding is consistent with neurogenic factors related to the pathogenesis of strabismus.

GTDC2 is known as *POMGNT2* (protein O-linked mannose N-acetylglucosaminyltransferase 2 (beta 1,4)), the pedigree coisolation gene in F10 (c.1181G>A (p.R394Q)). The variant was not detected in 422 sporadic concomitant exotropia samples or 239 unaffected samples (Table 4). Subsequent capture and sequencing results revealed that four different SNP variants were acquired from 220 sporadic samples (Table 6). The results revealed that *GTDC2* has a variety of variants associated with concomitant exotropia, and it could be an underlying pathogenic gene. *GTDC2* is a protein-coding gene associated with muscular dystrophy–dystroglycanopathy [44]. This finding is consistent with muscular factors related to the pathogenesis of strabismus.

HERC2 (HECT and RLD domain containing E3 ubiquitin protein ligase 2), *CDH3* (cadherin 3), and *LOXHD1* (lipoxygenase homology PLAT domains 1) are protein-coding genes. Diseases associated with *HERC2* include intellectual developmental disorders [45], autosomal recessive diseases and skin/hair/eye pigmentation [46]. Diseases associated with *CDH3* include ectodermal dysplasia, ectrodactyly, and macular dystrophy syndrome [47]. Diseases associated with *LOXHD1* include deafness, autosomal recessive 77 and autosomal recessive nonsyndromic sensorineural deafness [48] and late-onset Fuchs' corneal dystrophy [49]. *LOXHD1* is also expressed in skeletal muscle, such as extraocular muscle [50].

Concomitant exotropia is a genetically heterogeneous disorder involving extraocular muscles [51] and neurophysiological [52] causes. Any factor that causes either of these changes may lead to this disease. Among the candidate genes we identified, *COL4A2*, *SYNE1*, *HERC2*, and *AUTS2* are related to nervous system development, and *CDH3*, *LOXHD1*, and *GTDC2* are related to muscle development.

Conclusions

Concomitant exotropia is characterized by genetic heterogeneity, presumably with numerous genes involved in its pathophysiology. This is the first study exploring the genetic mechanism of concomitant exotropia, and 7 candidate genes (*COL4A2*, *SYNE1*, *LOXHD1*, *AUTS2*, *GTDC2*, *HERC2*, and *CDH3*) that may be candidate genes causing concomitant exotropia were identified. Among these genes, 5 genes (*COL4A2*, *SYNE1*, *AUTS2*, *HERC2*, and *CDH3*) intersected with the HPO strabismus gene list (HP:0000486).

The limitation of this study is that the genes that cosegregated in pedigrees have not been thoroughly studied at the levels of mRNA transcription and protein translation, including in animal model experiments. Larger sample sizes and functional studies are needed to validate these findings and explore the mechanisms by which these genes may contribute to concomitant exotropia. With the discovery of an increasing number of genes and loci, we can better understand the genetic mechanisms that cause strabismus, such as concomitant exotropia. Soon, genetic testing for strabismus may allow for earlier diagnosis or prenatal diagnosis and even lead to the development of

new therapeutic options. Currently, there is no particularly effective treatment for concomitant exotropia, and surgical intervention is recommended for constant and frequent intermittent exotropia [53]. We hope that further studies of pathogenic genes will lead to new insights, potentially guiding the development of more effective treatments for concomitant strabismus.

Acknowledgements
The authors thank the patients and all family members for their participation in this study. The experiment was completed in the central laboratory of the Affiliated Hospital of Yunnan University.

Author contributions
WD and XH carried out the experiments. WD and TZ drafted the manuscript. TZ and MH designed this study. MH, DH and WD funded this study. All the authors have read and approved the final manuscript. Wenhua Duan and Taicheng Zhou contributed equally to this work as co-first authors.

Funding
This study was supported by the Leading the Charge of Yunnan Province Health System (L-2018018), the Yunnan University "Double first-class" construction - Children Low Vision Prevention and Control Innovation Team (CY22624106), the Kunming University of Science and Technology medical joint project (KUST-KH2022036Y), and the Science and Technology plan project of the First People's Hospital of Yunnan Province (2024-KHRCBZ-B06).

Data availability
The relevant data were generated during this study and included in this article. Requests for data from this study should be made to the corresponding author, Wenhua Duan (drduanwh@163.com).

Declarations

Ethics approval and consent to participate
This study was performed in accordance with the Declaration of Helsinki. It was reviewed by the research unit's professional ethics committee, and informed consent was obtained and signed by the investigator. Our study was approved by the Ethics Committee of The Affiliated Hospital of Yunnan University (The Second People's Hospital of Yunnan Province), No. 20180774. Informed written consent was obtained from all the participants and the legal guardians of the children.

Consent for publication
Written informed consent was obtained from the patients or their guardians (parents), and they consented to the publication of their medical information.

Competing interests
The authors declare no competing interests.

Received: 30 October 2024 / Accepted: 23 December 2024
Published online: 07 January 2025

References

1. McKean-Cowdin R, Cotter SA, Tarczy-Hornoch K, Wen G, Kim J, Borchert M, Varma R. Multi-ethnic Pediatric Eye Disease Study G: prevalence of amblyopia or strabismus in Asian and non-hispanic white preschool children: multi-ethnic pediatric eye disease study[J]. Ophthalmology. 2013;120(10):2117–24.
2. Pan CW, Zhu H, Yu JJ, Ding H, Bai J, Chen J, Yu RB, Liu H. Epidemiology of intermittent Exotropia in Preschool Children in China[J]. Optom Vis Sci. 2016;93(1):57–62.
3. Wang Y, Zhao A, Zhang X, Huang D, Zhu H, Sun Q, Yu J, Chen J, Zhao X, Li R, Han S, Dong W, Ma F, Chen X, Liu H. Prevalence of strabismus among preschool children in eastern China and comparison at a 5-year interval: a population-based cross-sectional study[J]. BMJ Open. 2021;11(10):e055112.
4. Hatt SR, Leske DA, Castaneda YS, Wernimont SM, Liebermann L, Cheng-Patel CS, Birch EE, Holmes JM. Association of Strabismus with Functional Vision and Eye-Related Quality of Life in Children[J]. JAMA Ophthalmol. 2020;138(5):528–35.
5. Zipori AB, Colpa L, Wong AMF, Cushing SL, Gordon KA. Postural stability and visual impairment: assessing balance in children with strabismus and amblyopia[J]. PLoS ONE. 2018;13(10):e0205857.
6. Yu CBFD, Wong VW, Wong CY, Lam DS. Changing patterns of strabismus:a decade of experience in Hong Kong[J]. Br J Ophthalmol. 2002;86(8):854–6.
7. Li J, Ma Y, Zhou W, Lyu W, Wang L, Mao S, Li J, Shi X. Novel variants identified in a three–generation family with concomitant exotropia[J]. Exp Ther Med. 2022;24(5):688.
8. Gong W, Chen H, Yang F, Lin S, Li C, Wang G. Inter-eye differences in Ocular Biometric Parameters of Concomitant Exotropia[J]. Front Med (Lausanne). 2021;8:724122.
9. Fiess A, Elflein HM, Urschitz MS, Pesudovs K, Munzel T, Wild PS, Michal M, Lackner KJ, Pfeiffer N, Nickels S, Schuster AK. Prevalence of Strabismus and its impact on Vision-Related Quality of Life: results from the German Population-based Gutenberg Health Study[J]. Ophthalmology. 2020;127(8):1113–22.
10. Kwon JM, Lee SJ. Factors affecting contrast sensitivity in intermittent Exotropia[J]. Korean J Ophthalmol. 2020;34(5):392–7.
11. Paul TO, Hardage LK. The heritability of strabismus[J]. OPHTHALMIC GENET. 1994;15(1):1–18.
12. Ye XC, Pegado V, Patel MS, Wasserman WW. Strabismus genetics across a spectrum of eye misalignment disorders[J]. Clin Genet. 2014;86(2):103–11.
13. An JY, Jung JH, Choi L, Wieben ED, Mohney BG. Identification of possible risk variants of familial Strabismus using exome sequencing Analysis[J]. Genes (Basel) 2021, 12(1).
14. Min X, Fan H, Zhao G, Liu G. Identification of 2 potentially relevant Gene mutations involved in Strabismus using whole-exome Sequencing[J]. Med Sci Monit. 2017;23:1719–24.
15. Gong HM, Wang J, Xu J, Zhou ZY, Li JW, Chen SF. Identification of rare paired box 3 variant in strabismus by whole exome sequencing[J]. Int J Ophthalmol. 2017;10(8):1223–8.
16. Plotnikov D, Shah RL, Rodrigues JN, Cumberland PM, Rahi JS, Hysi PG, Atan D, Williams C, Guggenheim JA, Eye UKB, Vision C. A commonly occurring genetic variant within the NPLOC4-TSPAN10-PDE6G gene cluster is associated with the risk of strabismus[J]. Hum Genet. 2019;138(7):723–37.
17. Zhang J, Matsuo T. MGST2 and WNT2 are candidate genes for comitant strabismus susceptibility in Japanese patients[J]. PeerJ. 2017;5:e3935.
18. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform[J]. Bioinformatics. 2010;26(5):589–95.
19. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, Philippakis AA, del Angel G, Rivas MA, Hanna M, McKenna A, Fennell TJ, Kernytsky AM, Sivachenko AY, Cibulskis K, Gabriel SB, Altshuler D, Daly MJ. A framework for variation discovery and genotyping using next-generation DNA sequencing data[J]. Nat Genet. 2011;43(5):491–8.
20. Liu X, Wu C, Li C, Boerwinkle E. dbNSFP v3.0: a one-stop database of functional predictions and annotations for human nonsynonymous and splice-site SNVs[J]. Hum Mutat. 2016;37(3):235–41.
21. Avetisov ES, Kashchenko TP, Tarastsova MM. [Results and characteristics of the treatment of concomitant strabismus in young children][J]. Oftalmol Zh 1987(6):325–8.
22. Wang F, Cao H, Zhang Y, Wang W. Analysis of Improvement Time and Influencing Factors of Diplopia after Intermittent Exotropia in Children[J]. J Healthc Eng 2022, 2022:2611225.
23. Choi YM, Kim SH. Comparison of clinical features between two different types of exotropia before 12 months of age based on stereopsis outcome[J]. Ophthalmology. 2013;120(1):3–7.
24. Wu H, Sun J, Xia X, Xu L, Xu X. Binocular status after surgery for constant and intermittent exotropia[J]. Am J Ophthalmol. 2006;142(5):822–6.
25. Tsoucalas G, Papaioannou T, Karamanou M. Michael Constantine Psellus (1020–1105 AD) and his definition of strabismus[J]. Strabismus. 2018;26(3):155–7.
26. Richter S. On the heredity of strabismus concomitans[J]. Humangenetik. 1967;3(3):235–43.
27. Bosley TM, Oystreck DT, Robertson RL, al Awad A, Abu-Amero K, Engle EC, Brain. 2006;129(Pt 9):2363–74.
28. Tischfield MA, Bosley TM, Salih MA, Alorainy IA, Sener EC, Nester MJ, Oystreck DT, Chan WM, Andrews C, Erickson RP, Engle EC. Homozygous HOXA1 mutations disrupt human brainstem, inner ear, cardiovascular and cognitive development[J]. Nat Genet. 2005;37(10):1035–7.

29. Jen JC, Chan WM, Bosley TM, Wan J, Carr JR, Rub U, Shattuck D, Salamon G, Kudo LC, Ou J, Lin DD, Salih MA, Kansu T, Al Dhalaan H, Al Zayed Z, MacDonald DB, Stigsby B, Plaitakis A, Dretakis EK, Gottlob I, Pieh C, Traboulsi EI, Wang Q, Wang L, Andrews C, Yamada K, Demer JL, Karim S, Alger JR, Geschwind DH, Deller T, Sicotte NL, Nelson SF, Baloh RW, Engle EC. Mutations in a human ROBO gene disrupt hindbrain axon pathway crossing and morphogenesis[J]. Science. 2004;304(5676):1509–13.
30. Miyake N, Chilton J, Psatha M, Cheng L, Andrews C, Chan WM, Law K, Crosier M, Lindsay S, Cheung M, Allen J, Gutowski NJ, Ellard S, Young E, Iannaccone A, Appukuttan B, Stout JT, Christiansen S, Ciccarelli ML, Baldi A, Campioni M, Zenteno JC, Davenport D, Mariani LE, Sahin M, Guthrie S, Engle EC. Human CHN1 mutations hyperactivate alpha2-chimaerin and cause Duane's retraction syndrome[J]. Science. 2008;321(5890):839–43.
31. Al-Baradie R, Yamada K, St Hilaire C, Chan WM, Andrews C, McIntosh N, Nakano M, Martonyi EJ, Raymond WR, Okumura S, Okihiro MM, Engle EC. Duane radial ray syndrome (Okihiro syndrome) maps to 20q13 and results from mutations in SALL4, a new member of the SAL family[J]. Am J Hum Genet. 2002;71(5):1195–9.
32. Shaaban S, Matsuo T, Fujiwara H, Itoshima E, Furuse T, Hasebe S, Zhang Q, Ott J, Ohtsuki H. Chromosomes 4q28.3 and 7q31.2 as new susceptibility loci for comitant strabismus[J]. Invest Ophthalmol Vis Sci. 2009;50(2):654–61.
33. Koene S, Peeters-Scholte C, Knijnenburg J, de Vries LS, van Scheltema PNA, Meuwissen ME, Steggerda SJ, Santen GWE. Intracerebral hemorrhage in a neonate with an intragenic COL4A2 duplication[J]. Am J Med Genet A. 2021;185(2):571–4.
34. Dahl S, Pettersson M, Eisfeldt J, Schroder AK, Wickstrom R, Tear Fahnehjelm K, Anderlid BM, Lindstrand A. Whole genome sequencing unveils genetic heterogeneity in optic nerve hypoplasia[J]. PLoS ONE. 2020;15(2):e0228622.
35. Karolak JA, Kulinska K, Nowak DM, Pitarque JA, Molinari A, Rydzanicz M, Bejjani BA, Gajecka M. Sequence variants in COL4A1 and COL4A2 genes in Ecuadorian families with keratoconus[J]. Mol Vis. 2011;17:827–43.
36. Neri S, Ferlazzo E, Africa E, Versace P, Ascoli M, Mastroianni G, Cianci V, Aguglia U, Gasparini S. Novel COL4A2 mutation causing familial malformations of cortical development[J]. Eur Rev Med Pharmacol Sci. 2021;25(2):898–905.
37. Baumann M, Steichen-Gersdorf E, Krabichler B, Petersen BS, Weber U, Schmidt WM, Zschocke J, Muller T, Bittner RE, Janecke AR. Homozygous SYNE1 mutation causes congenital onset of muscular weakness with distal arthrogryposis: a genotype-phenotype correlation[J]. Eur J Hum Genet. 2017;25(2):262–6.
38. Synofzik M, Smets K, Mallaret M, Di Bella D, Gallenmuller C, Baets J, Schulze M, Magri S, Sarto E, Mustafa M, Deconinck T, Haack T, Zuchner S, Gonzalez M, Timmann D, Stendel C, Klopstock T, Durr A, Tranchant C, Sturm M, Hamza W, Nanetti L, Mariotti C, Koenig M, Schols L, Schule R, de Jonghe P, Anheim M, Taroni F, Bauer P. SYNE1 ataxia is a common recessive ataxia with major non-cerebellar features: a large multi-centre study[J]. Brain. 2016;139(Pt 5):1378–93.
39. Yoshinaga T, Nakamura K, Ishikawa M, Yamaguchi T, Takano K, Wakui K, Kosho T, Yoshida K, Fukushima Y, Sekijima Y. A novel frameshift mutation of SYNE1 in a Japanese family with autosomal recessive cerebellar ataxia type 8[J]. Hum Genome Var. 2017;4:17052.
40. Indelicato E, Nachbauer W, Fauth C, Krabichler B, Schossig A, Eigentler A, Dichtl W, Wenning G, Wagner M, Fanciulli A, Janecke A, Boesch S. SYNE1-ataxia: novel genotypic and phenotypic findings[J]. Parkinsonism Relat Disord. 2019;62:210–4.
41. Zhang X, Xu R, Zhu B, Yang X, Ding X, Duan S, Xu T, Zhuang Y, Han M. Syne-1 and Syne-2 play crucial roles in myonuclear anchorage and motor neuron innervation[J]. Development. 2007;134(5):901–8.
42. Pang W, Yi X, Li L, Liu L, Xiang W, Xiao L. Untangle the Multi-facet functions of Auts2 as an Entry Point to understand Neurodevelopmental Disorders[J]. Front Psychiatry. 2021;12:580433.
43. Biel A, Castanza AS, Rutherford R, Fair SR, Chifamba L, Wester JC, Hester ME, Hevner RF. AUTS2 syndrome: Molecular mechanisms and Model Systems[J]. Front Mol Neurosci. 2022;15:858582.
44. Manzini MC, Tambunan DE, Hill RS, Yu TW, Maynard TM, Heinzen EL, Shianna KV, Stevens CR, Partlow JN, Barry BJ, Rodriguez J, Gupta VA, Al-Qudah AK, Eyaid WM, Friedman JM, Salih MA, Clark R, Moroni I, Mora M, Beggs AH, Gabriel SB, Walsh CA. Exome sequencing and functional validation in zebrafish identify GTDC2 mutations as a cause of Walker-Warburg syndrome[J]. Am J Hum Genet. 2012;91(3):541–7.
45. Puffenberger EG, Jinks RN, Wang H, Xin B, Fiorentini C, Sherman EA, Degrazio D, Shaw C, Sougnez C, Cibulskis K, Gabriel S, Kelley RI, Morton DH, Strauss KA. A homozygous missense mutation in HERC2 associated with global developmental delay and autism spectrum disorder[J]. Hum Mutat. 2012;33(12):1639–46.
46. Suarez P, Baumer K, Hall D. Further insight into the global variability of the OCA2-HERC2 locus for human pigmentation from multiallelic markers[J]. Sci Rep. 2021;11(1):22530.
47. Kjaer KW, Hansen L, Schwabe GC, Marques-de-Faria AP, Eiberg H, Mundlos S, Tommerup N, Rosenberg T. Distinct CDH3 mutations cause ectodermal dysplasia, ectrodactyly, macular dystrophy (EEM syndrome)[J]. J Med Genet. 2005;42(4):292–8.
48. Kim BJ, Jeon HW, Jeon W, Han JH, Oh J, Yi N, Kim MY, Kim M, Kim JN, Kim BH, Hyon JY, Kim D, Koo JW, Oh DY, Choi BY. Rising of LOXHD1 as a signature causative gene of down-sloping hearing loss in people in their teens and 20s[J]. J Med Genet. 2022;59(5):470–80.
49. Riazuddin SA, Parker DS, McGlumphy EJ, Oh EC, Iliff BW, Schmedt T, Jurkunas U, Schleif R, Katsanis N, Gottsch JD. Mutations in LOXHD1, a recessive-deafness locus, cause dominant late-onset Fuchs corneal dystrophy[J]. Am J Hum Genet. 2012;90(3):533–9.
50. Zhou Y, Xu B, Wu S, Liu Y. Prognostic Immune-related genes of patients with Ewing's Sarcoma[J]. Front Genet. 2021;12:669549.
51. Kim CZ, Lee SJ. Increased myofiber size and reduced satellite cell numbers in medial rectus muscle of patients with intermittent exotropia[J]. Strabismus. 2020;28(4):201–7.
52. Rudell JC, Fleuriet J, Mustari MJ, McLoon LK. Childhood Onset Strabismus: a neurotrophic factor Hypothesis[J]. J Binocul Vis Ocul Motil. 2021;71(2):35–40.
53. Thorisdottir RL, Malmsjo M, Tenland K, Blohme J, Hesgaard HB. The success of unilateral surgery for constant and intermittent exotropia and factors affecting it in a large scandinavian Case Series[J]. J Pediatr Ophthalmol Strabismus. 2021;58(1):34–41.

Publisher's note