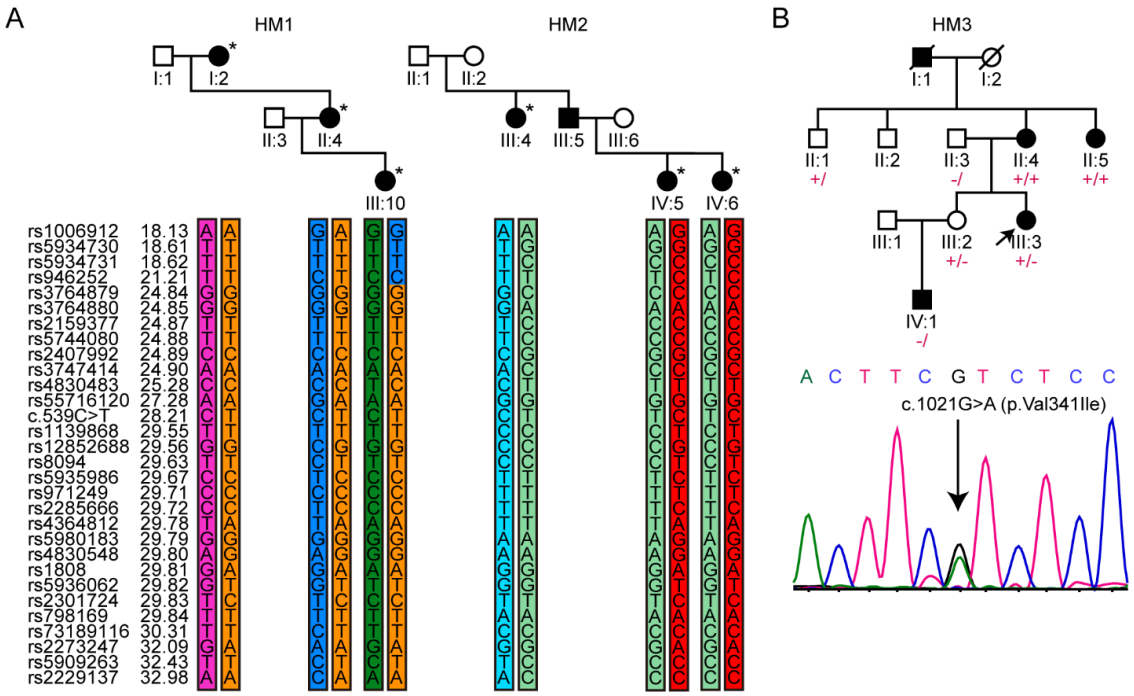
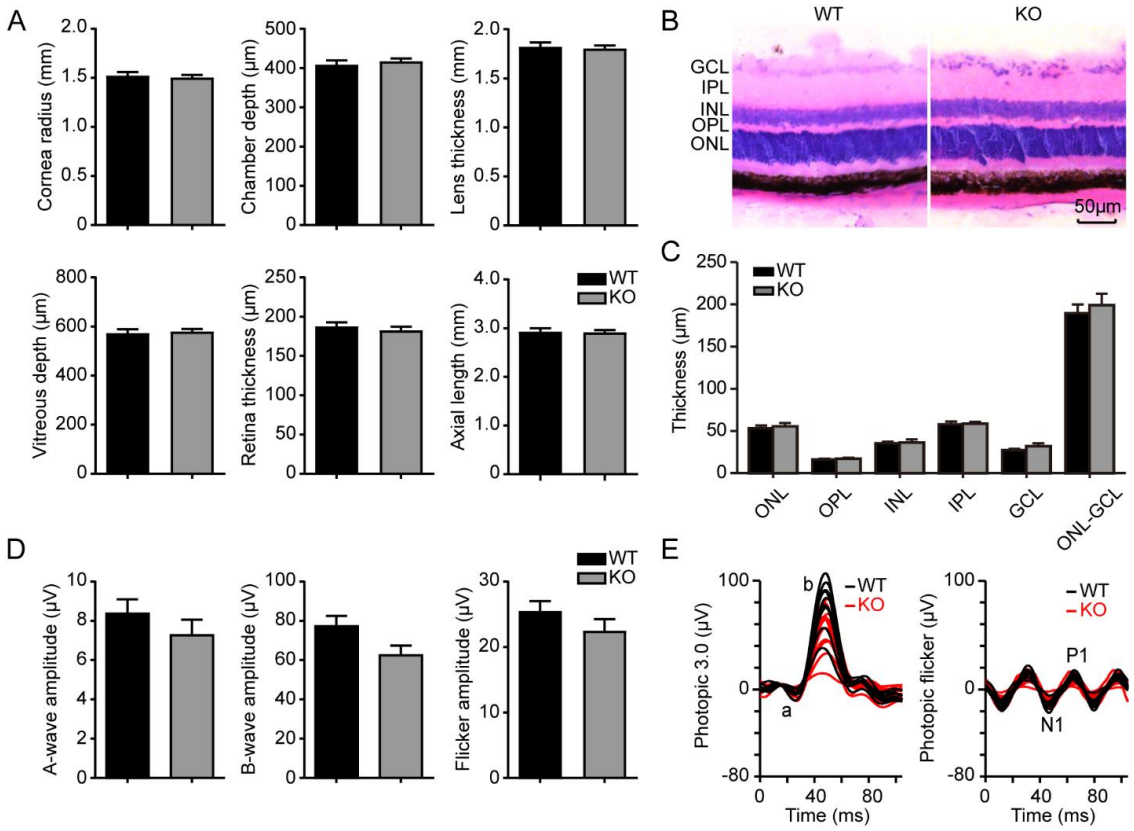
Online Supplemental material

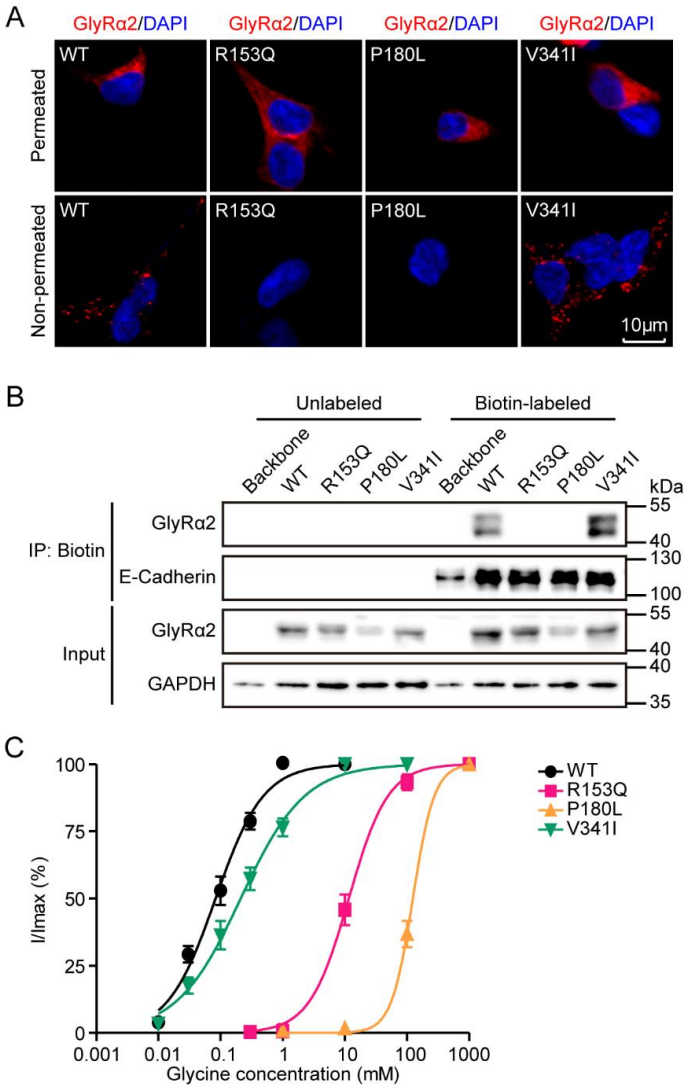
Supplemental Figures and legends



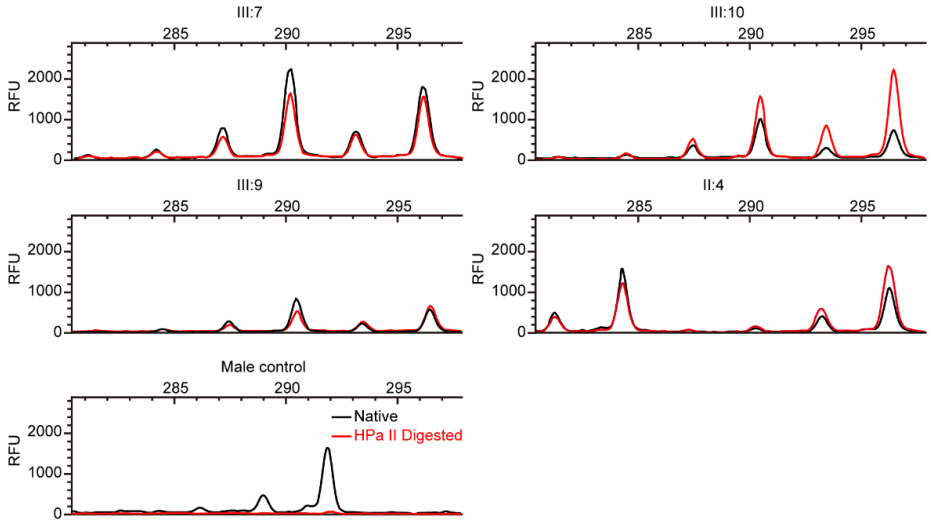
Supplemental Figure 1. Haplotype of HM1 and HM2 families and *GLRA2* variants detected in HM3 family. (A) The haplotype of HM1 and HM2 families shows no founder effect. (B) Co-segregation status of variant c.1021G>A (p.Val341Ile) in HM3 family. □' and '○' symbols present asymptomatic male and female subjects, respectively; '■' and '●' characters stand for male and female patients, respectively. Samples selected for ES were marked with '*'. '+' stands for wild-type allele and '-' refers to c.1021G>A variant in *GLRA2* gene.



Supplemental Figure 2. Assessment of ocular parameters, retinal stratification, and photopic-electroretinography response of *Glra2* knockout mice. (A) Normalized ocular parameters measured using optical coherence tomography. (B) Retinal stratification observed using hematoxylin and eosin staining. (C) Statistical results of (B). (D, E) Light-adapted electroretinography responses of *Glra2* knockout and wild-type mice. *Glra2* knockout mice show a decreased amplitude of photopic 3.0 a and b waves and flicker response, but the differences have no statistical significance. Data are represented as mean±SEM.



Supplemental Figure 3. Functional assessment of HM-related variants. (A) Immunofluorescent staining of wild type and mutated plasmids overexpressed in HEK293 cells with anti-GlyRα2. (B) Isolation of biotinylated membrane protein of wild-type and mutated GlyRα2. (C) Electrophysiological characteristics of wild-type and mutated GlyRα2.



Supplemental Figure 4. X-inactivation proportion of asymptomatic subject III:9 in HM1 family. The results show no skewed inactivation of X chromosome between subject III:9 and other female patients.

Supplemental tables

Supplemental Table 1. Primers were used in this study.

Primer Name	Primer sequence 5'-3'	Product size	Note
GLRA2-E1F	CCAACTCCCTTTGCATGGTG	631bp	Amplify *GLRA2* exon 1
GLRA2-E1R	CGTTGGCTGTGAAAATGTGTG		
GLRA2-E2F	GTGACGCGACTCAGGATTTA	633bp	Amplify *GLRA2* exon 2
GLRA2-E2R	TCAGCCACACTCCCACTTAC		
GLRA2-E3F	ACTCTTCAGGGTAAGTTGCCA	553bp	Amplify *GLRA2* exon 3
GLRA2-E3R	GAGGCGAGCAAAGTTGGAAA		
GLRA2-E5F	AAAAGCACTGCCCTGAGTTG	680bp	Amplify *GLRA2* exon 5
GLRA2-E5R	CCCTTCCTGCCAGAATTCCT		
GLRA2-E5S	GGGGTTGGTCAGTATATAGGGA	—	*GLRA2* exon 5 sequencing primer
GLRA2-E4F	ACTCGGACACCAAAGCTGTA	488bp	Amplify *GLRA2* exon 4
GLRA2-E4R	GGGACTTCTGACACTCTCCA		
GLRA2-E6F	TGGCTCCAATGACACAGAGT	458bp	Amplify *GLRA2* exon 6
GLRA2-E6R	TTTGAGCCAAATCAGGTCCG		
GLRA2-E7F	CGTAGGGTGAACATTTTGTGC	528bp	Amplify *GLRA2* exon 7
GLRA2-E7R	TTCCCCATGTTCCCAGATCC		
GLRA2-E8F	GCGTGTGACTTTCAGTGCTC	691bp	Amplify *GLRA2* exon 8
GLRA2-E8R	GGAGCCCAGTTACTTCCGAA		
GLRA2-E9F	CCTCCCACACCACCAGTTAA	721bp	Amplify *GLRA2* exon 9
GLRA2-E9R	TGACCCCGCATATCATGTCT		
mGlra2-E2F	AGCAAGTGAGAAAATAAGCATGT	279bp	Amplify *Glra2* knockout allele
mGlra2-E2R	TGCAAGCAAACTCTATCATTGG		
mGlra2-E2Fb	ACTTGGTTGACATTGCTCAGG	440bp	Amplify *Glra2* wild type allele
mGlra2-E2Rb	CAGGGAGGCTGAAATTGTGT		
mSRY-F	GTGACACTTTAGCCCTCCGA	334bp	Amplify sex determine gene
mSRY-R	TAGTGTTCAGCCCTACAGCC		
ARF	HEX-GCTGTGAAGGTTGCTGTTCCTCAT	288bp	ChrX-inactivation detection
ARR	TCCAGAATCTGTTCCAGAGCGTGC		

Supplemental Table 2. Genome-wide multi-point parameter linkage analysis results of HM1 family.

Cytoband	Markers	LOD_Score	Size
	rs7066674	-0.07	
	rs4830891	2.88	
	...		
	rs4825340	2.88	
	...		
	rs6633421	2.88	
Xp22.2-p11.4	...		24.7Mb
	rs4332301	1.89	
	...		
	rs5971622	0.88	
	...		
	rs1801686	0.31	
	rs6609813	-2.07	

Supplemental Table 3. *Glra2* knockout mice ocular biometry measured by OCT.

Measurements	Wild type (mm, mean ±SEM)	Knockout (mm, mean ±SEM)	n, p-value
Cornea thickness	0.1259±0.0037	0.1167±0.0037	p=0.049*, n=8pairs
Cornea radius	1.524±0.0370	1.504±0.0251	p=0.6857, n=8pairs
Chamber depth	0.4086±0.0107	0.4174±0.0064	p=0.5422, n=8pairs
Lens thickness	1.821±0.0440	1.804±0.0296	p=0.7580, n=8pairs
Vitreous depth	0.5731±0.0155	0.5793±0.0102	p=0.7618, n=8pairs
Retina thickness	0.1876±0.0051	0.1825±0.0046	p=0.4658, n=8pairs
Axial length	2.929±0.0721	2.913±0.04755	p=0.8609, n=8pairs

Supplemental Table 4. Photopic 3.0 ERG results of *Glra2* knockout mice.

Measurements	Wild-type (μV, mean ±SEM)	Knockout (μV, mean ±SEM)	n, p-value
Photopic 3.0 a wave	8.386±0.7088	7.284±0.7756	p=0.291, n=11pairs
Photopic 3.0 b wave	77.45±5.081	62.61±4.763	p=0.093, n=11pairs
Photopic 3.0 flicker	25.51±1.516	22.48±1.796	p=0.296, n=11pairs

Supplemental Table 5. Nonsynonymous variants on the *GLRA2* gene were reported previously and in the current study.

No.	Patient_ID	Sex	Variation	Inheritance	Phenotype
1	12724	F	c.16G>C[1]	de novo	Autism.
2	11842	M	c.407A>G[1, 2]	de novo	Autism.
3	Patient 2	M	c.458G>A[2]	de novo	Non-syndromic autism, severe language delay with functional language, mild intellectual disability, generalized tonic-clonic seizures; But his autistic elder sister does not carry the variant.
4	AGP: 6323_3	M	E8-9del[2, 3]	Maternal	Autism, average IQ, language delay, bilateral HM, normal physical exam, no epilepsy; His mother and maternal grandfather are also myopic.
5	S00125-ASD-GT	F	c.1049G>T[4, 5]	Maternal	Autism.
6	M21227	M	c.458G>A[a]	NA	HM.
7	HM1-II:4 HM2-III:5	F	c.539C>T[a]	Familial	Among these two families, 18 patients with HM, two heterozygotes with normal phenotype.
8	HM3-III:3	F	c.1021G>A[a]	Familial	Two heterozygotes with HM, two heterozygotes with normal phenotype, and two patients without the variant.

a, this study; NA, not available

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