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Presence of Copy Number Variants Associated With Esotropia in Patients With Exotropia

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IMPORTANCE Strabismus is a common ocular disorder of childhood. There is a clear genetic component to strabismus, but it is not known if esotropia and exotropia share genetic risk factors.

OBJECTIVE To determine whether genetic duplications associated with esotropia are also associated with exotropia.

DESIGN, SETTING, AND PARTICIPANTS This was a cross-sectional study conducted from November 2005 to December 2023. Individuals with constant or intermittent exotropia of any magnitude or a history of surgery for exotropia were recruited from pediatric ophthalmic practices. Data were analyzed from March to December 2023.

EXPOSURE Genetic duplication.

MAIN OUTCOMES AND MEASURES Presence of genetic duplications at 2p11.2, 4p15.2, and 10q11.22 assessed by digital droplet polymerase chain reaction. Orthoptic measurements and history of strabismus surgery were performed.

RESULTS A total of 234 individuals (mean [SD] age, 19.5 [19.0] years; 127 female [54.3%]) were included in this study. The chromosome 2 duplication was present in 1.7% of patients with exotropia (4 of 234; $P = .40$), a similar proportion to the 1.4% of patients with esotropia (23 of 1614) in whom it was previously reported and higher than the 0.1% of controls (4 of 3922) previously reported (difference, 1.6%; 95% CI, 0%-3.3%; $P < .001$). The chromosome 4 duplication was present in 3.0% of patients with exotropia (7 of 234; $P = .10$), a similar proportion to the 1.7% of patients with esotropia (27 of 1614) and higher than the 0.2% of controls (6 of 3922) in whom it was previously reported (difference, 2.8%; 95% CI, 0.6%-5.0%; $P < .001$). The chromosome 10 duplication was present in 6.0% of patients with exotropia (14 of 234; $P = .08$), a similar proportion to the 4% of patients with esotropia (64 of 1614) and higher than the 0.4% of controls (18 of 3922) in whom it was previously reported (difference, 5.6%; 95% CI, 2.5%-8.6%; $P < .001$). Individuals with a duplication had higher mean (SD) magnitude of deviation (31 [13] vs 22 [14] prism diopters [PD]; difference, 9 PD; 95% CI, 1-16 PD; $P = .03$), were more likely to have constant (vs intermittent) exotropia (70% vs 29%; difference, 41%; 95% CI, 20.8%-61.2%; $P < .001$), and had a higher rate of exotropia surgery than those without a duplication (58% vs 34%; difference, 24%; 95% CI, 3%-44%; $P = .02$).

CONCLUSIONS AND RELEVANCE In this cross-sectional study, results suggest that the genetic duplications on chromosomes 2, 4, and 10 were risk factors for exotropia as well as esotropia. These findings support the possibility that esotropia and exotropia have shared genetic risk factors. Whether esotropia or exotropia develops in the presence of these duplications may be influenced by other shared or independent genetic variants or by environmental factors.

Invited Commentary

Supplemental content

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Monogenic causes have been identified for specific paralytic strabismus syndromes,[1] but genetic causes for the common, concomitant forms of developmental horizontal strabismus have not been identified. Twin, family, and population studies all support a genetic contribution to developmental strabismus.[2] Several environmental risk factors for common horizontal strabismus have also been identified, including low birth weight, prematurity, maternal smoking during pregnancy, and advanced maternal age. The heritability factor remains substantial after correction for these environmental risk factors.[3] It is unknown whether esotropia and exotropia result from unique genetic risk factors, shared genetic risk factors, or a combination of both. Although in most families the affected individuals all have either esotropia or exotropia, there are families in which both subtypes are present.[4]

In search of common variation contributing to risk of strabismus, 2 genome-wide association studies (GWASs) (with different inclusion criteria) have reported different risk alleles.[5,6] A GWAS of nonaccommodative esotropia identified 1 risk allele, a functional single nucleotide variation (SNV) in an intron of the *WRB* gene, which affects expression of *WRB* and neighboring genes.[5] A second GWAS, using self-reported strabismus in the UK Biobank, identified a locus on chromosome 17q25, which extends across the NPLOC4-TSPAN10-PDE6G gene cluster.[6] The association of these alleles with strabismus was replicated in the FinnGen cohort, which included all subtypes of strabismus.[7] The *WRB* gene variation was associated with any strabismus and divergent strabismus, and the *TSPAN10* gene variation was associated with any strabismus, convergent strabismus, and divergent strabismus.[7] This suggests that these common variants confer risk for strabismus in general, rather than a specific subtype of strabismus.

In addition to SNVs, genetic variation can also include copy number variants (CNVs), which are large deletions or duplications of areas of the genome. CNVs have been shown to contribute to genetic risk of multiple neurodevelopmental and neuropsychiatric disorders.[8-10] In addition, several CNVs have been associated with syndromic presentations that include strabismus.[11-13] In a previous study, we identified 3 rare, recurrent duplications that increase the risk of esotropia in White populations.[14] The duplications include a 23kb duplication on chromosome 4 (hg38|4:25,554,985-25,578,843) that includes 1 long noncoding RNA (lncRNA); a 464kb duplication on chromosome 2 (hg38|2:87,201,554-87,665,840) that includes 1 lncRNA and 1 microRNA; and a 344kb duplication on chromosome 10 (hg38|10:46,172,086-46,516,407) that includes 2 lncRNAs and 3 protein-coding genes. The duplications were found in individuals with accommodative, nonaccommodative, and infantile esotropia. To determine whether these CNVs confer risk specifically for esotropia or for strabismus in general, we have examined our cohort of patients with exotropia for each of these duplications.

Key Points

Question Are genetic risk factors for esotropia also risk factors for exotropia?

Findings In this cross-sectional study including 234 individuals, genetic duplications associated with esotropia on chromosomes 2, 4, and 10 were found to be present in 1.7%, 3.0%, and 6.0%, respectively, of individuals with exotropia. These are similar proportions as reported in individuals with esotropia, and higher than controls; individuals with a duplication had a larger magnitude of exotropia, a higher frequency of constant (vs intermittent) deviation, and higher rates of strabismus surgery.

Meaning Results suggest that genetic risk factors for esotropia also were risk factors for exotropia, supporting a common genetic risk for both forms of strabismus.

Methods

This cross-sectional study, conducted from November 2005 to December 2023, was approved by the institutional review board of Boston Children's Hospital, Boston, Massachusetts. Written informed consent was obtained from all participants. Participants received no incentive to participate. All investigations were conducted in accordance with the principles of the Declaration of Helsinki. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines.

Participants

Individuals with exotropia were enrolled into an ongoing study of strabismus genetics at Boston Children's Hospital. Participants were recruited from orthoptic clinics and pediatric ophthalmology practices. Patients with and without a family history of strabismus were recruited. Participants were included if they had constant or intermittent exotropia of any magnitude or a history of surgery for exotropia. Exclusion criteria included strabismus with a nonheritable etiology or structural ocular abnormality causing acquired vision loss, structural brain abnormalities on neuroimaging, deprivation amblyopia, molecularly defined genetic syndromes or other diagnoses associated with strabismus (such as trisomy 21 or craniosynostosis). Patients with paralytic strabismus were also excluded. Participants self-identified with the following race and ethnicity categories: Asian, Hispanic Black, non-Hispanic Black, multiracial, Native American, Hispanic White, non-Hispanic White, and race not reported. Race and ethnicity information was included in this study because the previous report identifying these duplications included only White individuals.

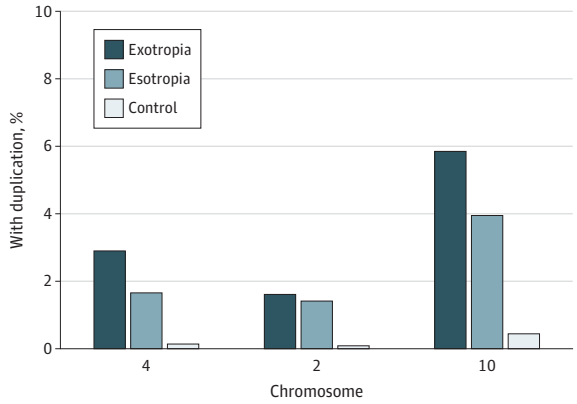
Strabismus Measurements

Strabismus measurements were obtained from medical records or dedicated orthoptic examinations. Alternate prism cover testing (APCT), with participants wearing their habitual correction (if any), was used, except for 3 participants who could not cooperate with APCT measurements. For those participants, Krimsky measurements were used. Only preoperative measurements were analyzed.

DNA Collection

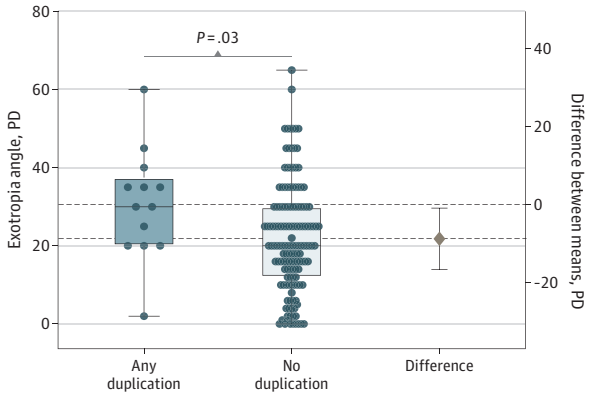
DNA was collected with Oragene saliva collection kits (DNA Genotek) and extracted via standard methods. All DNA samples

Figure 1. Percentage of Participants With Each Duplication



Data from esotropia and control participants are from Whitman et al, 2020.[14]

Figure 2. Magnitude of Deviation



Prism diopters (PDs) of exotropia at distance in individuals with any of the duplications (dark blue) or no duplication (light blue). Whiskers show minimum to maximum values; center line is mean. Points overlayed over the box and whiskers represent individual values for each participant. Dashed horizontal lines are the mean for each group. Error bars represent 95% CI of the difference. Data were evaluated using *t* test.

were quantified, and quality was assessed with Genomic DNA ScreenTape assay (Agilent). Only samples with a DNA integrity number score of 7 or higher and a concentration of 20 ng or higher were included.

Digital Droplet Polymerase Chain Reaction

Duplications were identified using digital droplet polymerase chain reaction (PCR; ddPCR [Bio-Rad]),[15] using the same probes used to confirm these CNVs in individuals with esotropia.[14] The probes were designed using a proprietary algorithm of Bio-Rad Laboratories. Locations of the probes were hg38|chr2:87,490,581-87,490,703, hg38|chr4:25,559,793-25,559,915, and hg38| chr10:46,449,597-46,449,719. The assay was performed in duplicate for each participant.

Statistical Analysis

Statistics (Fisher exact test, *t* test) were performed using GraphPad Prism, version 10. All *P* values were 2-sided, and a *P* value < .05 was considered statistically significant. Data were analyzed from March to December 2023.

Results

A total of 234 participants (mean [SD] age, 19.5 [19.0] years; range, 1-77.8 years; 127 female [54.3%]; 107 male [45.7%]) had high-quality DNA available and were included. Participants self-identified with the following race and ethnicity categories: 9 Asian (3.8%), 15 non-Hispanic Black (6.4%), 4 multiracial (1.7%), 1 Native American (0.4%), 13 Hispanic White (5.1%), 130 non-Hispanic White (55.6%), and 62 race not reported (26.4%). The chromosome 2 duplication at 2p11.2 was present in 1.7% of patients with exotropia (4 of 234; *P* = .40), a similar proportion to the 1.4% of patients with esotropia (23 of 1614) in whom it was previously reported and higher than the 0.1% of controls (4 of 3922) previously reported (difference, 1.6%; 95% CI, 0%-3.3%; *P* < .001) (**Figure 1**).[14] This corresponds to a substantially increased exotropia risk (odds ratio [OR], 17.0; 95% CI, 4.9-58.7), similar to the risk for esotropia (OR, 14.2; 95% CI, 5.4-38.1).

The chromosome 4 duplication at 4p15.2 was present in 3.0% of patients with exotropia (7 of 234; *P* = .10), a similar proportion to the 1.7% of patients with esotropia (27 of 1614) and higher than the 0.2% of controls (6 of 3922) in whom it was previously reported (difference, 2.8%; 95% CI, 0.6%-5.0%; *P* < .001). This corresponds to a substantially increased exotropia risk (OR, 20.1; 95% CI, 7.4-60.4), similar to the risk for esotropia (OR, 11.1; 95% CI, 4.6-25.2).

The chromosome 10 duplication at 10q11.22 was present in 6.0% of patients with exotropia (14 of 234; *P* = .08), a similar proportion to the 4% of patients with esotropia (64 of 1614) and higher than the 0.4% of controls (18 of 3922) in whom it was previously reported (difference, 5.6%; 95% CI, 2.5%-8.6%; *P* < .001). This corresponds to a substantially increased exotropia risk (OR, 13.7; 95% CI, 6.7-28.4), similar to the risk for esotropia (OR, 9.0; 95% CI, 5.4-14.9). One individual had both a chromosome 2 and chromosome 10 duplication; no others had more than 1 duplication.

Individuals with exotropia with a duplication had a higher rate of strabismus surgery (58% vs 34%; difference, 24%; 95% CI, 3%-44%; *P* = .02; evaluated using Fisher exact test) and a higher rate of constant (vs intermittent) exotropia than those without a duplication (70% vs 29%; difference, 41%; 95% CI, 20.8%-61.2%; *P* < .001 evaluated using Fisher exact test). For the 161 participants for whom preoperative orthoptic measurements were available, there was an association between presence of 1 of these 3 duplications and the measured mean (SD) magnitude of exotropia at distance (31 [13] vs 22 [14] prism diopters [PD]; difference, 9 PD; 95% CI, 1-16 PD; *P* = .03; evaluated using *t* test) (**Figure 2**).

Among the 24 participants with 1 (or more) of the duplications, race and ethnicity was self-reported as follows: 1 Hispanic White (4%), 19 non-Hispanic White (79%), and 4 race not reported (17%). A total of 12 were male and 12 were female (biologic sex), and mean (SD) age at enrollment was 15.5 (12.8) years,

Table. Demographic Characteristics of Participants With Exotropia With and Without Duplications

Characteristic	Any duplication (n = 24)	Chromosome 2 duplication (n = 4)	Chromosome 4 duplication (n = 7)	Chromosome 10 duplication (n = 14)	No duplication (n = 210)
Race and ethnicity, No. (%)					
Asian	0	0	0	0	9 (4.3)
Black	0	0	0	0	15 (7.1)
Multiracial	0	0	0	0	4 (1.9)
Native American	0	0	0	0	1 (0.5)
White, Hispanic	1 (4.1)	0	0	1 (7.1)	12 (5.7)
White, non-Hispanic	19 (79.2)	3 (75.0)	5 (71.4)	12 (85.8)	111 (52.9)
Not reported	4 (16.7)	1 (25.0)	2 (28.6)	1 (7.1)	58 (27.6)
Age at enrollment, mean (SD), y	15.5 (12.8)	8.3 (6.5)	10.3 (6.4)	18.2 (14.2)	19.9 (14.1)
Sex, No. (%)					
Male	12 (50.0)	1 (25.0)	5 (71.4)	6 (42.9)	95 (45.2)
Female	12 (50.0)	3 (75.4)	2 (28.6)	8 (57.1)	115 (54.8)

ranging from 2 to 46 years. Detailed demographic information for individuals with each duplication separately are presented in the **Table**. The 210 participants without a duplication had a larger range of racial identifications, including 15 Black (7%), 4 multiracial (2%), 1 Native American (0.5%), 11 Hispanic White (5%), 111 non-Hispanic White (53%), and 58 not reported (28%). A total of 95 were male (45.2%) and 115 female (54.8%), and mean (SD) age at enrollment was 19.9 (14.1) years (range, 1-77 years). The difference in mean age was 4.4 years (95% CI, −4.65 to 13.46; $P = .34$; evaluated using unpaired *t* test).

Discussion

Study results suggest that specific genetic duplications on chromosomes 2, 4, and 10 were risk factors for exotropia as well as for accommodative, nonaccommodative, and infantile esotropia. Overall, 10% of participants had 1 or more of these duplications. This is further evidence that common forms of esotropia and exotropia have shared genetic risk factors. Although each duplication is present in a small proportion of individuals with strabismus, the presence of each duplication confers substantially increased odds of having strabismus. Additionally, the presence of one of these duplications was associated with an increased magnitude of deviation, an increased rate of constant rather than intermittent exotropia, and a higher rate of exotropia surgery, indicating these duplications may be associated with more severe strabismus.

All the individuals identified with a duplication were White or unreported race. Notably, none of the 29 participants who reported race as Asian, Black, multiracial, or Native American had a duplication. Although this raises the question of whether these duplications may be risk factors only in White populations, the sample size is not large enough to exclude the presence of these duplications in other populations. The previous study of esotropia identified these duplications in White participants and did not examine individuals of other racial groups. Further studies are needed to determine whether these duplications are risk factors for strabismus in persons of African or Asian ancestry.

The mechanisms by which these duplications increase risk of strabismus are unknown. The chromosome 4 duplication includes exon 1 of the uncharacterized lncRNA *LOC101929161*. The duplication on chromosome 2p11.2 spans the lncRNA *CYTOR* and microRNA *miR4435* and contains several putative regulatory regions. The duplication on chromosome 10q11.22 spans 2 lncRNAs: *LINC00842* and *LOC105378577*; 3 protein-coding genes: *ANTXRL*, *ANXA8L1,* and *NPY4R*; and 3 transcribed pseudogenes: *ANTXRLP1*, *FAM25BP*, and *HNRNPA1P33*. Studies are ongoing to identify how these duplications affect gene expression and neuronal development.

Limitations

This study has several limitations. The study design does not allow us to assert that these duplications cause exotropia. Given the rarity of these duplications, the associations are imprecise, with very wide CIs. Potential sources of bias in recruitment include that recruitment sites have a majority of White patients, participants with a family history may have been more motivated to participate, and participants who were older at recruitment may have been more likely to provide a high-quality DNA sample. Although participants were recruited without regard to family history of strabismus, participants with a family history may have been more motivated to enroll. The conclusions that these duplications were associated with more severe strabismus should also be judged in the context of several limitations. Measurements were from medical records from a variety of examiners, performed at a wide range of ages. For many participants, preoperative orthoptic measurements were not available; these individuals were not included in analyses of strabismus angle. Indications for exotropia surgery vary across time and place, therefore, rates of surgery across a large age group should be compared with caution. Additionally, some participants who enrolled as young children may require surgery in the future. We do not have data about age of onset of strabismus for our participants, there-

fore, age at onset cannot be used as a measure or correlation of strabismus severity.

Conclusions

Results of this cross-sectional study suggest that presence of any of 3 specific genetic duplications was associated with an increased risk of developing either esotropia or exotropia. Our results, coupled with the fact that the common SNVs associated with strabismus are also associated with multiple types of strabismus, support a shared underlying genetic predisposition to esotropia and exotropia. This indicates that there are shared developmental mechanisms underlying both forms of horizontal strabismus. Development of esotropia vs exotropia in the presence of these duplications or SNVs may be influenced by specific genetic variants and/or by environmental factors.

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Concept and design: Chan, Whitman.
Acquisition, analysis, or interpretation of data: Martinez Sanchez, MacKinnon, Barry, Hunter, Engle, Whitman.
Drafting of the manuscript: Martinez Sanchez, Whitman.
Critical review of the manuscript for important intellectual content: Chan, MacKinnon, Barry, Hunter, Engle, Whitman.
Statistical analysis: Martinez Sanchez, Whitman.
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Administrative, technical, or material support: Chan, MacKinnon, Barry, Engle.
Supervision: Hunter, Whitman.

Conflict of Interest Disclosures: Ms Chan reported being an employee of Howard Hughes Medical Institute during the conduct of the study. Ms Barry reported being an employee of Howard Hughes Medical Institute during the conduct of the study. Dr Hunter reported holding equity in Rebion and Luminopia. Dr Engle reported receiving grants from the National Eye Institute and being a Howard Hughes Medical Institute Investigator. Dr Whitman reported receiving grants from National Eye Institute during the conduct of the study. No other disclosures were reported.

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