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Genome-wide search for strabismus susceptibility loci.

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Abstract

The purpose of this study was to search for chromosomal susceptibility loci for comitant strabismus. Genomic DNA was isolated from 10mL blood taken from each member of 30 nuclear families in which 2 or more siblings are affected by either esotropia or exotropia. A genome-wide search was performed with amplification by polymerase chain reaction of 400 markers in microsatellite regions with approximately 10 cM resolution. For each locus, non-parametric affected sib-pair analysis and non-parametric linkage analysis for multiple pedigrees (Genehunter software, http://linkage.rockefeller.edu/soft/) were used to calculate multipoint lod scores and non-parametric linkage (NPL) scores, respectively. In sib-pair analysis, lod scores showed basically flat lines with several peaks of 0.25 on all chromosomes. In non-parametric linkage analysis for multiple pedigrees, NPL scores showed one peak as high as 1.34 on chromosomes 1, 2, 4, 7, 10, 15, and 16, while 2 such peaks were found on chromosomes 3, 9, 11, 12, 18, and 20. Non-parametric linkage analysis for multiple pedigrees of 30 families with comitant strabismus suggested a number of chromosomal susceptibility loci. Our ongoing study involving a larger number of families will refine the accuracy of statistical analysis to pinpoint susceptibility loci for comitant strabismus.

KEYWORDS: chromosomal susceptibility locus, esotropia, exotropia, genome-wide search, strabismus

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Original Article

Genome-Wide Search for Strabismus Susceptibility Loci

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The purpose of this study was to search for chromosomal susceptibility loci for comitant strabismus. Genomic DNA was isolated from 10 mL blood taken from each member of 30 nuclear families in which 2 or more siblings are affected by either esotropia or exotropia. A genome-wide search was performed with amplification by polymerase chain reaction of 400 markers in microsatellite regions with ~10 cM resolution. For each locus, non-parametric affected sib-pair analysis and non-parametric linkage analysis for multiple pedigrees (Genehunter software, http://linkage.rockefeller.edu/soft/) were used to calculate multipoint lod scores and non-parametric linkage (NPL) scores, respectively. In sib-pair analysis, lod scores showed basically flat lines with several peaks of 0.25 on all chromosomes. In non-parametric linkage analysis for multiple pedigrees, NPL scores showed one peak as high as 1.34 on chromosomes 1, 2, 4, 7, 10, 15, and 16, while 2 such peaks were found on chromosomes 3, 9, 11, 12, 18, and 20. Non-parametric linkage analysis for multiple pedigrees of 30 families with comitant strabismus suggested a number of chromosomal susceptibility loci. Our ongoing study involving a larger number of families will refine the accuracy of statistical analysis to pinpoint susceptibility loci for comitant strabismus.

Key words: chromosomal susceptibility locus, esotropia, exotropia, genome-wide search, strabismus

Strabismus is the misalignment of both eyes and classified largely into paralytic and non-paralytic (comitant) strabimsus. Predominant types of comitant strabismus are divergent deviation (exotropia) and convergent deviation (esotropia). Comitant strabismus is sometimes associated with other systemic diseases such as Down syndrome and brain tumors, but most strabismus occurs in isolation. Major clinical categories of comitant strabismus include intermittent or constant exotropia, infantile esotropia, and accommodative or partially accommodative esotropia. Epidemiological studies [1-3] and twin studies [4-6] revealed hereditary background for these major types of comitant strabismus. However, no genes or even chromosomal loci related to comitant strabismus have yet been elucidated. In this study, we tried to discover chromosomal susceptibility loci for comitant strabismus through a genome-wide search.

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Patients and Methods

Genomic DNA was isolated from 10 mL peripheral blood obtained from 2 or 3 children, together with one or both biological parents in each of 30 nuclear families in which at least 2 children had been afflicted by either esotropia or exotropia (Table 1). This study was approved by the Ethical Committee of Okayama University Hospital, and informed consent was obtained from one or both parents. Briefly, leukocytes were separated by

Table I Clinical features of sib-pairs

sib-pair number	Sex	Age(years)*	Diagnosis	Others features
1	M	7	XpT	
	F	5	ET	
2	M	14	Infantile ET	
	F	11	AccET	
3	F	7	XpT	two second cousins (male): strabismus
	F	10	XpT	paternal grandfather's sister: amblyopia
4	F	4	XpT	father : XpT
	F	6	vertical strabismus	
5	F	11	XpT	
	F	10	XpT	
6	F	10	XpT	
	M	12	XpT	
7	M	15	ET	
	M	13	ET	
8	F	11	XpT	persistent pupillary membrane (OD)
	M	13	XpT	
9	F	7	ET	dizygotic twins, paternal grandfather : XT
	F	7	ET	
10	F	10	Infantile ET	second cousin (female) : ET
	F	12	XpT	
11	M	10	Hyperopia	mother : ET
	F	8	AccET	
12	F	12	ET	
	F	14	AccET	
13	M	8	AccET	monozygotic twins
	M	8	AccET	
14	M	9	AccET	
	F	10	AccET	
15	F	3	EpT	dizygotic twins, father : amblyopia
	F	3	ET	
16	F	12	XpT	
	F	14	XpT	
17	F	7	XpT	
	F	6	XpT	
18	F	6	XpT	second cousin (female) : XpT
	F	10	XpT	
19	M	8	AccET	cousin (male) : hyperopia
	F	6	InfantileET	
20	M	7	InfantileET	
	F	3	InfantileET	
21	F	17	XpT	
	F	18	XpT	
22	M	19	XpT	mother : XpT
	F	17	XpT	small for date birth (39weeks, 1720g)
23	F	4	XT	paternal grandfather : XpT
	M	6	XpT	congenital camptodactyly
24	M	13	XpT	cousin (male) : ET
	M	15	AccET	
25	F	6	Infantile ET	
	F	4	Infantile ET	
26	M	8	XpT	
	M	6	XT	
27	M	8	XpT	
	M	11	XpT	
28	F	12	XpT	
	F	15	XpT	
29	F	9	AccET	
	F	13	AccET	
30	M	10	ET	father : amblyopia, second cousin (female) : ET
	F	11	AccET	

AccET, accomodative esotropia; EpT, intermittent esotropia; ET, esotropia; XpT, intermittent exotropia; XT, exotropia.
*The Age when the blood was taken.

gradient centrifugation using Mono-Poly Resolving medium (Dainippon Pharmaceutical, Osaka, Japan), suspended in extraction buffer (10 mM Tris, 100 mM EDTA, 0.5% SDS, pH 8), and digested with proteinase K (Merck, Darmstadt, Germany). Genomic DNA was purified by phenol/chloroform extraction and ethanol precipitation.

The genome-wide search was performed with amplification by polymerase chain reaction (PCR) of 400 markers in microsatellite regions with ~10 cM resolution. Each marker site was amplified by AmpliTaq Gold polymerase (PE Applied Biosystems, Foster City, CA, USA) from 60 ng of genomic DNA with fluorescent dye-labeled primers (ABI PRISM Linkage Mapping Set Version 2, PE Applied Biosystems) at the manufacturer's recommended conditions for GeneAmp PCR System 2400. DNA fragments were then mixed with size standards (GenScan 400 HD [ROX]) in formamide, applied to ABI PRISM 310 Genetic Analyzer, and analyzed by GeneScan Analysis Software. For each locus, non-parametric affected sib-pair analysis and non-parametric linkage analysis for multiple pedigrees in Genehunter software version 2.0 beta (http://linkage.rockefeller.edu/soft/) were used to calculate non-parametric multipoint lod scores and non-parametric linkage (NPL) scores, respectively.

Results

Clinical characteristics. Table 1 summarizes clinical characteristics of each sib-pair with strabismus. Exotropia, including intermittent exotropia and constant exotropia, was found as a common manifestation in 13 sib-pairs, while esotropia, including infantile esotropia, accommodative esotropia, esophoria, and unclassified esotropia, was found in common in 12 sib-pairs. In three sib-pairs, one sibling had exotropia while the other had esotropia. One sib-pair consisted of one sibling with exotropia and the other with vertical comitant strabismus. One sib-pair comprised of one sibling with accommodative esotropia and the other with hyperopia was included in this analysis since hyperopia is a well-known risk factor for the development of accommodative esotropia.

Statistical analysis. In sib-pair analysis, multipoint lod scores showed basically flat lines with several peaks only as high as 0.25 on all chromosomes (data not shown). In non-parametric linkage analysis for multiple pedigrees, a single peak as high as 1.34 was found on chromosomes 1, 2, 4, 7, 10, 15, and 16, while 2 such peaks were found on chromosomes 3, 9, 11, 12, 18, and 20 (Fig. 1, Table 2). These NPL scores, however, did not reach a statistical significance (Table 2). The remaining chromosomes showed no peaks with an NPL score of 1 or greater.

Discussion

Affected sib-pair analysis did not detect any suggestive chromosomal loci because of the small number of pairs used in this study. In contrast, non-parametric linkage analysis for multiple pedigrees produced scores that distributed into positive peaks sandwiched by negative troughs on several chromosomes. Because of the small number of pedigrees, these NPL scores were not sufficiently high to identify definite chromosomal susceptibility loci for comitant strabismus. This is the weakness of this particular application of non-parametric analysis. Its strength, and the reason we used it, is that non-parametric linkage analysis does not require assumptions about the mode of inheritance [7], and the mode of inheritance of comitant strabismus is uncertain at present [6].

For instance, it remains unknown whether comitant strabismus including exotropia and esotropia is a single-gene disease or multi-gene disease, much less how many genes are responsible for the development of comitant strabismus if it is a multi-gene disease. The present study suggests the presence of several chromosomal susceptibility loci for comitant strabismus. The mode of inheritance of comitant strabismus may be recessive or dominant [6]. Furthermore, the phenotypes of comitant strabismus, such as the extent of deviation and the levels of binocular function, vary greatly from patient to patient, even within the 2 categories of exotropia and esotropia.

In the search for the keyword "strabismus" in the OMIM (Online Mendelian Inheritance in Man) database (http://ncbi.nlm.nih.gov), a large number of diseases are "hit". Strabismus in most of these diseases results secondarily from poor vision, which is caused by abnormalities in the eyeball structure. In the present study, cases of exotropia and esotropia that were secondary to poor vision, usually called "sensory exotropia or esotropia," were, of course, excluded from the analysis. Patients with exotropia or esotropia in isolation, such as those involved in this study, do not have any abnormalities in eye structure. Presumably, the gene or genes

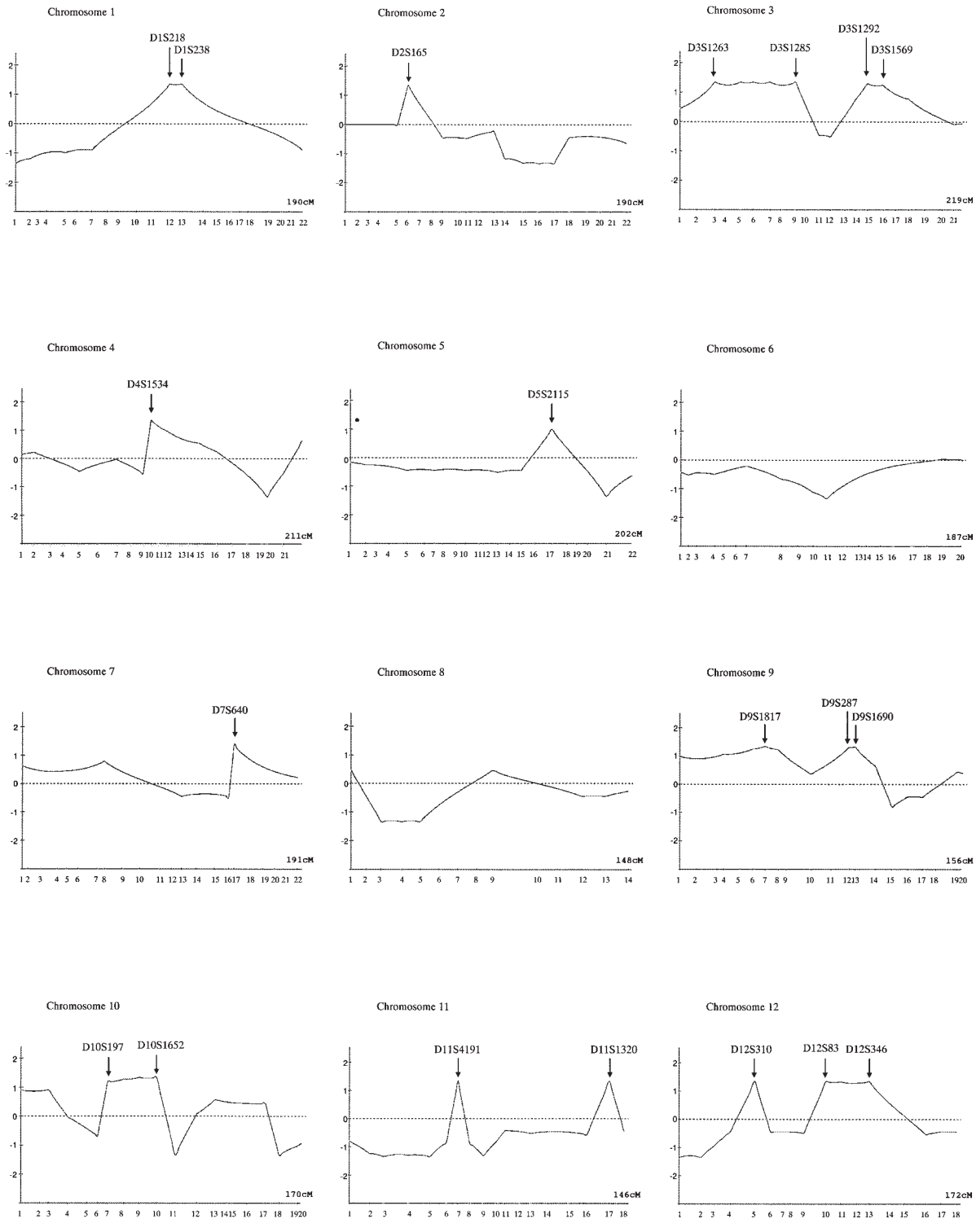

Fig. 1 Non-parametric linkage (NPL) scores for microsatellite markers on all chromosomes. NPL scores are given in the ordinate, and the "location" (arbitrary numbering) of microsatellite markers are given in the abscissa.

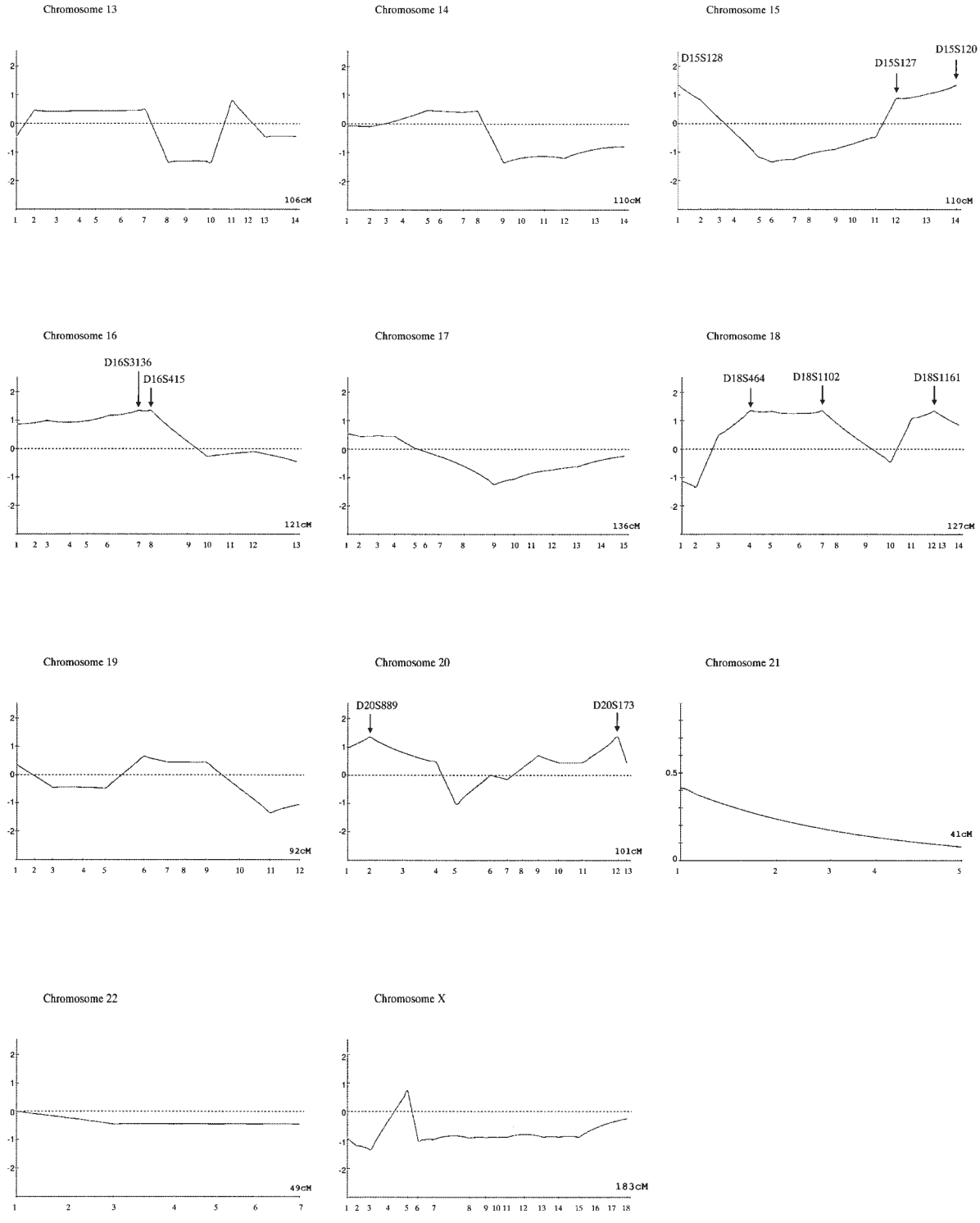
Chromosome 13
106cM
Chromosome 14
110cM
Chromosome 15
D15S128
D15S127
D15S120
110cM
Chromosome 16
D16S3136
D16S415
121cM
Chromosome 17
136cM
Chromosome 18
D18S464
D18S1102
D18S1161
127cM
Chromosome 19
92cM
Chromosome 20
D20S889
D20S173
101cM
Chromosome 21
41cM
Chromosome 22
49cM
Chromosome X
183cM

Table 2 NPL score and *P* value of chromosomal susceptibility loci suggested by non-parametric linkage analysis for multiple pedigrees of exotropia and esotropia

Chromosome No.	Locus	NPL score	*P* value
1	D1S218	1.34	0.25
	D1S238	1.34	0.25
2	D2S165	1.34	0.25
3	D3S1263	1.34	0.25
	D3S1285	1.34	0.25
	D3S1292	1.29	0.25
	D3S1569	1.24	0.25
4	D4S1534	1.34	0.25
5	D5S2115	0.99	0.25
7	D7S640	1.34	0.25
9	D9S1817	1.34	0.25
	D9S287	1.32	0.25
	D9S1690	1.33	0.25
10	D10S197	1.34	0.25
	D10S1652	1.34	0.25
11	D11S4191	1.34	0.25
	D11S1320	1.34	0.25
12	D12S310	1.34	0.25
	D12S83	1.34	0.25
	D12S346	1.34	0.25
15	D15S127	0.85	0.25
	D15S120	1.34	0.25
16	D16S3136	1.34	0.25
	D16S415	1.34	0.25
18	D18S464	1.34	0.25
	D18S1102	1.32	0.25
	D18S1161	1.34	0.25
20	D20S889	1.34	0.25
	D20S173	1.34	0.25

NPL score, non-parametric linkage score.

responsible for strabismus would be expressed in the central nervous system since eye alignment and binocular function, which are disturbed in strabismus, are controlled by the brain. The chromosomal loci for exotropia or esotropia suggested in the present study overlapped with several chromosomal loci found in the OMIM database associated with the keyword "strabismus," even after the exclusion of "sensory strabismus" as mentioned above (Table 3). The chromosomal loci for strabismus associated with other genetic diseases, so-called "syndromic strabismus," will help in selecting candidate chromosomal loci for exotropia or esotropia from among those initially suggested in this study.

It should be noted that all 3 chromosomal loci (FEOM 1 on 12cen, FEOM2 on 11q13, and FEOM3 on 16q24) for congenital fibrosis of the extraocular muscles (CFEOM) happened to correspond to strabismus susceptibility loci suggested in this study (Table 3). CFEOM is characterized by strabismus and ptosis, and considered to result from developmental defects of the oculomotor, trochlear, and abducens nuclei in the brainstem and hence, their nerves, leading to fibrosis of the extraocular muscles and the levator muscle [8]. Recently, homozygous mutations in ARIX, a homeobox-containing gene, were found in patients with CFEOM type 2, which was initially mapped to FEOM2 on chromosome 11q13 [9]. If these FEOM loci survive the analysis of a significantly larger number of pedigrees with exotropia and esotropia, it may be concluded that strabismus in isolation is due to subclinical changes of the ocular motility nerves and their brainstem nuclei.

Until now in the ophthalmological field, genome-wide searches have been performed only for age-related macular degeneration [10, 11] and glaucoma [12]. Although responsible genes were not identified in age-related macular degeneration, a locus on chromosome 1 found by the genome-wide search coincided with the results of pedigree analysis in one large family with age-related macular degeneration [11]. In contrast, the results of the genome-wide search for glaucoma were not necessarily consistent with several chromosomal loci (GLC1 A to 1F) found by the previous pedigree studies [12]. These contrasting facts show the reliability and the limitation of genome-wide searches.

Strabismus is probably better suited to genome-wide searches because strabismus such as infantile esotropia, accommodative esotropia, and intermittent exotropia is known for its early onset in childhood and its high rate of family history, ranging from 20 to 40% [13]. The prevalence of comitant strabismus is as high as about 2% among Japanese children [14]. Furthermore, parents who bring children with strabismus to the hospital usually have the affected children's siblings in tow. Given these circumstances, ophthalmologists can easily examine siblings and parents in addition to patients themselves.

As another advantage for genome-wide searches, the Japanese population may be more homogeneous than populations of other countries with a wide variety of ethnic backgrounds, as is the case, for example, in North America. To accentuate this effect, the Okayama area in Japan, from which the patients were mainly recruited, has experienced less immigration than large cities like Tokyo and Osaka in Japan. These features suggest that the families analyzed in this study may have common ancestors: another rationale for linkage analysis including

Table 3 Strabismus associated diseases found in the OMIM (Online Mendelian Inheritance in Man) detabase which correspond to chromosomal susceptibility loci for exotropia and esotropia suggested by non-parametric linkage analysis for multiple pedigrees

Chromosomal locus	Strabismus associated disease	OMIM No.
D1S218		
D1S238		
D2S165		
D3S1263	Fanconi anemia complementation group D2	*227646
D3S1285	Bardet-Biedl syndrome	#209900
	Combined pituitary hormone deficiency	*173110
D3S1292		
D3S1569		
D4S1534		
D5S2115	Sotos syndrome	#117550
	Corneal dystrophy granular type	#121900
D7S640		
D9S1817	Rufous oculocutaneous albinism	#278400
D9S287	Fanconi anemia complementation group C	*227645
	Basal cell nevus syndrome	#109400
D9S1690	Oculocutaneous albinism type III	#203290
D10S197	Cockayne syndrome type B	*133540
D10S1652	Crouzon syndrome	#123500
	Saethre-Chotzen syndrome	#101400
	Bannayan-Riley-Ruvalcaba syndrome	#153480
	Apert syndrome	#101200
	Infantile-onset spinocerebellar ataxia	*271245
D11S4191	Ocular albinism with sensorineural deafness	#103470
	Smith-Lemli-Opitz syndrome	#270400
	Bardet-Biedl syndrome	#209900
	Oculocutaneous albinism type I	#203100
	Congenital fibrosis of extraocular muscles 2	#602078
D11S1320	Jacobsen syndrome	#147791
	Ataxia-Telangiectasia	*208900
	Gilles de la tourette syndrome	*137580
D12S310	Congenital fibrosis of extraocular muscles I	*135700
D12S83	Acrocallosal syndrome	*200990
D12S346	Cardiofaciocutaneous syndrome	115150
	Holt-Oram syndrome	#142900
D15S127	Bardet-Biedl syndrome	#209900
	Prader-Willi syndrome	#176270
	Shprintzen-Goldberg craniosynostosis syndrome	#182212
	Bartter syndrome type I	*600839
D15S120		
D16S3136	Congenital disorder of glycosylation	#212065
	Bilateral frontoparietal polymicrogyria	*606854
D16S415	Fanconi anemia	#227650
	Bardet-Biedl syndrome	#209900
	Congenital fibrosis of extraocular muscles 3	*600638
D18S464		
D18S1102		
D18S1161		
D20S889	Bardet-Biedl syndrome	#209900
	Alagille syndrome	#118450
D20S173		

multiple small families.

In this study, the patients were not stratified into 2 groups having either esotropia or exotropia, mainly because of the limited number of sib-pairs available. One rationale for including both esotropia and exotropia in this kind of analysis is that esotropia and exotropia might have a common etiology such as binocular vision abnormalities or fusion deficit, notwithstanding the variations in the deviation. Furthermore, this overall approach takes into account clinical and genetic heterogeneity; division into esotropia and exotropia might adversely affect the detection of linkage. Other studies have indeed shown that such clinical stratification almost never increases the power of a study to detect linkage [15].

This study is the first genome-wide search to be completed for comitant strabismus. The number of sib-pairs analyzed in this study was too small to obtain reliable chromosomal loci; thus, the results should be interpreted as preliminary. Recently, the limitation of genome-wide screening has been shown for a schizophrenia locus using a large number of multicenter blood samples [16]. Ongoing research is underway in our laboratory to include a larger number of sib-pairs with comitant strabismus. Based on the preliminary results of this study, linkage analysis for multiple pedigrees may be the method of choice, rather than affected sib-pair analysis. Accordingly, not only affected sib-pairs, but also small families without affected sib-pairs will be included in the future study.

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