IJMCM
Summer 2019, Vol 8, No 3
DOI: 10.22088/IJMCM.BUMS.8.3.179

Original Article

Identification of PROS1 as a Novel Candidate Gene for Juvenile Retinitis Pigmentosa

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Retinitis Pigmentosa (RP)

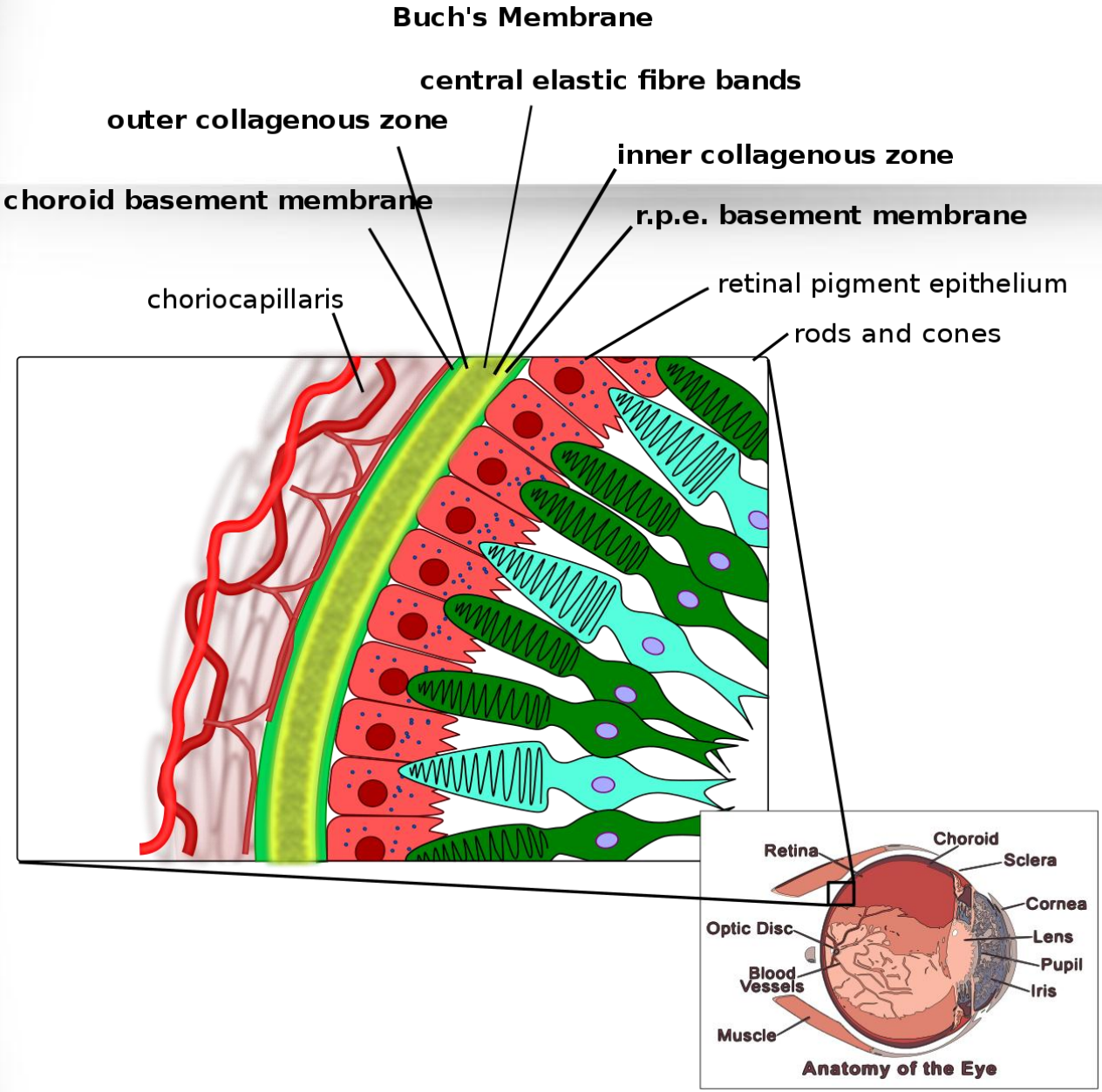
- Heterogeneous group of genetic disorders.
- Degeneration of rod and cone photoreceptors.
- The earliest clinical manifestation of RP is night blindness, progressing to reduction in mid-peripheral visual field.
- As the disease becomes worse, the patients develop tunnel vision and eventually lose central vision.

Retinitis Pigmentosa (RP)

- 1 in 3500-4000 in most populations.
- Most cases result from one of a series of monogenic disorders inherited in an autosomal-dominant, autosomal-recessive, or X-linked manner.
- Oligogenic inheritance and mitochondrial inheritance have been established in a small proportion of RP cases.

In clinical practice

- Electroretinogram (ERG)
- Optical coherence tomography (OCT)
- Fundus Autofluorescence (FAF) imaging
- Retinal Pigmented Epithelium (RPE)/Bruch's membrane complex thinning

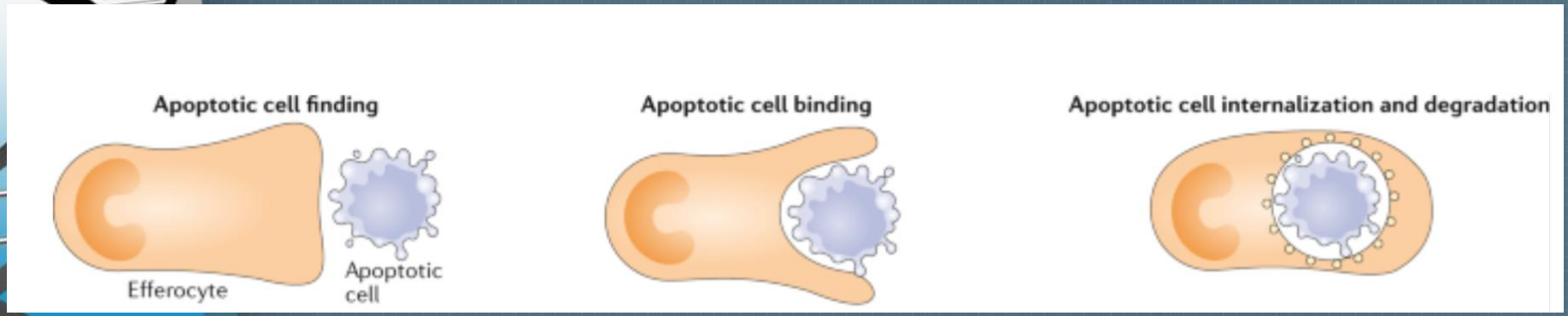

Buch's Membrane
central elastic fibre bands
outer collagenous zone
inner collagenous zone
choroid basement membrane
r.p.e. basement membrane
retinal pigment epithelium
choriocapillaris
rods and cones
Retina
Choroid
Sclera
Cornea
Optic Disc
Lens
Pupil
Blood Vessels
Iris
Muscle
Anatomy of the Eye

Retinal Pigmented Epithelium Task

- Nourishing retinal, light sensitive cells.
- Phagocytosis of outer segment (OS) membrane debris

and

- Clearance of apoptotic cells, a phenomenon known as efferocytosis.



The protein S (PS) gene (PROS1)

- Protein S is a vitamin K-dependent plasma glycoprotein synthesized in the liver.
- The best characterized function of Protein S is its role in the anti coagulation pathway, where it functions as a cofactor to Protein C in the inactivation of Factors Va and VIIIa.
- PROS1 variants, with loss of function effect, might be associated with an increased risk of thrombosis.
- Heterozygotes are at risk of recurrent venous thrombosis and cardiovascular accidents during adolescence.

The protein S (PS) gene (PROS1)

- Homozygotes suffer from purpura fulminans during infancy necessitating fresh frozen plasma administration.
- PROS1 encodes a peculiar and biologically relevant ligand of Tyro3/Axl/Mer (TAM) receptors
- Promoting efferocytosis meticulously in high oxygen consumptive tissues like RPE cells.

- It is hypothesized that the proper visual function requires the correct functioning of TAM receptors and their downstream pathways where PS plays a role as well.

The protein S (PS) gene (PROS1)

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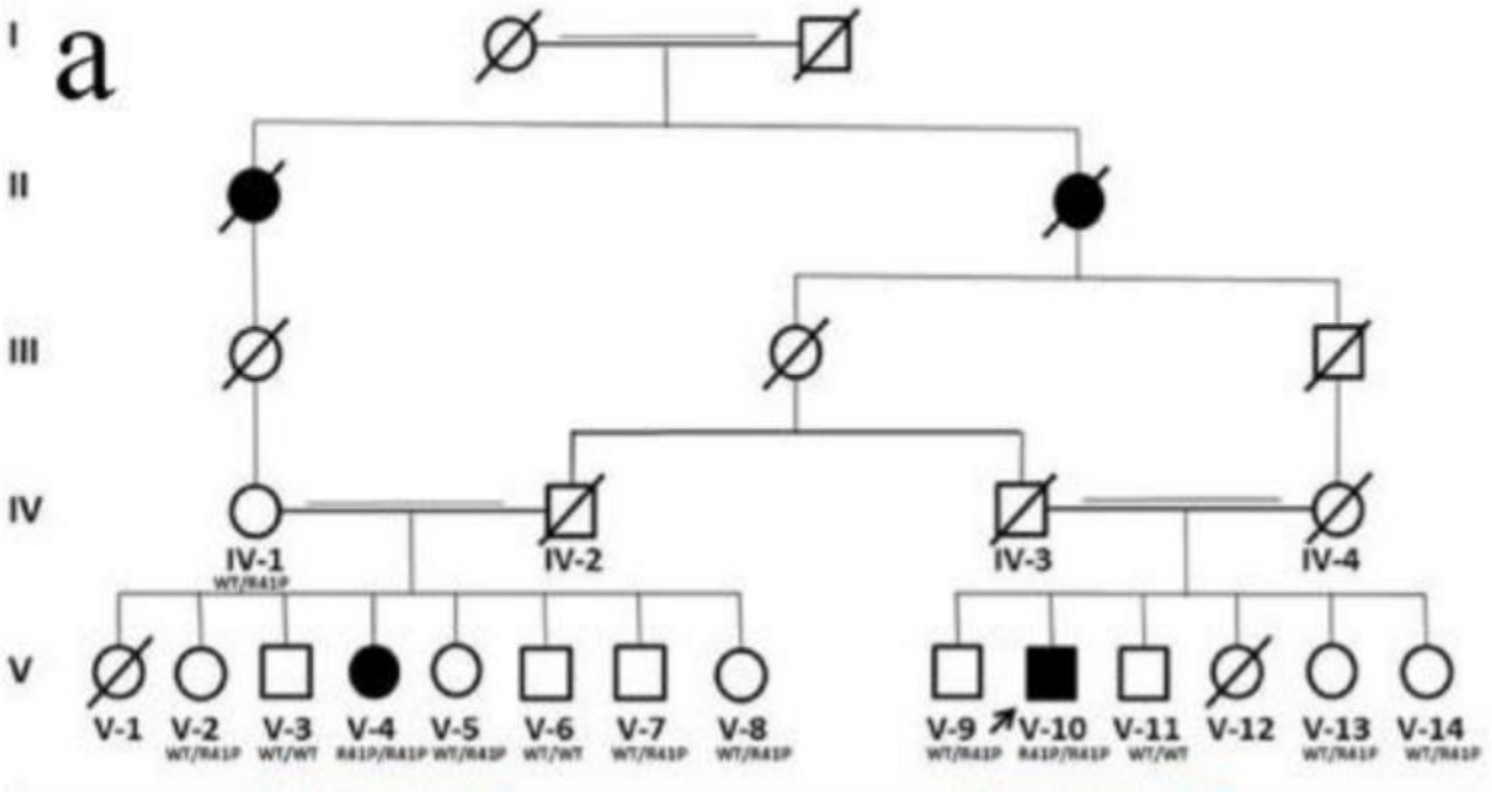
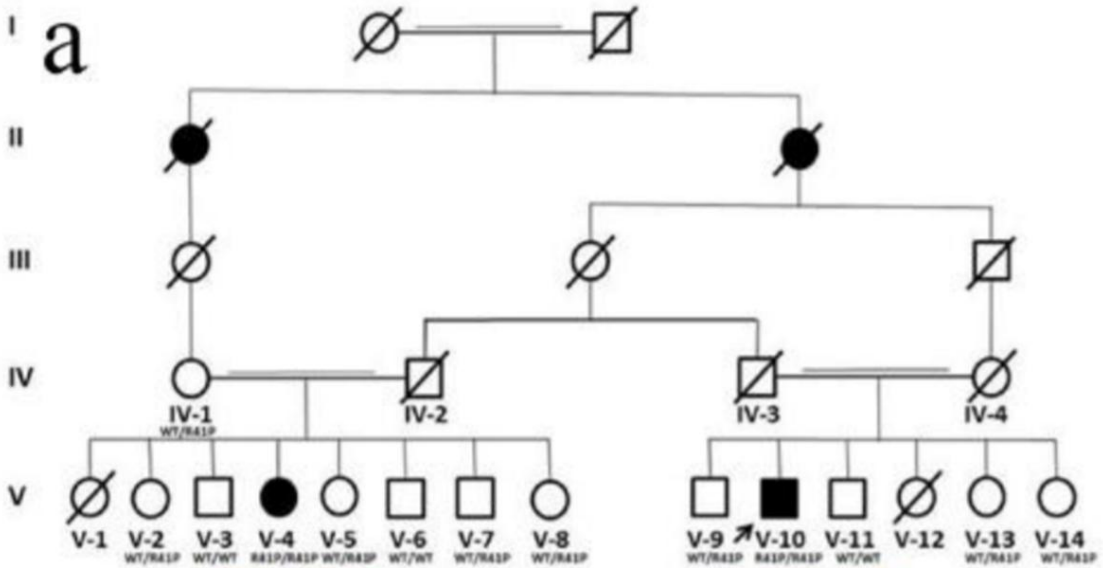
In this study

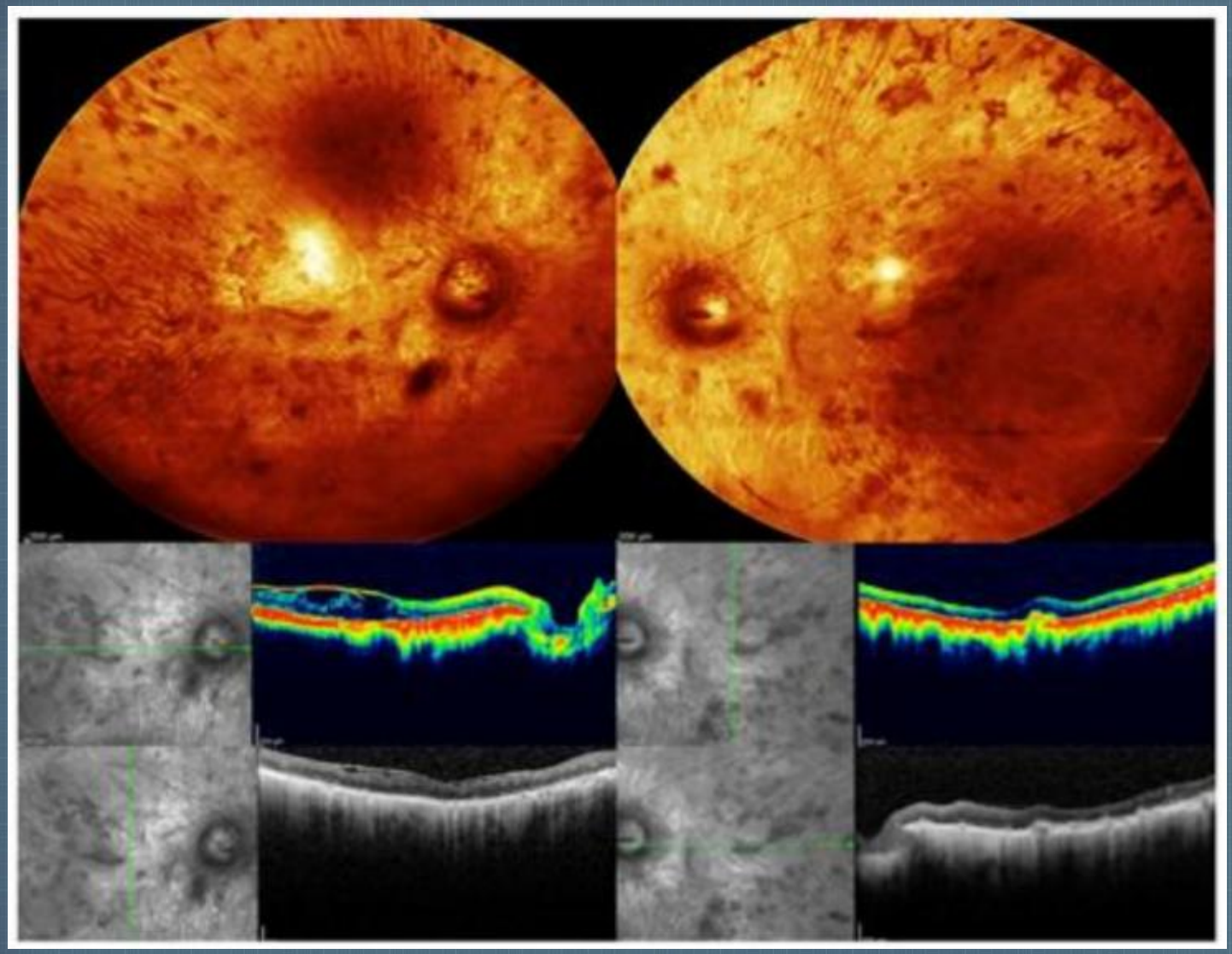
- A novel homozygous missense variant in PROS1 gene
- Deleterious variant p.R41P
- Two unrelated consanguineous families associated with non- syndromic RP

Family A



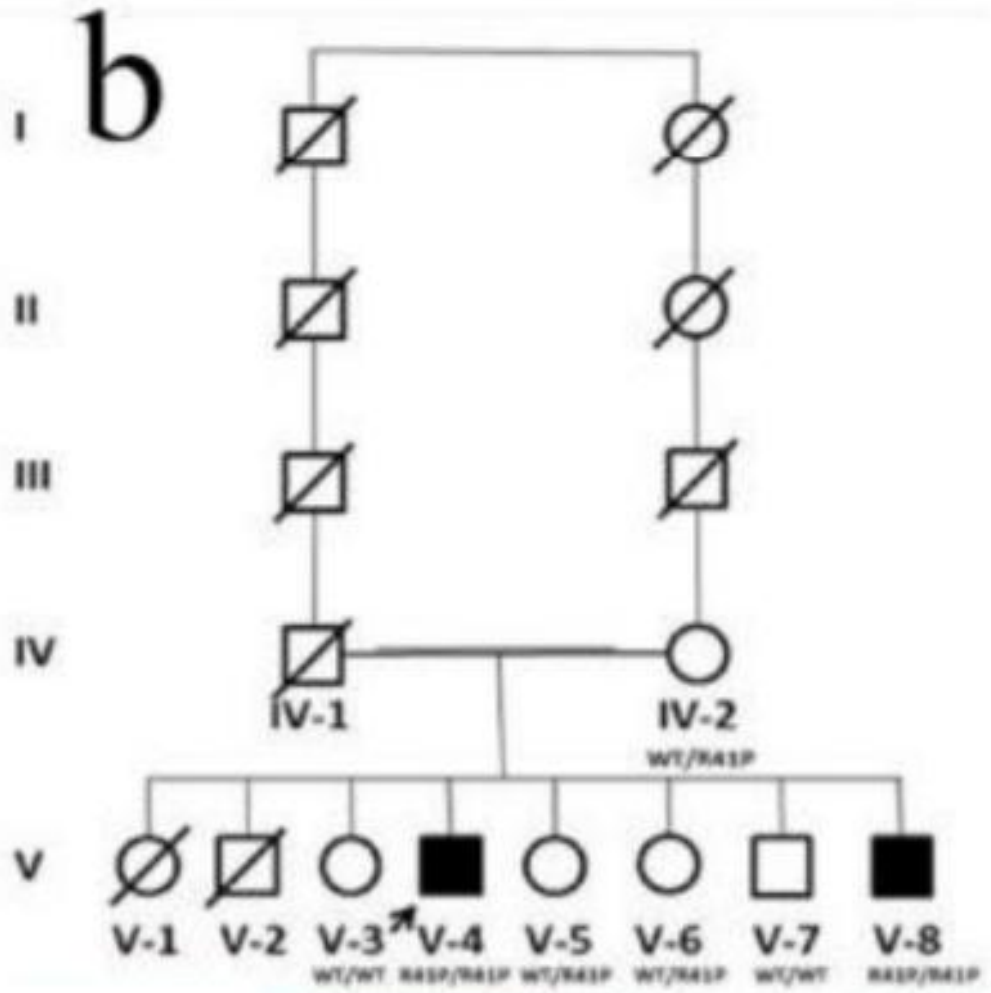


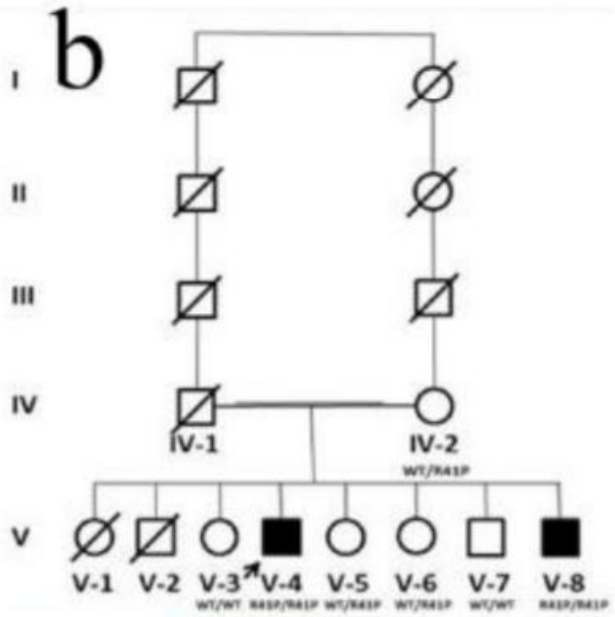
No.	Status	*PROS1*:NM_000313:exon2:c.G122C:p.R41P
V-10	Proband	
V-4	Affected cousin	
V-2	Non-affected sister	
V-3	Non-affected brother	
V-5	Non-affected sister	
V-6	Non-affected brother	
V-7	Non-affected brother	
V-8	Non-affected sister	
V-9	Non-affected brother	
V-11	Non-affected brother	
V-13	Non-affected sister	
V-14	Non-affected sister	
IV-1	Non-affected mother	



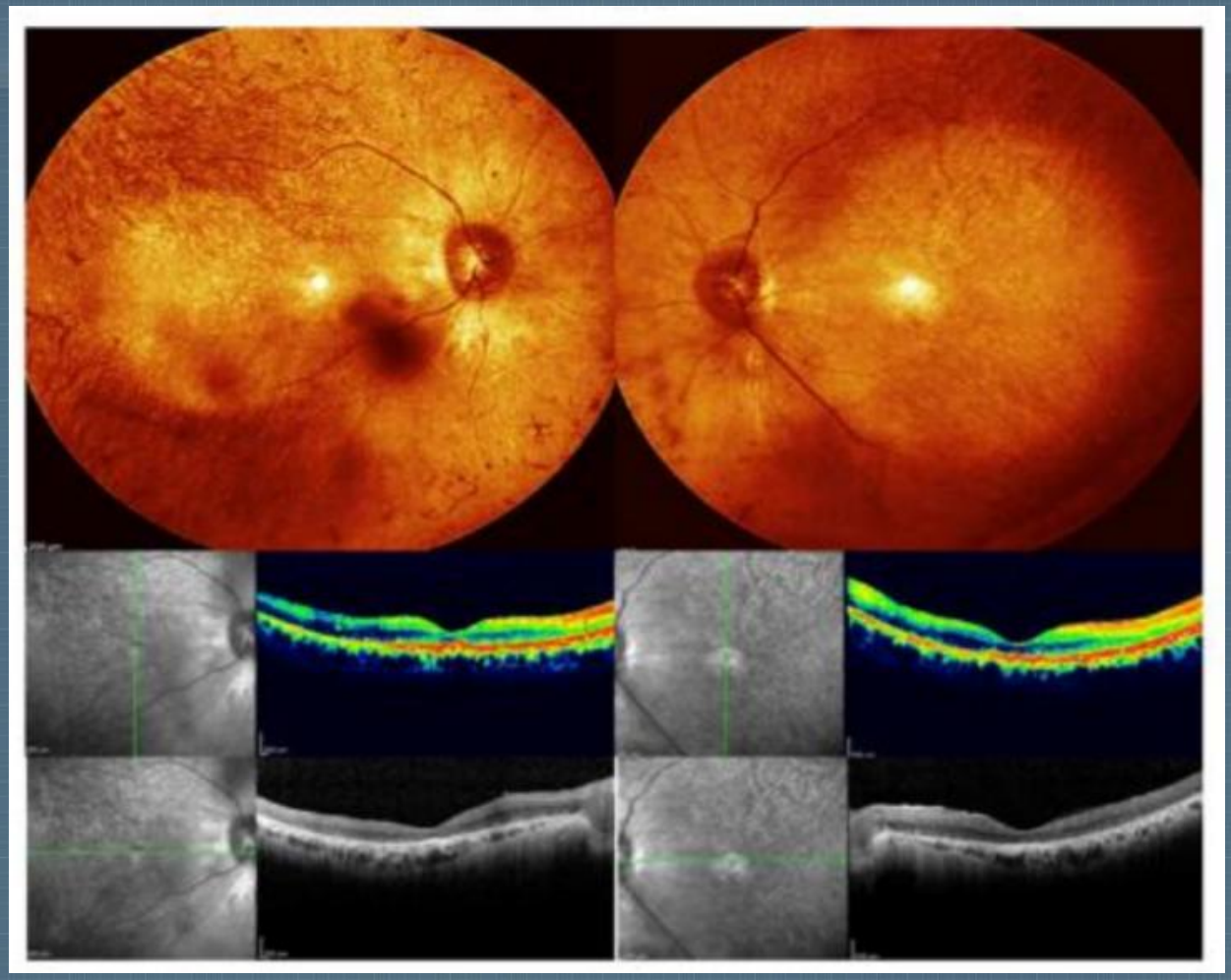
Retinal imaging of patient V-10 (proband) from family A

Family B





No.	Status	*PROS1*:NM_000313:exon2:c.G122C:p.R41P
V-4	Proband	
V-8	Affected brother	
V-3	Non-affected sister	
V-5	Non-affected sister	
V-6	Non-affected sister	
V-7	Non-affected brother	
IV-1	Non-affected mother	



Retinal imaging of patient V-4 (proband) from family B

Table 1. Clinical description of the patients

Family.Subject ID/Gender. Age at last review	A.V-10/M.62	A.V-4/F.60	B.V-4/M.50	B.V-8/M.42
Symptom (Onset)	IDA (9) NB (10) TVF (mid 20s) VAD (28) CS (28) Photophobia Photopsia	NB (11) TVF (mid 20s) VAD (late 20s) CS (30) Photophobia Photopsia	NB (early teen ages) TVF (early 20s) VAD (mid 20s) CS (27) Photophobia Photopsia	NB (11) TVF (early 20s) VAD (26) CS (26) Photophobia Photopsia
VF to confrontation	NA	NA	5°-10° Central	10°-20° Central
BCVA	OD: LP OS: LProj	OD: LProj OS: LProj	OD: 0.001 (HM@2') OS: 0.001 (HM@2')	OD: 0.1 OS: 0.1
Fundoscopy	VDLP Severe RVA Intensive RL Fiddling BSC WODP AFR	VDLP Severe RVA Intensive RL Fiddling BSC WODP AFR	VDLP RVA Diffuse RL Slight BSC WODP AFR	VDLP RVA RL Slight BSC ODP AFR
Additional findings	PSC CV:NA RE: Plano Fixation: NA	PSC CV: NA RE: Plano Fixation: NA	PSC CV: Achromat RE: Plano Fixation: NA	PSC CV: Protanopia RE: Plano Fixation: No
Pattern of functional disorder	RCD	RCD	RCD	RCD
OCT	Retinal atrophy Epi-retinal membrane (OD)	Not performed	Retinal atrophy	Not performed
ERG	Non-recordable	Not performed	Non-recordable	Not performed

AFR: absent foveal reflex; BCVA: best corrected visual acuity; BSC: bone spicule configuration; CS: central scotoma; CV: color vision; HM: hand motion; IDA: impaired dark adaptation; LProj: recognition of light projection; LP: light perception; NA: not applicable; NB: night blindness; OCT: optical coherence tomography; ODP: optic disc pallor; OD: right eye; OS: left eye; PSC: posterior sub-capsular cataract; RE: refractive errors; RL: RPE loss; RVA: retinal vessels attenuation; TVF: Tunnel visual field; VAD: visual acuity disturbance; VDLP: vitreous dust like particles; VF: visual field; WODP: waxy optic disc pallor;

Molecular genetics investigation

- Written informed consent was obtained from all participants.
- Following obtaining 5 ml blood samples from all family members
- Genomic DNA was extracted using salting out method.
- Whole- exome sequencing (WES) was performed on two patients, one from each family.
- Concerning validity, confirmation of candidate variants and segregation analysis were performed on 4 subjects, 2 mothers and 14 non- affected siblings.

Results

- **PROS1:NM_000313:exon2:c.G122C:p.R41P**

It converts

- Arginine (R), which is an amino acid with hydrophilic and amphipathic moiety and contains a highly polar positively charged guandino group,

to

- Proline (P), a hydrophobic amino acid with an exceptional conformational rigidity owing to the distinctive cyclic structure of its side chain.

Options Display: Highlights

PROTEIN S PSEUDOGENE, INCLUDED; PROSP, INCLUDED
PROTEIN S, BETA, INCLUDED; PSB, INCLUDED
PROS2, INCLUDED

HGNC Approved Gene Symbol: PROS1

Cytogenetic location: 3q11.1 *Genomic coordinates (GRCh38): 3:93,873,050-93,973,895* (from NCBI)

Gene-Phenotype Relationships

View clinical synopses as a table

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key
3q11.1	Thrombophilia due to protein S deficiency, autosomal dominant	612336	AD	3
	Thrombophilia due to protein S deficiency, autosomal recessive	614514	AR	3

PheneGene Graphics

TEXT

Description

varsome | PROS1(NM_000313.4):c.122G>C | hg19 | Search | Editions | About | Community | Blog

chr3-93646206-C-G (PROS1:p.R41P)

Link a publication | Classify | Community contributions 1 | Favorites

Variant

Explain

Chromosome	Position	REF Sequence	ALT Sequence	Variant type	Cytoband	HGVS	Gene symbol
chr3	93646206	C	G	SNV	3q11.1	PROS1(NM_000313.4):c.122G>C (p.Arg41Pro)	PROS1

UCSC genome browser | Mastermind | TraP Score

Connect with past and future viewers of this variant...

ACMG Classification - Educational use only Version: 8.4.6

Terms of use | Documentation | Options

Verdict

Likely Pathogenic

Transcript NM_000313.4, canonical, protein length 677, gene PROS1, missense variant

Rules

PVS1	PS1	PS2	PS3	PS4	PM1 Moderate	PM2 Moderate	PM3
PM4	PM5 Moderate	PM6	PP1	PP2 Supporting		PP4	PP5 Supporting
BA1	BS1	BS2	BS3	BS4			
BP1	BP2	BP3	BP4	BP5	BP6	BP7	

Very Strong | Strong | Moderate | Supporting

The verdict will update automatically if you enable or disable rules or change their strength. The blue question marks displays details about the rule, including why it was not triggered.

Users of VarSome Premium benefit from additional data sources included in the automated classification.

Automated criteria

Table 2a. Conserved 18-amino acid propeptide sequences upstream of human vitamin K-dependent (VKD) proteins

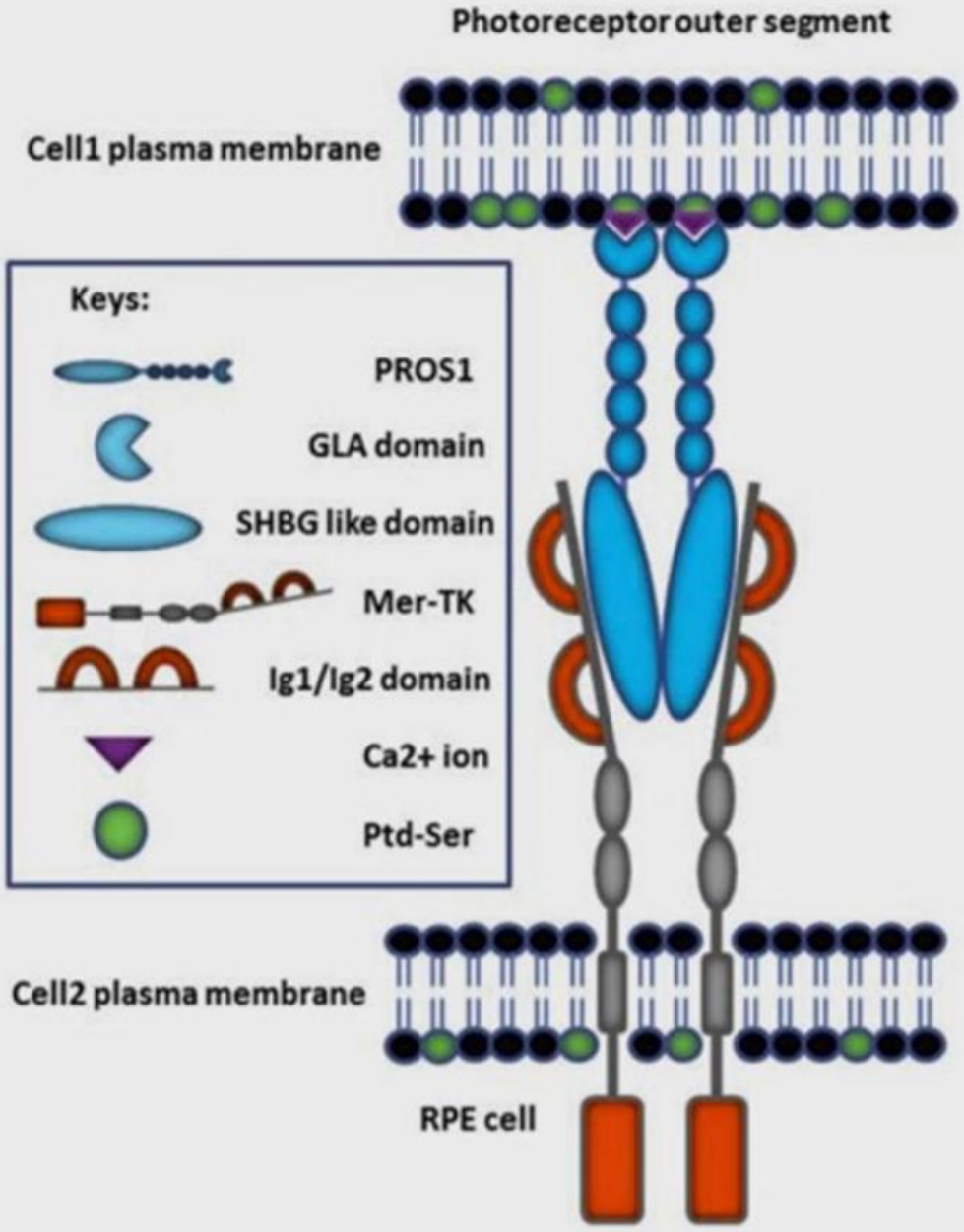
VKD propeptide	-18	-17	**-16**	-15	-14	-13	-12	-11	**-10**	-9	-8	-7	**-6**	-5	-4	-3	-2	**-1**
Protein S	A	N	**F**	L	S	K	Q	Q	**A**	S	Q	V	**L**	V	R	K	R	**R**
Protein C	S	V	**F**	S	S	S	E	R	**A**	H	Q	V	**L**	R	I	R	K	**R**
Factor X	S	L	**F**	I	R	R	E	Q	**A**	N	N	I	**L**	A	R	V	T	**R**
Factor IX	T	V	**F**	L	D	H	E	N	**A**	N	K	I	**L**	N	R	P	K	**R**
Factor VII	R	V	**F**	V	T	Q	E	E	**A**	H	G	V	**L**	H	R	R	R	**R**
Prothrombin	H	V	**F**	L	A	P	Q	Q	**A**	R	S	L	**L**	Q	R	V	R	**R**
PRGP1	R	V	**F**	L	T	G	E	K	**A**	N	S	I	**L**	K	R	Y	P	**R**
Matrix Gla Protein	N	P	**F**	I	N	R	R	N	**A**	N	T	F	**I**	S	P	Q	Q	**R**
Bone Gla Protein	K	A	**F**	V	S	K	Q	E	**G**	S	E	V	**V**	K	R	P	R	**R**

Highly conserved amino acids (F -16, A -10, L -6 and R -1) are highlighted in yellow.

Table 2b. High degree of similarity of propeptide of PS among vertebrates

Propeptide of PS	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	Similarity
Human	A	N	F	L	S	K	Q	Q	**A**	S	Q	V	**L**	V	**R**	K	**R**	**R**	**100%**
Chimpanzee	A	N	F	L	S	K	Q	Q	**A**	S	Q	I	**L**	V	**R**	K	**R**	**R**	**94%**
Gorilla	A	N	F	F	S	K	Q	Q	**A**	S	Q	V	**L**	V	**R**	K	**R**	**R**	**94%**
Marmoset	A	N	F	L	S	K	Q	Q	**A**	S	Q	V	**L**	I	**R**	K	**R**	**R**	**94%**
Mouse	T	N	F	L	S	K	E	R	**A**	S	Q	V	**L**	V	**R**	K	**R**	**R**	**83%**
Sheep	A	N	F	L	S	R	Q	H	**A**	S	Q	V	**L**	V	**R**	R	**R**	**R**	**83%**
Armadillo	D	S	I	L	S	K	Q	Y	**A**	S	Q	V	**L**	F	**R**	K	**R**	**R**	**72%**
Chicken	A	T	F	L	S	H	Q	Y	**A**	S	E	F	**L**	A	**R**	K	**R**	**R**	**67%**
Chinese turtle	M	F	L	L	S	Q	Q	Y	**A**	S	E	F	**L**	V	**R**	K	**R**	**R**	**61%**
Xenopus	R	T	F	L	S	P	Q	Y	**A**	S	E	F	**L**	N	**R**	R	**R**	**R**	**56%**
Zebrafish	Q	R	F	L	P	Q	S	K	**A**	S	E	F	**L**	L	**R**	H	**R**	**R**	**44%**
Asian bonytongue	Q	H	F	L	Q	Q	S	T	**A**	L	Q	F	**L**	A	**R**	R	**R**	**R**	**44%**
Big-finned mudskipper	S	L	F	L	G	R	S	S	**A**	S	Q	F	**L**	S	**R**	Q	**R**	**R**	**44%**

Highly evolutionary conserved amino acids (A -10, L -6, R -4, -2 and -1) are highlighted in yellow.

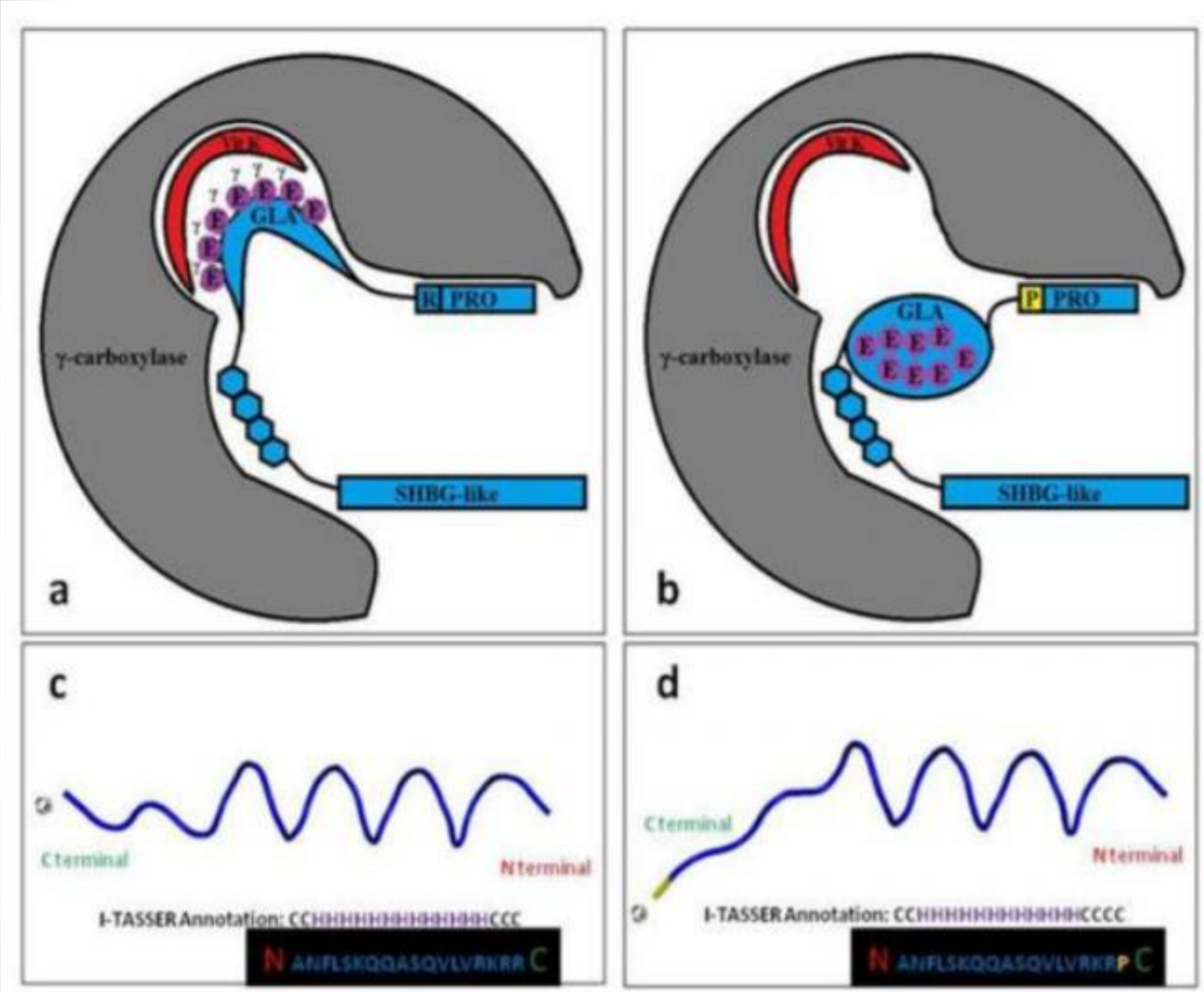

Photoreceptor outer segment
Cell1 plasma membrane
Keys:
PROS1
GLA domain
SHBG like domain
Mer-TK
Ig1/Ig2 domain
Ca2+ ion
Ptd-Ser
Cell2 plasma membrane
RPE cell

PtdSer-PS-MerTK

- PtdSer-PS-MerTK signaling clears apoptotic cells and debris, and prohibits the chronic immune response.
- Mutations of MERTKgene, encoding MerTK protein, are known as the cause of inherited RP (RP38; OMIM: 613862).
- Defect in activation of MerTk results in efferocytosis failure of RPE cells.
- As revealed, TAM triple knockout mice (TKO) developed visual impairment in adulthood. Intriguingly, the phenotype of TKO infant mice are significantly similar to that of wild types

PtdSer-PS-MerTK

- AsTAM receptors expression is dramati- cally increased after birth and kept at high levels in adulthood, it indicates its critical role in postnatal maintenance of visual function.
- In fact, elimination of MerTK phosphorylation causes adult onset retinal degeneration through accum- ulation of debris and subsequent innate immunity inducement.


γ-carboxylase
GLA
R PRO
SHBG-like
a
γ-carboxylase
GLA
P PRO
SHBG-like
b
c
Cterminal
Nterminal
I-TASSER Annotation: CCHHHHHHHHHHHHHHCCC
N ANFLSKQQASQVLVRKRR C
d
Cterminal
Nterminal
I-TASSER Annotation: CCHHHHHHHHHHHHHCCCC
N ANFLSKQQASQVLVRKRP C

- It has been cleared that the retina and particularly RPE cells have the highest metabolic expenditure among body tissues, far higher than brain and kidney in terms of oxygen consumption levels.

- inadequate function of PS at demanding RPE, ensuing cumulative failure of MerTK mediated efferocytosis, pursuant progressive expansion of innate immunity in the retina and subsequent drusen formation in Bruck’s membrane finally result in adult onset rod-cone dystrophy.

- Previous reports have noted the existence of retinopathy accompanied with recurrent venous thromboses in patients with PS deficiency, mainly due to thrombosis-mediated retinal veno-occlusion, starting very early in utero.

- In the case of our patients, it is hypothesized that the mechanism of pathogenicity is different. In fact, degeneration of photoreceptors begins sometime after birth due to dysregulation of innate immunity as a sequel of impaired efferocytosis.

Taken together

- Based on co-segregation of this mutation, particularly in two unrelated families, an intriguing similar RP-like phenotype and the intimate interaction of PS and MerTK in maintaining photoreceptor and RPE homeostasis, led the role of some mutations in the PROS1 gene in the pathogenesis of RP to be raised.