Potential linkage of different phenotypic forms of childhood strabismus to a recessive susceptibility locus (16p13.12-p12.3)

Arif O. Khan,1,2 Jameela Shinwari,2 Nada Abu Dhaim,2 Dania Khalil,2 Latifa Al Sharif,2 Nada Al Tassan2

1Division of Pediatric Ophthalmology, King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia; 2Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

Purpose: To perform linkage analysis on an inbred family with members who exhibit different phenotypic forms of childhood strabismus.

Methods: Prospective clinical examination and linkage analysis.

Results: Three of the ten siblings and their cousin each had a different phenotypic form of childhood strabismus: infantile esotropia with convergence excess, esotropia associated with anisometropic amblyopia, unilateral esotropic Duane syndrome, and monocular elevation deficiency. Linkage analysis for the four strabismic individuals, an unaffected sibling, and the unaffected parents identified a single disease locus on chromosome 16p13.12-p12.3 (Ensembl cytogenetic band) with a 2.5 maximum logarithm of odds score. The region is 6 MB in size and comprises 80 genes.

Discussion: Linkage analysis in this unique family suggests that childhood strabismus can be recessive and that different phenotypic forms of childhood strabismus can share the same underlying genotype.

Strabismus (ocular misalignment) is usually comitant (the same in different gaze positions) rather than incomitant (varying with different gaze positions) and affects up to 4% of the general population [1-3]. Most isolated strabismus is sporadic, but familial cases have been described for virtually all types and some types are more commonly familial than others [2,3]. Although all forms of early childhood strabismus have in common a disruption of normal binocular vision, there are distinct phenotypes. Comitant esotropia (inward deviation) is most common form in most populations [1-4] and includes infantile esotropia, convergence excess esotropia, and sensory esotropia. Infantile esotropia is a large-angle esotropia not typically associated with significant refractive error. Convergence excess esotropia refers to a significantly larger esotropia during near fixation that is not related to uncorrected hyperopic refractive error (unlike refractive accommodative esotropia, which is often familial and is the most common form of childhood esotropia) [3]. Sensory esotropia is from poor vision in one eye and often is related to significant uncorrected refractive error in the non-preferred eye. Congenital incomitant strabismus is less common than comitant strabismus and includes phenotypes such as monocular elevation deficiency and Duane retraction syndrome [4]. In monocular elevation deficiency, the affected eye is hypotropic (lower than the fellow eye) and unable to elevate beyond the midline, although elevation can sometimes be elicited via an ocular reflex that occurs during forced lid closure (Bell phenomenon). In Duane retraction syndrome, the most common form of congenital incomitant strabismus [4], the lateral rectus muscle of the affected eye has subnormal innervation from the sixth cranial nerve and variable inappropriate innervation from the ipsilateral third cranial nerve.

Despite the frequency of strabismus in the general population, the genetics of common forms of comitant and incomitant strabismus are not well described [1-3]. One confounding variable in previous genetic studies of strabismus is potential heterogeneity of cause when unrelated patients are studied [1-3]. Different genotypes can underlie the same strabismus phenotype and environmental factors such as prematurity can also play a strong independent role. The identification and study of multiple cases from a single family is a strategy that could minimize this issue. Also confounding previous studies of the genetics of common strabismus is the fact that studies sometimes group different strabismus phenotypes together (e.g., infantile esotropia and refractive accommodative esotropia) when in fact they may be distinct conditions rather than phenotypic variability for the same underlying cause [5-7]. Again, the identification and study of multiple cases from a single family is a strategy that would minimize this issue as in a single family it is more likely for different strabismus phenotypes to have the same underlying etiology.

In this study, we describe a single unique inbred family that has multiple members affected by different forms of childhood strabismus and perform linkage analysis to explore the possibility that a single recessive susceptibility locus underlies their phenotypes.
METHODS

This study was approved by our institutional review boards and adhered to research adhered to the tenets of the Declaration of Helsinki. Full informed consent was obtained from the family after explanation of the nature of the study.

**Clinical:** A consanguineous family with four members affected by childhood strabismus was identified from the pediatric ophthalmology practice of one of the authors (A.O.K.) and was invited to participate in the study. Each available family member underwent complete ophthalmic examination with attention to ocular motility both before and after pharmacologic cycloplegia (cyclopentolate 1%) by an ophthalmologist with strabismus experience (A.O.K.).

**Genetic:** Blood samples were obtained with informed consent from family members. DNA was extracted from 3 ml of whole blood using Gentra Systems (QIAGEN, Valencia, CA) according to the manufacturer’s conditions. The 10K single nucleotide polymorphism (SNP) genotyping was performed as detailed by Affymetrix (Santa Clara, CA) on the GeneChip® Human Mapping 10K Array Xba 142 2.0. The SNP genotypes were called using Affymetrix GCOS 1.4 software with an overall SNP call rate of 95%–99%. Multipoint logarithmic odds (LOD) score calculations were performed with the Allegro module of the EasyLinkage software package [8] assuming an autosomal recessive mode of inheritance with 100% penetrance and disease allele frequency of 0.01%.

RESULTS

**Clinical:** The family pedigree is shown in Figure 1 (starred individuals participated in the study). The asymptomatic parents (III:6, III:4) and one asymptomatic sibling (IV:10) were examined and confirmed to have no significant ophthalmic findings. The four individuals with childhood strabismus (IV:1, IV:3, IV:4, and IV:6) are summarized below:

**IV:1** This 18-year-old girl (Figure 2) had inward ocular deviation noted within the first few months of life and an unremarkable birth history. She had no treatment before presentation. Best corrected visual acuity was 20/50 in her right eye (OD) and 20/20 in her left eye (OS). Ophthalmic examination was significant for 45 prism diopters (PD) esotropia at distance and 75 PD esotropia at near withoutduction limitations. Cycloplegic refraction and fundus examination were unremarkable. Her history and examination were consistent with infantile esotropia combined with convergence excess and amblyopia OD.

**IV:3** This 19-year-old girl had inward ocular deviation noted within the first few months of life and an unremarkable birth history. She had undergone strabismus surgery OS for her eye turn a few years before presentation. Best-corrected visual acuity was 20/20 OD and 20/200 OS. Ophthalmic examination was significant for 10 PD esotropia at distance and 25 PD esotropia at near withoutduction limitations. Cycloplegic refraction and fundus examination were unremarkable. Her history and examination were consistent with infantile esotropia combined with convergence excess and amblyopia OD.

**IV:11** This 18-year-old girl (Figure 2) had inward ocular deviation noted within the first few months of life and an unremarkable birth history. She had no treatment before presentation. Best corrected visual acuity was 20/50 in her right eye (OD) and 20/20 in her left eye (OS). Ophthalmic examination was significant for 45 prism diopters (PD) esotropia at distance and 75 PD esotropia at near withoutduction limitations. Cycloplegic refraction and fundus examination were unremarkable. Her history and examination were consistent with infantile esotropia combined with convergence excess and amblyopia OD.
examination was significant for 8 PD esotropia at distance and 15 PD esotropia at near without duction limitations. Cycloplegic refraction revealed +1.50 diopters OD and +5.00 diopters OS. Fundus examination was unremarkable. Her history and examination were consistent with childhood sensory esotropia secondary to anisohyperopic amblyopia OS.

IV:4 This 18-year-old girl (Figure 3) had inward ocular deviation noted within the first few years of life and an unremarkable birth history. She had no treatment before presentation. Best-corrected visual acuity was 20/20 OD and 20/400 OS with eccentric fixation OS. Ophthalmic examination was significant for 45 PD esotropia at distance and near with abduction limitation OS (−3, with −4 being no abduction), globe retraction during adduction OS, and true mild ptosis OS. Cycloplegic refraction and fundus examination were unremarkable. Her history and examination were consistent with congenital Duane retraction syndrome OS with mild true ptosis and amblyopia OS.

IV:6 This 17-year-old boy (Figure 4) had misaligned eyes noted in the first few years of life and an unremarkable birth history. He had no treatment before presentation. He preferred...
a moderate chin-down head position with a slight right head
tilt. Best-corrected visual acuity was 20/30 OD and 20/20 OS.
Ophthalmic examination was significant for 25 PD esotropia
and 45 PD right hypotropia at distance and near with −2
supraduction limitation OD. There was a good Bell
phenomenon to forced lid closure in both eyes. Cycloplegic
refraction and fundus examination were unremarkable with
no evidence for torsion in either eye. His history and
examination were consistent with congenital monocular
elevation deficiency OD.

Genetic: Multipoint linkage analysis of family (both
unaffected parents [III:6 and III:4 ], all four strabismic
individuals [IV:1, IV:3, IV:4, and IV:6], and one unaffected
sibling [IV:10]) identified a single disease locus on
chromosome 16p13.12-p12.3 (Ensembl project genome
database cytogenetic band) with a maximum logarithm of
odds (LOD) score of 2.5 (Figure 5). The region is 6 MB in
size and comprises 80 genes.

DISCUSSION
Careful ophthalmic phenotyping and linkage analysis in this
unique inbred family with hereditary strabismus revealed four
different forms of childhood strabismus – infantile esotropia
combined with convergence excess, sensory esotropia from
anisometropia, monocular elevation deficiency, and esotropic
Duane retraction syndrome. Linkage analysis suggested that
the four strabismic phenotypes linked to a single recessive 6
MB band on chromosome 16p13.12-p12.3. These results
suggest that childhood strabismus can be recessive and that
distinct childhood strabismus phenotypes can be related to the
same underlying genetic locus.

Most advances in understanding the genetics of ocular
motility have not been for common forms of comitant or
incomitant strabismus but rather have been for rarer forms of
congenital incomitant strabismus known as congenital cranial
dysinnervation disorders [1,9]. For the recessive forms of
these disorders, genetic studies of affected consanguineous
families have allowed successful identification of novel genes
involved in ocular motility such as paired-like homeobox 2a
(PHOX2A; recessive congenital fibrosis of the extraocular
muscles) [10] and roundabout homolog 3 (ROBO3; recessive
horizontal gaze palsy with progressive scoliosis) [11]. Consanguineous families more commonly have
recessive cause for familial ocular disease and also are not
likely to be confounded by non-allelic genetic heterogeneity
among affected family members [12]. Thus genetic analysis
of such families offers a unique opportunity to uncover
recessive loci associated with a familial ocular phenotype,
as was the case for the family described in the current study.
In addition to being amenable for genetic study, the inbred
family that we describe was unique in that multiple family
members each had different phenotypic forms of common
childhood strabismus, evidence that they can represent
phenotypic variability for the same disorder.

Prior genetic studies of common forms of childhood
strabismus are limited. Both recessive and dominant linkage
of childhood esotropia to 7p22.1 have been reported in one
family each, but without differentiation of accommodative
esotropia from infantile esotropia [5,6]. Additional
susceptibility loci for comitant strabismus such as 4q28.3 and
7q31.2 have been reported, but again various subtypes of
strabismus were grouped as a single phenotype among
unrelated families who often did not have a large number of affected family members and typically were not consanguineous [7]. Infantile esotropia may best fit a codominant inheritance model [13]. Regarding monocular elevation deficiency, to the best of our knowledge no genetic studies have been published to date. Regarding Duane retraction syndrome, which can be considered a congenital cranial dysinnervation disorder [9], a dominant familial form (DURS2) was linked in a large pedigree to 2q31 [14] and is now known to be caused by heterozygous mutation in chimerin 1 (CHN1) [15]. However, heterozygous CHN1 mutation is a rare cause of Duane syndrome and is not relevant for most of Duane syndrome patients encountered in pediatric ophthalmology clinical practice [15]. There have been other genes such as sal-like 4 (SALL4) [16,17] and homeobox a1 (HOXA1) [18] that when mutated cause Duane retraction syndrome with systemic manifestation but again these genes are not associated with the common isolated form of Duane retraction syndrome [19,20]. One previous analysis of pedigrees with familial strabismus suggested that isolated Duane retraction syndrome may in some families be allelic to infantile esotropia [21], a hypothesis that is consistent the findings of our study.

In summary, we suggest that childhood strabismus can be related to a recessive susceptibility locus (16p13.12-p12.3). In addition, our results suggest that distinct childhood strabismus phenotypes (infantile esotropia combined with convergence excess, sensory esotropia from anisometropia, monocular elevation deficiency, and esotropic Duane retraction syndrome) can share a similar underlying genotype.

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