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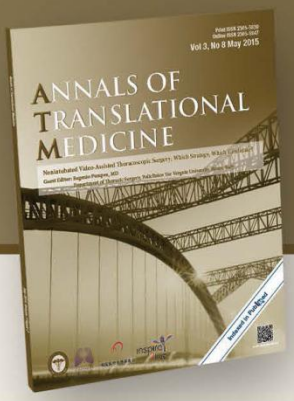

Peer Review File

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Reviewer Comments

In this manuscript the authors report four Chinese families with a p.Arg130Cys mutation and congenital cataracts. While the data appear solid and the association with cataracts are almost certainly correct, the associated findings are inconstant and their relationship to the mutation is less clear. In addition, the possibility that these families might have a common founder needs to be checked. Specific comments follow:

Comment 1:

Abstract, "Congenital cataract is a congenital abnormality with lens opacity at birth, associated with highly heterozygous clinical manifestations.": Maybe 'opacity present at birth' and 'highly heterogeneous clinical'.

Reply 1:

Thank you for your comment. We have modified our text as advised (see Page 3, line 34-35).

Comment 2:

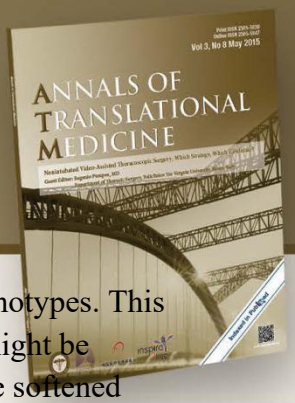
Introduction p. 6, lines 96-97, "We present the following article in accordance with the STROBE reporting checklist.": This checklist is really for epidemiological studies and is not necessary here, although it probably doesn't hurt to follow it.

Reply 2:

Thank you. We follow the STROBE reporting checklist at the request of the editorial department of ATM. Thus, we keep this statement. Thank you all the same.

Comment 3:

Results, Clinical Data, p. 8-9, lines 139-161, "He had elongated eye axes in both eyes...bilateral optic disc hypoplasia and a leopard-shaped fundus.": From the descriptions here, it looks like some individuals in two of the four families described had a mixture of extralenticular phenotypes. These do not appear to be consistently present in all cataract patients, and there is

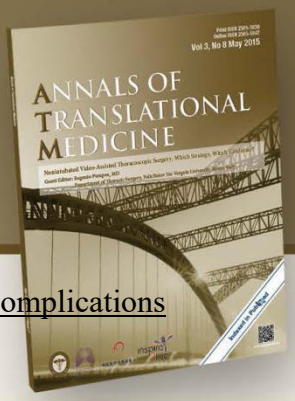


some variation in phenotype among those individuals with extralenticular phenotypes. This suggests that the association of *LIM2* mutations and PFV is incomplete, and might be conincidental, so that the statement about the phenotype in the paper should be softened considerably. Have you checked the ATOH7 gene in those individuals with PFV?

Reply 3:

Thank you for your valuable comments.

Firstly, all available patients of the four families in this study indeed have various extralenticular phenotypes, including elongated axial length, fundus anomalies (leopard-shaped fundus, patchy chorioretinal atrophy and optic disc changes), congenital nystagmus, esotropia, persistent fetal vasculature (PFV) and eyeball atrophy. Of them, elongated axial length and/or myopia related fundus anomalies are consistently present in all available cataract patients. Congenital nystagmus, esotropia and eye atrophy might be the complications of cataracts and myopia. For only one patient in Family 1 had PFV, the association of the *LIM2* mutation and PFV is indeed incomplete. It could be coincidental. To make the presentation of the ocular phenotypes clearly, we tried out best to represent the cataract and extralenticular phenotypes of all available patients in Table 1. We modified the title of this manuscript into "Elongated axial length and myopia-related fundus changes associated with the Arg130Cys mutation in the *LIM2* gene in four Chinese families with congenital cataracts". We also revised related description in the Result section of the manuscript as following: "In addition to congenital cataracts, all eleven patients in the four families had elongated axial length and/or myopia related fundus changes (including leopard-shaped fundus, patchy chorioretinal atrophy and optic disc change). In Family 1, all patients were diagnosed with bilateral cataracts before age 10 years. IV:1 was the proband. He was referred to the ophthalmic department for bilateral leukocoria at four months of age and was diagnosed with nuclear cataracts. His cataract phenotype was bilateral nuclear cataract surrounded by pulverulent cortical opacity. After undergoing cataract extraction and intraocular lens implantation surgery at six months old, he was diagnosed with elongated axial length (23.17mm OD, 23.07mm OS), leopard-shaped fundus and optic disc tilt in both eyes at four years and five months old. IV:2 was the younger brother of IV:1. Differing from the nuclear cataract phenotype of IV:1, he had bilateral lamellar cataracts with a relatively transparent central area. He also had myopia related fundus changes including patchy chorioretinal atrophy and optic disc tilt. What's more, there were plaques of remnant tissue sitting on the posterior face of bilateral lens of IV:2. He was diagnosed with persistent fetal vasculature (PFV) at seven months old" (see Page 9-10, line 175-189). We also revised this description in the Discussion section: "New phenotypes detected in the four families included elongated axial length, myopia related fundus anomalies (leopard-shaped fundus, patchy chorioretinal atrophy and optic disc change), congenital nystagmus, esotropia, PFV and eyeball atrophy. Of them, elongated axial length and/or myopia related fundus anomalies were consistently present in all available patients. The occurrence of other phenotypes, including congenital nystagmus, esotropia, and



eyeball atrophy in multiple members of two or three of the families might be complications associated with the elongated axial length" (see Page 12, line 229-236).

Lastly, besides cataract related genes, we have double-checked all genes reported associated with PFV in the whole-exome sequencing result of the member IV:2 in Family 1. It included the *ATOH7* gene. No candidate variant was found in those genes. All genes associated with PFV was listed in Supplementary Table S1.

Comment 4:

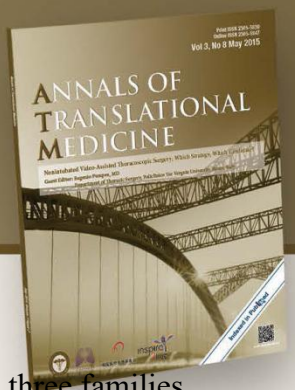
Results, general: It would be helpful if a more complete description of the cataract phenotype could be provided for as many patients as possible.

Reply 4:

Thank you. To provide more cataract phenotypes associated with this mutation, we contacted the four families and found a new affected member in Family 4. He was the younger brother of the proband in Family 4, member III:2. He was also a patient with congenital cataracts and elongated axial length. He had irregular lamellar cataracts surround by pulverulent cortical opacity in both eyes, which was different from other affected members. His bilateral axial lengths were 21.10mm of the right eye and 21.07mm of the left eye at 11 months old. His cataract phenotype images were presented in Figure 2G. He is also a carrier of the same mutation c.C388T in the *LIM2* gene. For other patients who visited our clinic before cataract surgery (IV:1, IV:2 in Family 1, the probands of Family 2,3 and 4), we provided a more detailed description of their cataract phenotype in the manuscript and Table 1 (see Page 10, line 180-181, line 185-186; Page 11, line 204-206, line 210-216). We also presented the cataract phenotype images of the probands in Family 2 & Family 3 and III:2 in Family 4 in Figure 2. For the remaining members, they had cataract surgery more than 10 years before coming to our clinic, thus we cannot get more information about their cataract phenotypes than the description in their medical records. Detailed descriptions of the cataract phenotype of all available patients was presented in Table 1.

Comment 5:

Results, p. 9, lines 177-178, "The proportion of probands with this mutation in our congenital cataracts database is 3.1% (4/130).": Is it possible that these families might be related? Were they all from Guangzhou or Guangdong Province? It should be possible to determine the disease haplotypes in 3 of the families, and enough information is present in Family 3 to exclude if it is not related. This should not be difficult and would strengthen the assertion about the frequency of *LIM2* mutations in the database, and thence the frequency in Han Chinese inherited cataract families.



Reply 5:

Thank you. Except for the proband in Family 3 which was a sporadic case, the three families were inherited in an autosomal dominant pattern of inheritance. Family 1 lived in Xinjiang Province for at least three generations in the Northwest China. Family 2 and 4 were from two different cities in Jiangxi Province. Xinjiang Province is at the Northwest China. Jiangxi Province is in the Southeast China. The distance between the two provinces is more than 3,600 kilometers. The geographical position of the two provinces was shown in Supplementary Figure S1. Although Family 1 lived in a different region from Family 2 and 4, it was still possible that they shared a common founder allele. Thus, we identified the haplotypes of the probands in the three families as following: "In the analysis result, the haplotypes shared by the three probands was between rs12461542 and rs3794984, which spanned 0.41 Mb pairs. The region shared between Family 1 and Family 2 was 0.26 Mb pairs. The region shared between Family 2 and 4 spanned 0.19 Mb pairs, which was even shorter than the shared region between Family 1 and 2. Considering that the shared haplotype region of the three probands was quite limited, as well as the geographical distance between Xinjiang and Jiangxi Province, the three families may have had founder events that occured independent from each other. The positions, intermarker distance and allele frequencies of the SNPs, and the haplotype analysis result of the probands in Family 1,2 and 4 are shown in Supplementary Table S2". The descriptions of the methods and results of the haplotype analysis was in the Methods section (see Page 8, line 139-147) and the Results section (see Page 9, line 165-174). The haplotype analysis result could support our inference that the frequency of this *LIM2* mutation in our congenital cataracts database was 3.1% (4/130).

Comment 6:

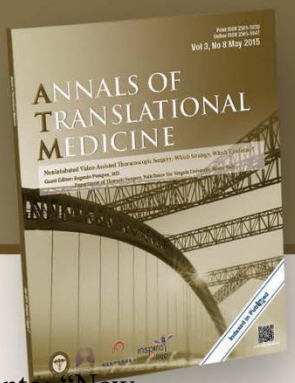
Conclusions, p. 10, lines 185-186, "This mutation might be a hot point for Chinese patients with congenital cataracts.": The meaning of this is unclear. Perhaps it might be better simply to say that the mutation appears to be a frequent cause of cataracts in the Han Chinese population.

Reply 6:

Thank you. We have modified our text as advised (see Page 4-5, line 54-55, Page 12, 228-229).

Comment 7:

Conclusions, p. 10, lines 16-189, "New phenotypes...*LIM2* mutations.": See comment 3 above. Association of these phenotypes with the mutation would be better if the occurrence were consistent in all patients. It might be better to say that the occurrence of these phenotypes in multiple members of two of the families suggests that they might be associated with the *LIM2* mutation.



Reply 7:

Thank you. Following our response in comment 3, we modified this sentence into: "New phenotypes detected in the four families included elongated axial length, myopia related fundus anomalies (leopard-shaped fundus, patchy chorioretinal atrophy and optic disc change), congenital nystagmus, esotropia, PFV and eyeball atrophy. Of them, elongated axial length and/or myopia related fundus anomalies were consistently present in all available patients. The occurrence of other phenotypes, including congenital nystagmus, esotropia, and eyeball atrophy in multiple members of two or three of the families might be complications associated with the elongated axial length" (see Page 12, line 229-236).

Comment 8:

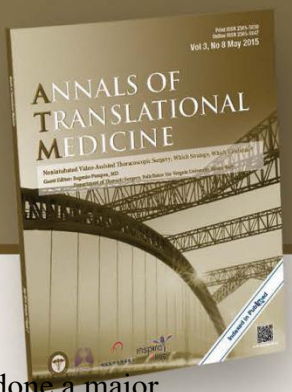
Conclusions, p. 11, lines 209-211, "The *LIM2* gene mutation detected in this study, p.Arg130Cys, was the only autosomal dominant mutation of *LIM2* associated with congenital cataract in humans, which indicated a dominant-negative effect.": Actually, any mutation causing a gain of a deleterious function would cause a dominant disease. Dominant-negative effects occur when the mutant gene product interacts with the remaining wild type protein and inhibits or inactivates it in some fashion. While *LIM2* does form hexamers, it is unclear whether this mutation acts through a dominant negative mechanism.

Reply 8:

Thanks for your valuable advice. Without a pathological mechanism study, it was indeed unclear whether this mutation acted through a dominant negative mechanism. It is also a limitation of this study. Further basic medical studies are needed to clarify the pathogenic mechanism of this mutation in the development of lens and eyeballs. We deleted the description in the Discussion section and added related limitation description as following: "The other limitation was the lack of basic experiments on the pathological mechanism of the relationship between cataracts, myopia and this mutation. The underlying molecular mechanism between this mutation in the LIM2 gene and myopia remains unknown. The function of the LIM2 gene might be related with the increased remodeling and creep rate of scleral matrix during the embryonic and postnatal period (49). It is necessary to further study the relationship between the p.Arg130Cys mutation in the LIM2 gene and myopia by cell or animal models" (see Page 14, line 275-282).

Comment 9:

General: While the manuscript is understandable, there are numerous problems with English usage that would benefit from further editing for usage and grammar.



Reply 9:

Thank you. We apologized for the poor language in the manuscript. We have done a major revision of this manuscript and carefully proof-read it to minimize typographical and grammatical errors. We have also involved native English speakers for language correction. We really hope that the flow and language level have been substantially improved.

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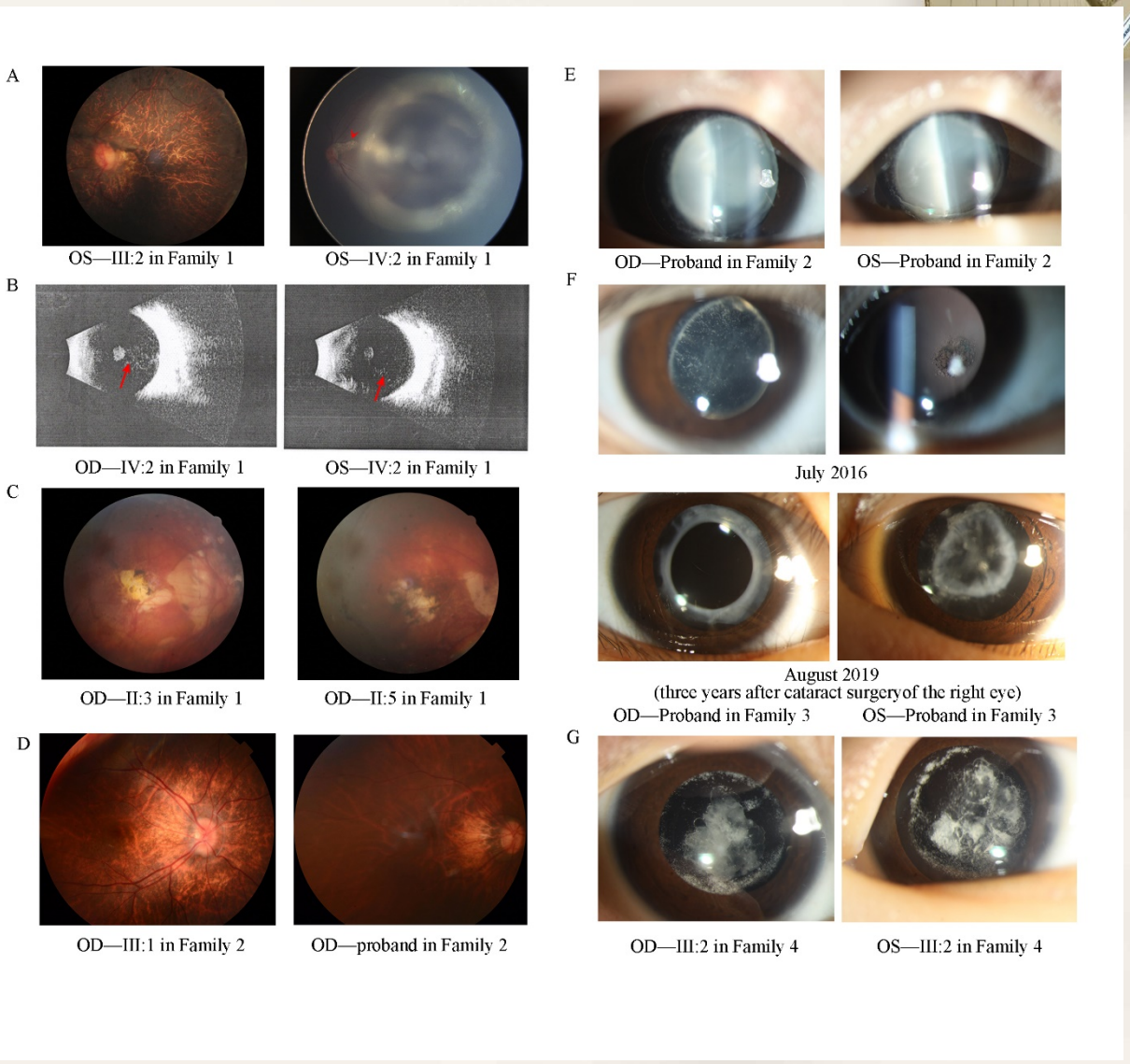

Figure 2. Ocular phenotypes of patients with congenital cataracts in the four families. A: Left panel: fundus photo of the left eye of III:2 in Family 1 showed severe leopard-pattern changes and an oval and tilt optic disc. Right panel: fundus photo of the left eye of IV:2 in Family 1 showed persistent fetal vasculature and posterior patchy chorioretinal atrophy (red arrow head). B: B scan of IV:2 in Family 1 showed fibrovascular stalk from the optic disc to the lens posterior capsule (red arrow). C: Fundus photo of II:3 and II:5 in Family 1, showed posterior patchy chorioretinal atrophy. D: Fundus photo of II:2 and III:1 (the proband) in Family 2, showed leopard-shaped fundus and optic disc changes. E: The total cataract of III:1 in Family 2. F: The development of cataract type from posterior subcapsular cataract to perinuclear cataract of II:1 (the proband) in Family 3. G: The irregular lamellar cataracts surround by pulverulent cortical opacity of III:2 in Family 4.

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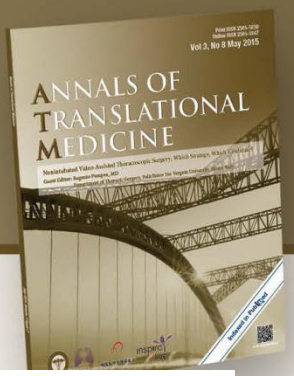

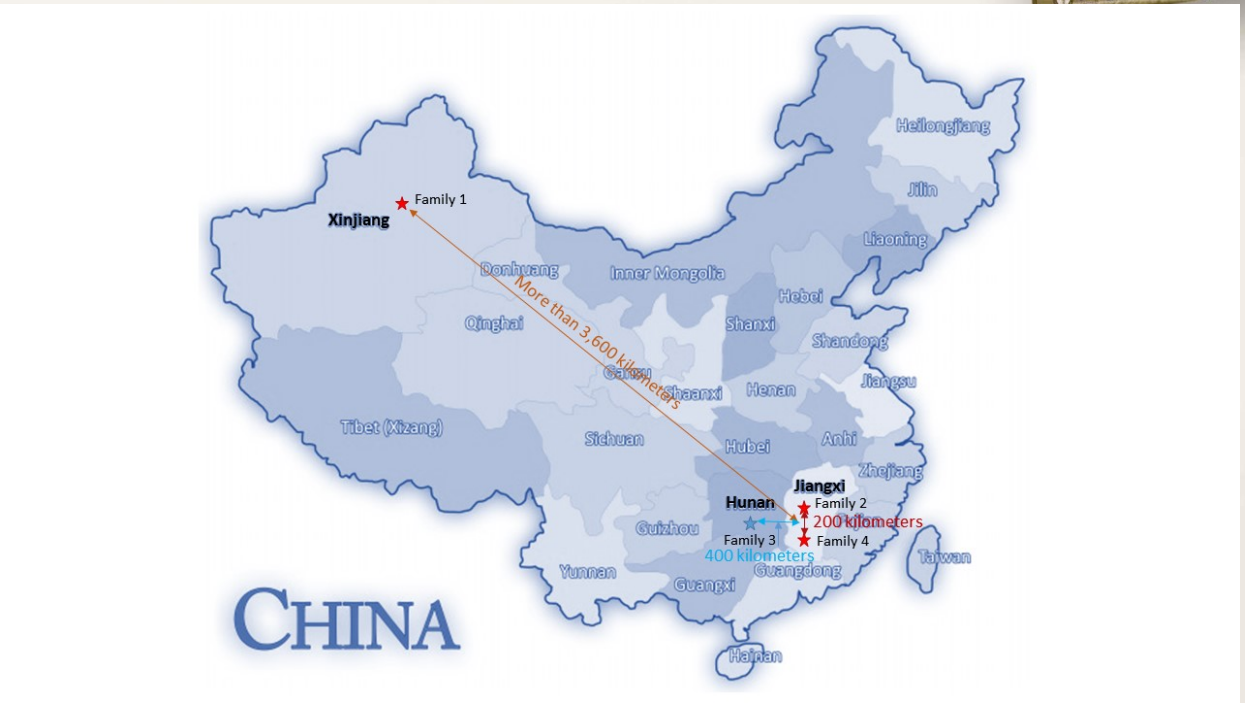

Figure S1. The geographical positions of Family 1, 2, 3 and 4. The position of Family 1 was in Urumqi, Xinjiang province. The position of Family 2 was in Jian, Jiangxi province. The position of Family 3 was in Changning, Hunan province. The position of Family 4 was in Ganzhou, Jiangxi province. The geographical distance between Family 1 and Family 2&4 is more than 3,600 kilometers. The geographical distance between Family 3 and Family 2&4 is around 400 kilometers. The geographical distance between Family 2 and Family 4 is around 200 kilometers.

Table 1. Clinical phenotypes of patients in the four families with the p.Arg130Cys mutation in *LIM2*.

ndividual ID	Inheritance patterns	Sex	Age at (year)		Lens morphology	Axis length at last-time examination (OD/OS mm)	Elongated axial length	Myopia related fundus changes			Others
			Presentation	On-set				leopard-shaped fundus	patchy chorioretinal atrophy	optic disc changes （tilt/torsion/PPA）	
amily 1-II:3	AD	M	65y	<10y	cataract OU	32.42/31.68	+	+	+	+	CN O
amily 1-II:5		M	62y	<10y	cataract OU	31.85/32.69	+	+	+	+	-
amily 1-III:2		M	35y	5y	cataract OU	NA/23.86	-	+ (OS)	-	+ (OS)	Eyeba atroph OD
amily 1-IV:1		M	4y5m	4m	nuclear cataract surrounded by pulverulent cortical opacity OU	23.17/23.07	+	+	-	+	CN OU; I OS
amily 1-IV:2		M	1y8m	7m	lamellar cataract with relatively transparent central area, surround by pulverulent cortical opacity OU	NA/NA	NA	-	+	+	PFV OU, CN OU, I OS
amily 2-roband	AD	M	3y9m	6m	nuclear cataract surrounded by pulverulent cortical opacity OU	25.43/25.57	+	+	-	+	-
amily 2-II:2		M	34y	infancy	cataract OU	27.88/NA	+ (OD)	+ (OD)	-	+ (OD)	Eyeba atroph OS, CN O
amily 3-roband	SC	F	4y8m	2y	pulverulent cataract OD, posterior polar cataract OS	21.20/21.13	-	+	-	+	-
amily 4-roband	AD	F	5y1m	7m	nuclear and posterior polar cataract OU	24.01/24.03	+	+	-	+	CN OU, AE O
amily 4-II:2		M	29y	10y	cataract OU	26.63/27.05	+	+	-	+	CN O

amily 4-III:2	M	11m	5m	irregular lamellar cataract surround by pulverulent cortical opacity OU	21.10/21.07	+	+	-	+	-

Footnote: AD=autosomal dominant inheritance; SC=sporadic case; +=*LIM2* mutation exists; -=*LIM2* mutation does not exist; M=male; F=female; y=years; m=months; OD=the right eye; OS=the left eye; OU= both eyes; NA=not available; PPA=parapapillary atrophy; CN= congenital nystagmus; IE= intermittent esotropia; PFV= persistent fetal vasculature; AE= alternating esotropia.

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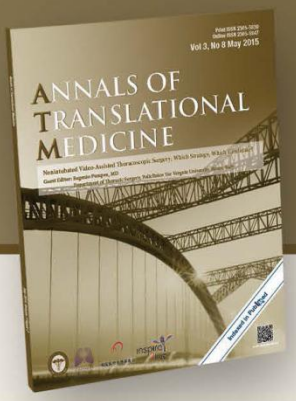

Table S1. Genomic information of the seven persistent fetal vasculature associated genes.

Gene	Cytogenetic Locus	gDNA	mRNA	Protein
NFKB1	4q24	NC_000004.11	NM_003998	NP_003989
VEGFA	6p21.1	NC_000006.11	NM_001025366	NP_001020537
EDN1	6p24.1	NC_000006.11	NM_001955	NP_001946
CDKN2A	9p21.3	NC_000009.11	NM_000077	NP_000068
ATOH7	10q21.3	NC_000010.10	NM_145178	NP_660161
TGFB1	19q13.2	NC_000019.9	NM_000660	NP_000651
NDP	Xp11.3	NC_000023.10	NM_000266	NP_000257

Note: The human genome information was based on NCBI build 37/hg19.

Table S2. The list of the SNPs and the haplotype analysis of the probands in Family 1, 2 and 4.

#Chromsome	Position	Intermarker distance (bp)	SNP ID	Haplotype of the mutant *LIM2* allele		
				F1-proband	F2-proband	F4-proband
19	51,608,074	-	rs2691249	C	C	A
19	51,689,700	81,626	rs12461542	T	C	C
19	51,837,332	147,632	rs10418551	G	G	G
19	51,883,831	46,499	Mutation	A	A	A
19	52,032,964	149,133	rs3976745	G	G	C
19	52,095,980	63,016	rs3794984	T	T	G
19	52,130,337	34,357	rs8105105	C	T	T
Distance of both ends of the shared haplotypes (bp)				258,648	406,280	194,131

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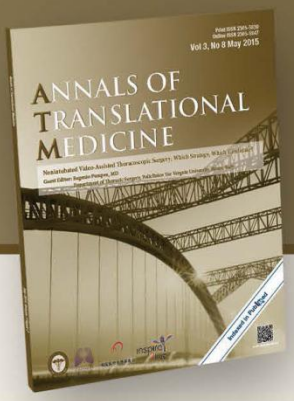

Note: Yellow highlighted regions in haplotypes were the haplotypes shared between the three families. The human genome information of SNPs was based on UCSC NCBI build 37/hg19.

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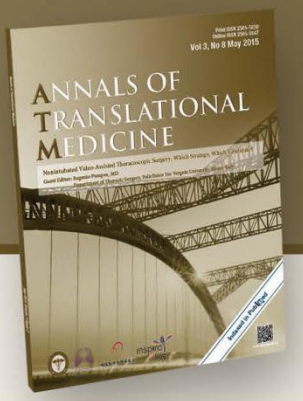

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