Functional Genomic Interactions in a Congenital Blinding Disease in the Developing World

POSTER No. 28

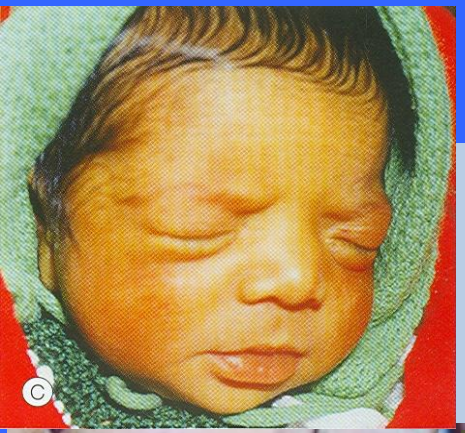
Subhabrata Chakrabarti, Ph.D.

Champalimaud Translational Eye Research Centre
LV Prasad Eye Institute
Hyderabad, INDIA
Email: SUBHO@LVPEI.ORG

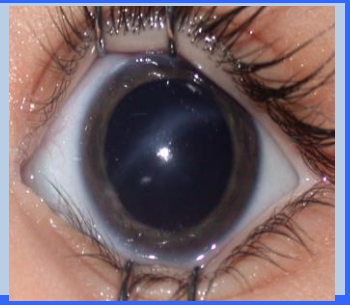
PRIMARY CONGENITAL GLAUCOMA

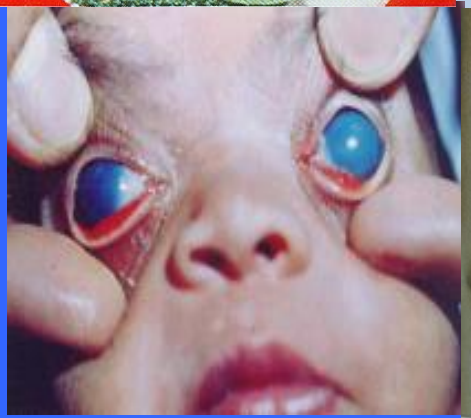
A severe form of childhood blindness

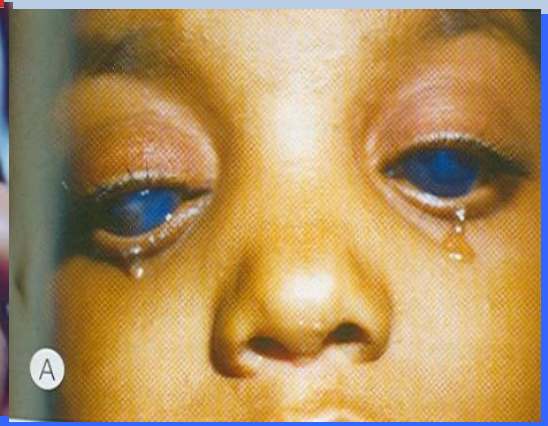
Developmental defect(s) of the trabecular meshwork and anterior chamber angle



Western countries	- 1: 10,000-30,000
Middle East	- 1: 2,500
Slovak gypsies	- 1: 1,250
India (Andhra Pradesh)	~ 1: 3,300

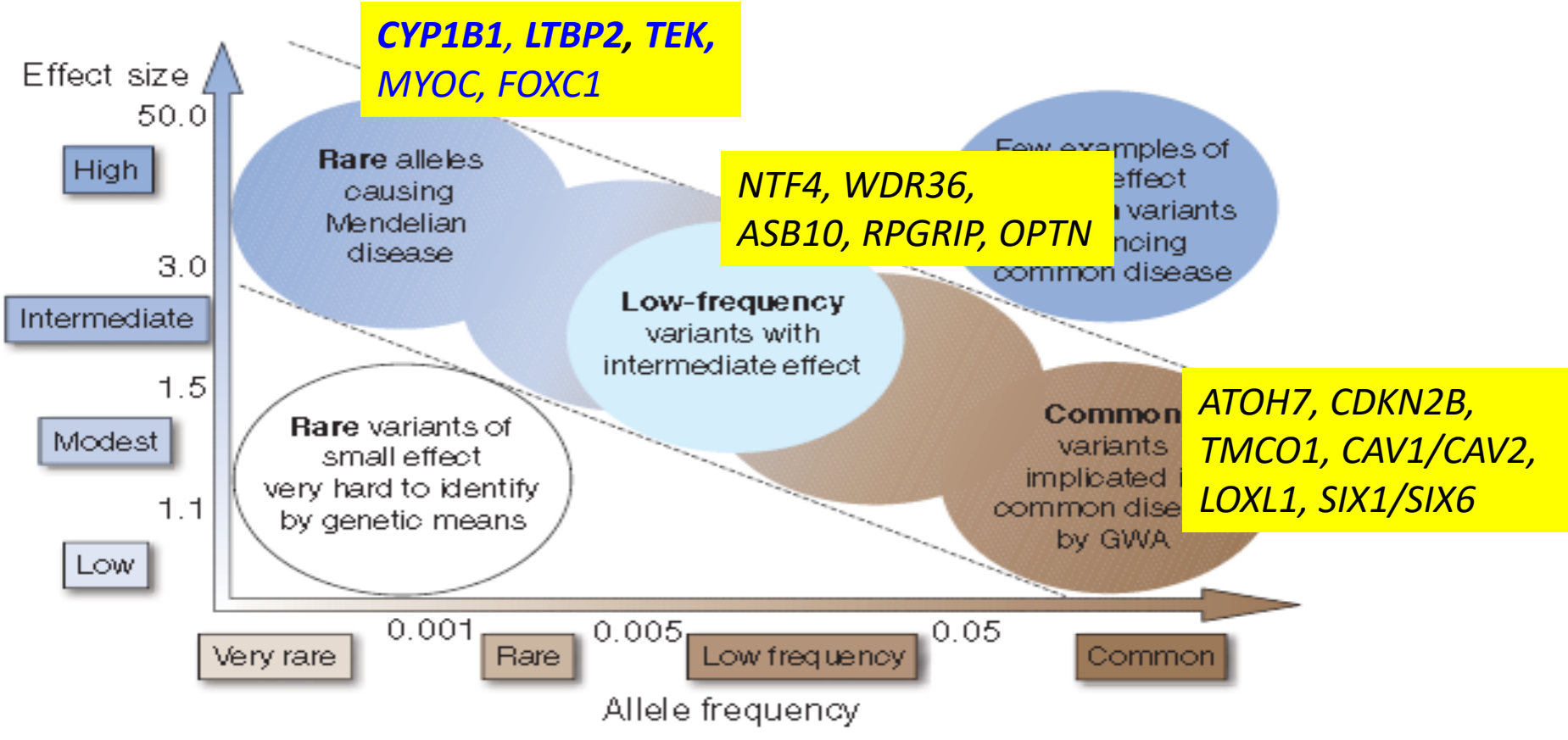






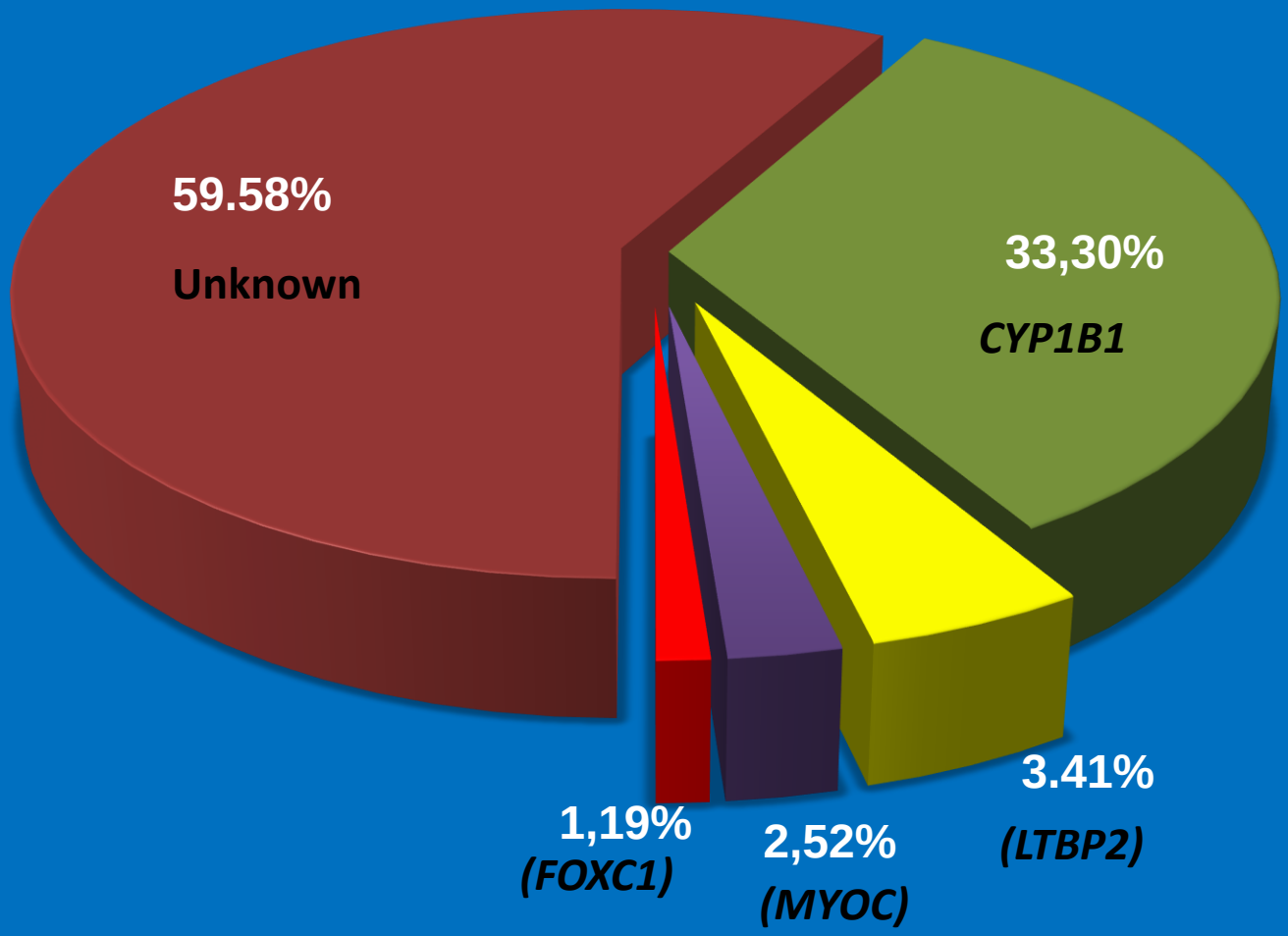
GLC3A **(2p21) -** ***CYP1B1***	**(~20-90%)**
GLC3B **(1p36)**	
GLC3C **(14q23)**	
GLC3D **(14q22) –** ***LTBP2***	**(~2%)**
GLC3E **(9p21.2) –** ***TEK***	**(~2%)**

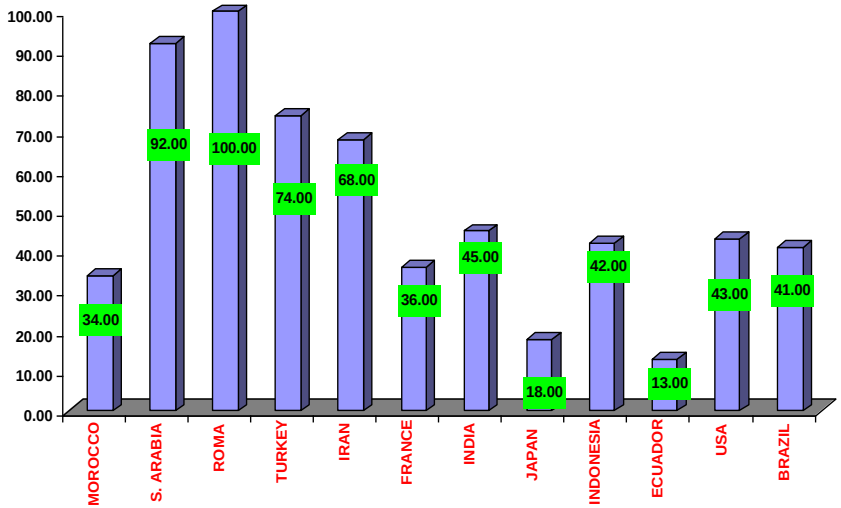
PCG results in 4.2% blindness in India

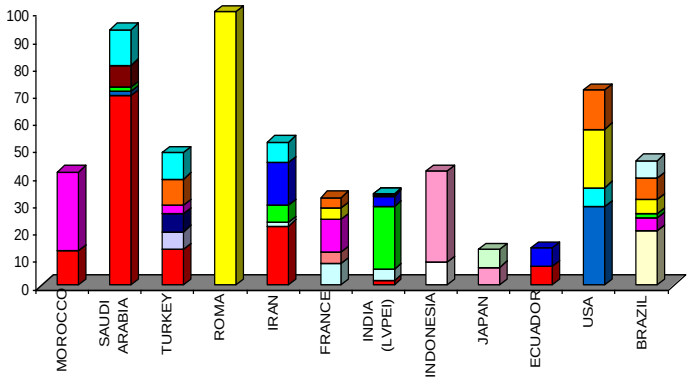

CYP1B1, LTBP2, TEK,
MYOC, FOXC1
NTF4, WDR36,
ASB10, RPGRIP, OPTN
ATOH7, CDKN2B,
TMCO1, CAV1/CAV2,
LOXL1, SIX1/SIX6
Effect size
50.0
High
3.0
Intermediate
1.5
Modest
1.1
Low
Rare alleles causing Mendelian disease
Low-frequency variants with intermediate effect
Rare variants of small effect very hard to identify by genetic means
Common variants implicated in common disease by GWA
0.001
0.005
0.05
Very rare
Rare
Low frequency
Common
Allele frequency

Mutation frequencies in our Indian cohort

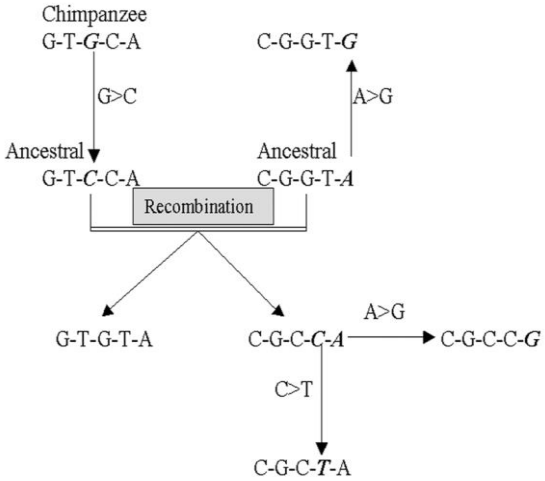
(n = 467)







COUNTRY	H	A	P	L	O	CYP1B1 MUTATIONS
ECUADOR	C	G	G	T	A	G61E 4340 delG
ALGERIA	C	G	G	T	A	4340 delG
MOROCCO	C	G	G	T	A	G61E 4340 delG
S. ARABIA	C	G	G	T	A	G61E R368H R469W 268del
INDIA	C	G	G	T	A	G61E R368H R469W
USA	C	G	G	T	A	268del 10bpdup
BRAZIL	C	G	G	T	A	R368H 4340 delG 10bpdup
ECUADOR	C	G	C	C	A	R390C
INDIA	C	G	C	C	A	R390C C250X
JAPAN	C	G	C	C	ND	C250X
S. ARABIA	C	G	C	C	G	L77P
INDIA	C	G	C	C	G	L77P
PORTUGAL	C	G	C	C	G	8182 del G
BRAZIL	C	G	C	C	G	8182 del G
ROME	G	T	C	C	A	E387K
USA	G	T	C	C	A	E387K
BRAZIL	G	T	C	C	A	E387K



Chakrabarti et al. Invest Ophthalmol Vis Sci 2006; 47: 43 - 47

Genotype – Phenotype Correlations in PCG

Mutation Profile	Type of mutation	India n, (%, [95%CI])	Brazil n, (%, [95%CI])	P value
Cases with *CYP1B1* Mutation	Total number of cases with any *CYP1B1* mutations	132 (43.85%, [95%CI, 38.36%-49.50%])	66 (44.00%, [95%CI, 36.30%-51.99%])	0.999
	Homozygous mutations	73 (24.25%, [95%CI, 19.75%-29.39%])	25 (16.66%, [95%CI, 11.55%-23.45%])	0.024[a]
	Heterozygous mutations	41 (13.62%, [95%CI, 10.20%-17.95%])	22 (14.66%, [95%CI, 9.88%-21.21%])	0.748
	Compound heterozygous mutations	18 (5.98%, [95%CI, 3.81%-9.25%])	19 (12.66%, [95%CI, 8.26%-18.94%])	0.012[a]
Cases without any *CYP1B1* mutation	-	169 (56.15%, [95%CI, 50.49%-61.64%])	84 (56.00%, [95%CI, 48.00–69.61%])	0.999

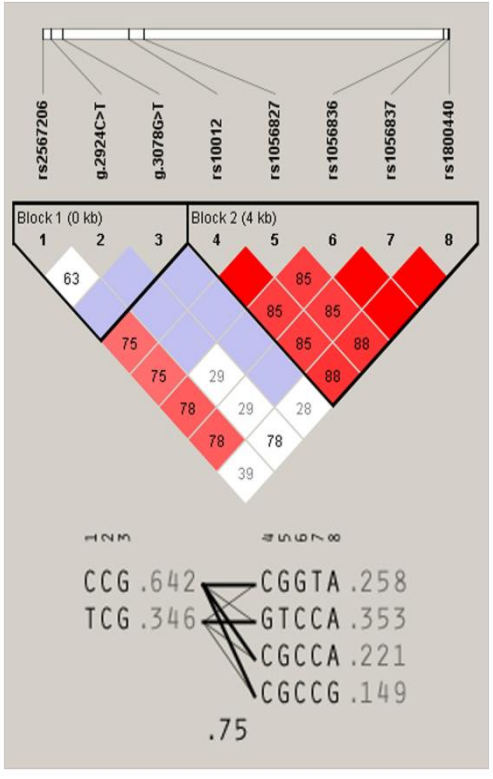
[a]Statistically significant (p<0.05)

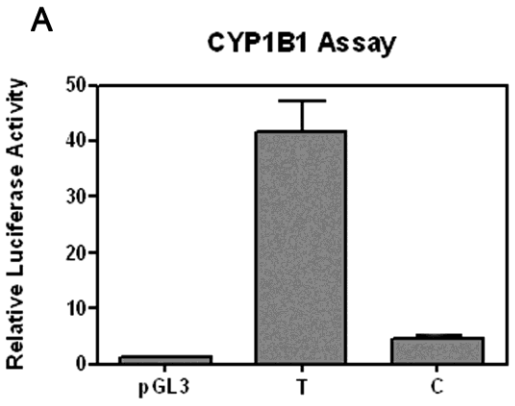
Variables	Parameters	"The prevalent mutation" versus "Other *CYP1B1* mutations"			
		India (R368H)		Brazil (4340delG)	
		Adjusted OR (95%CI)	*P value*	*Adjusted OR (95%CI)*	*P value*
Binary	Gender (Male)	1.96 (0.50-7.62)	0.334	1.45 (0.17-12.56)	0.734
	History of consanguinity	3.24 (0.88-11.95)	0.078	0.98 (0.06-14.8)	0.986
Continuous	IOP	0.95 (0.83-1.08)	0.410	1.06 (0.90-1.26)	0.471
	CD	1.84 (0.98-3.46)	0.059	0.96 (0.22-4.25)	0.957

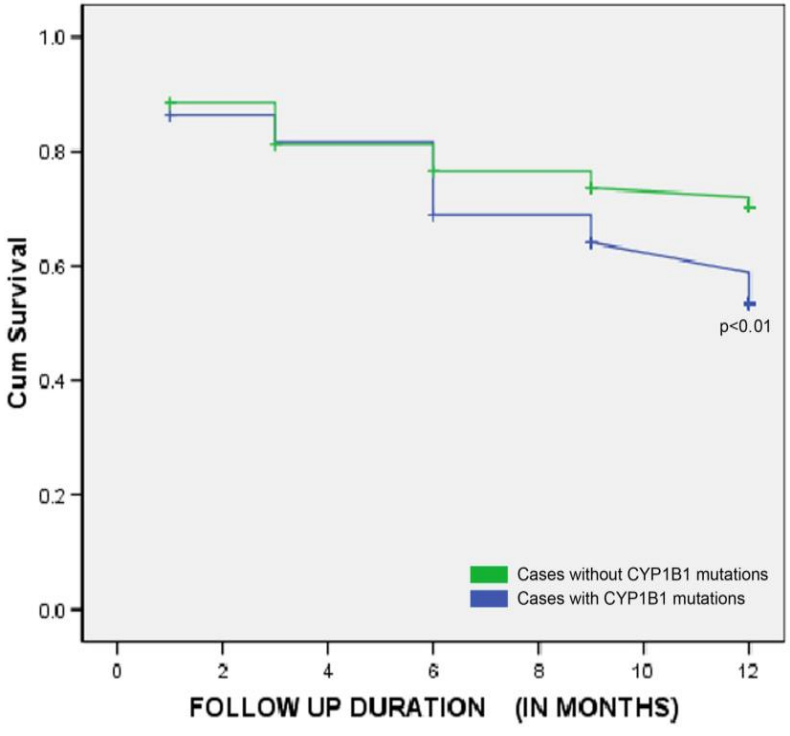
Variables	Parameters	"All *CYP1B1* mutations" versus "No *CYP1B1* mutations"				All "Homozygous *CYP1B1* mutations" versus "Heterozygous *CYP1B1* mutations"			
		India		Brazil		India		Brazil	
		Adjusted OR (95%CI)	*P value*	*Adjusted OR (95%CI)*	*P value*	*Adjusted OR (95%CI)*	*P value*	*Adjusted OR (95%CI)*	*P value*
Binary	Gender (Male)	0.55 (0.25-1.99)	0.131	0.42 (0.13-1.41)	0.160	0.35 (0.10-1.28)	0.112	1.96 (0.28-13.83)	0.502
	History of consanguinity	0.84 (0.39-1.81)	0.650	0.34 (0.02-5.58)	0.448	0.40 (0.10-1.52)	0.178	0.24 (0.03-2.28)	0.215
Continuous	IOP	1.02 (0.96-1.09)	0.481	1.05 (0.95-1.15)	0.284	0.98 (0.86-1.11)	0.751	0.93 (0.79-1.10)	0.395
	CD	0.73 (0.50-1.06)	0.099	1.36 (0.65-2.82)	0.416	1.10 (0.59-2.07)	0.755	0.67 (0.17-2.69)	0.573

de Melo *et al*. *PLoS One* 2015: e0127147

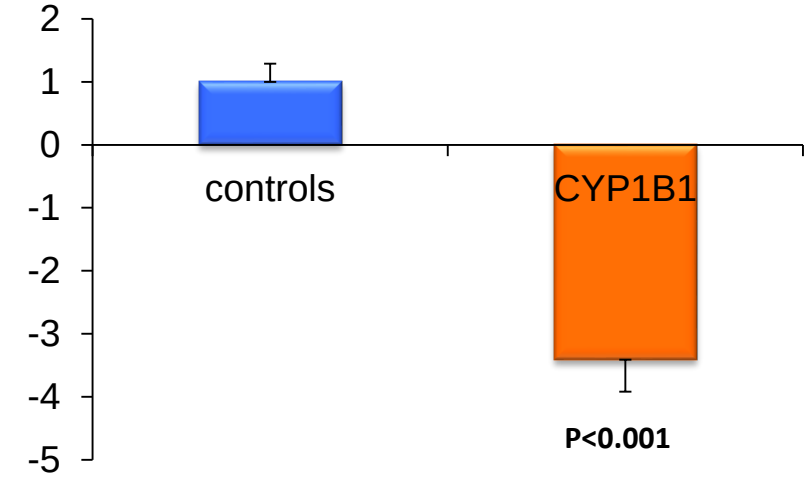
Continuing with *CYP1B1*…….

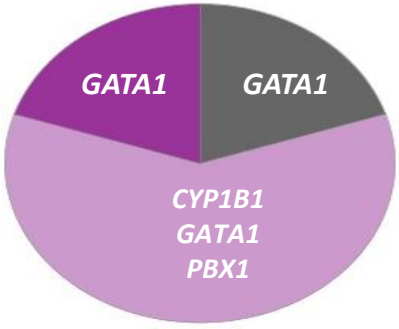


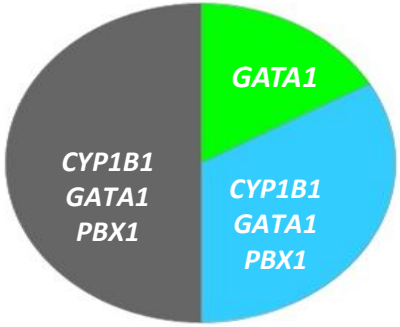




Chakrabarti et al. Hum Mol Genet 2010; 19: 4083 - 90

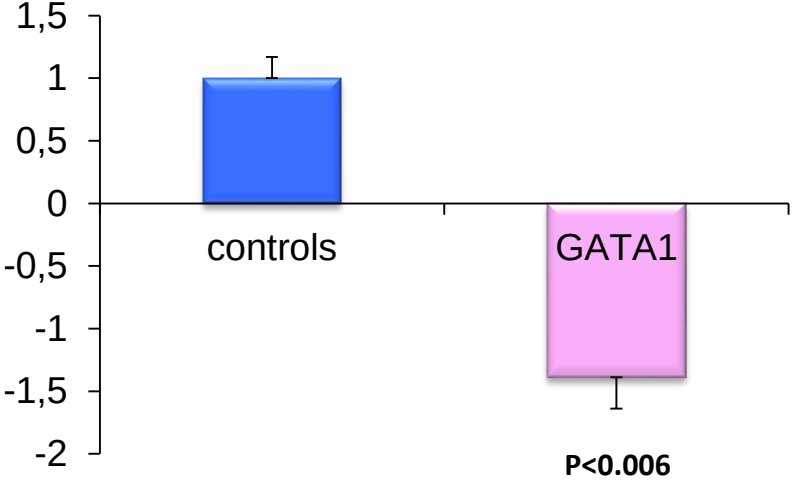


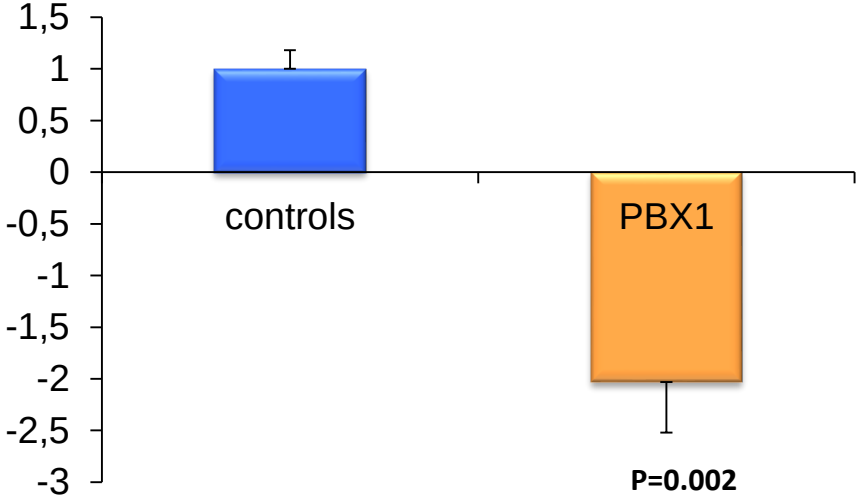




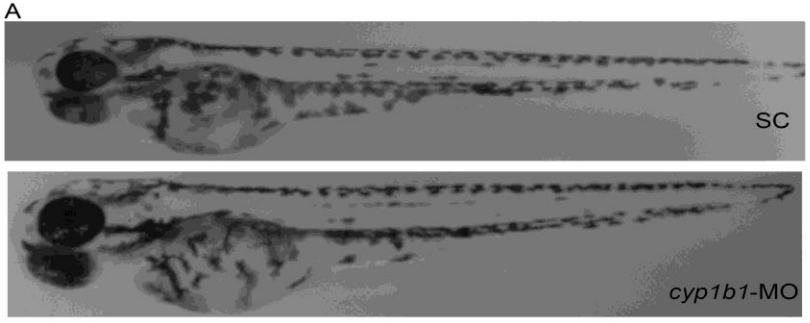
binding (GO:0005488)
catalytic activity (GO:0003824)
nucleic acid binding transcription factor activity (GO:0001071)

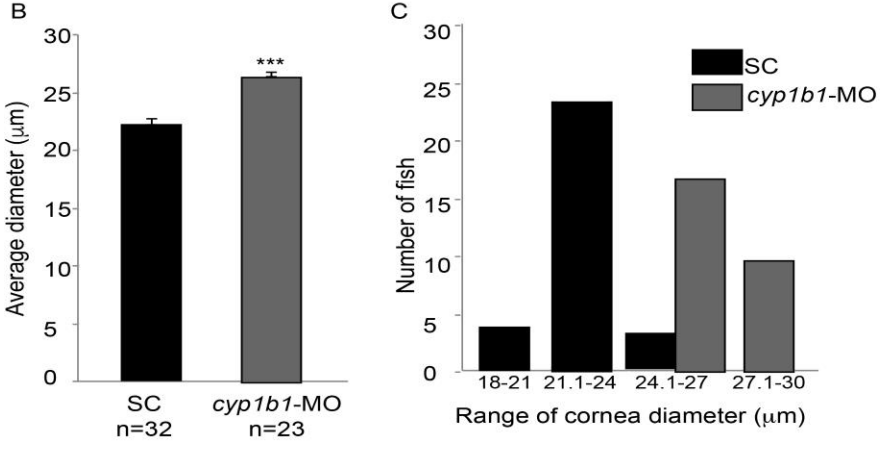
biological regulation (GO:0065007)
developmental process (GO:0032502)
metabolic process (GO:0008152)

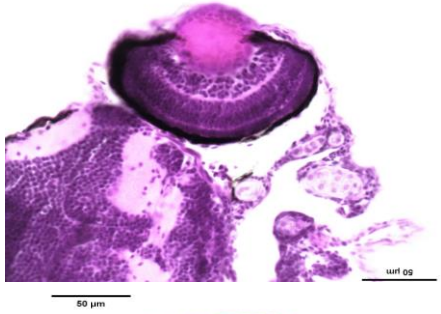


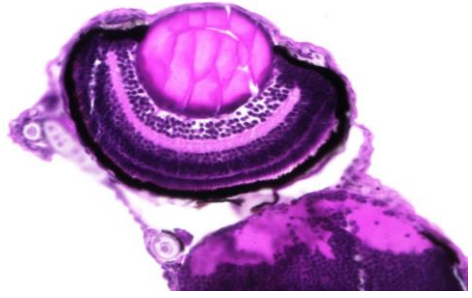


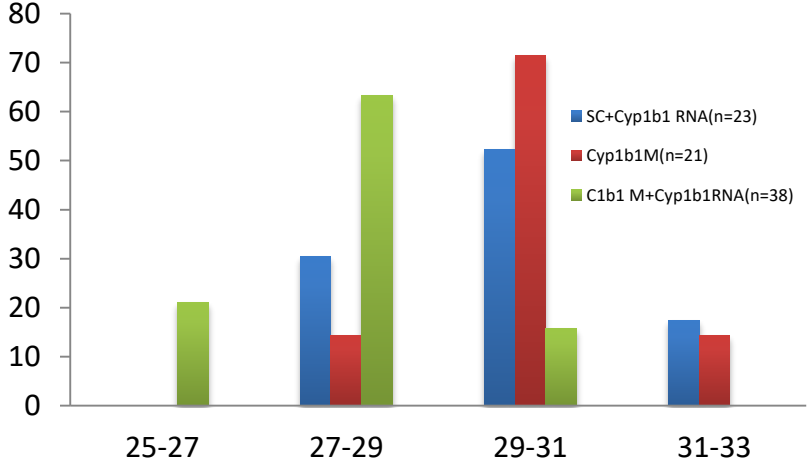
In vivo analysis











HUNT FOR OTHER GENES

Non-Mutated (*CYP1B1+LTBP2+MYOC+FOXC1*) PCG cases

Targeted sequencing

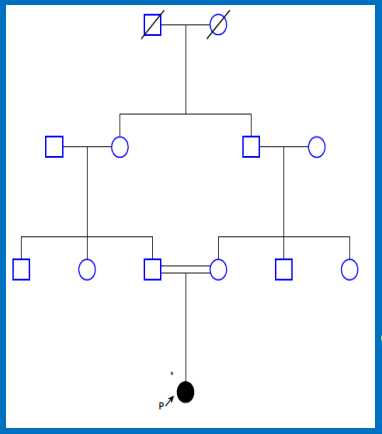
Disease panel in Cases

Exome sequencing

Disease variants in Trios

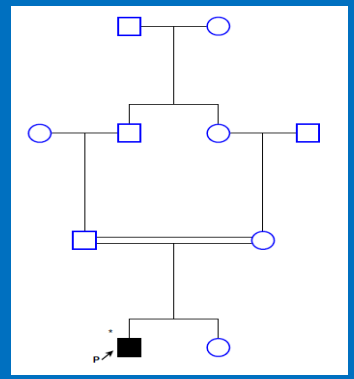
Transcriptome analysis

Expression in the AqH, TM, Iris

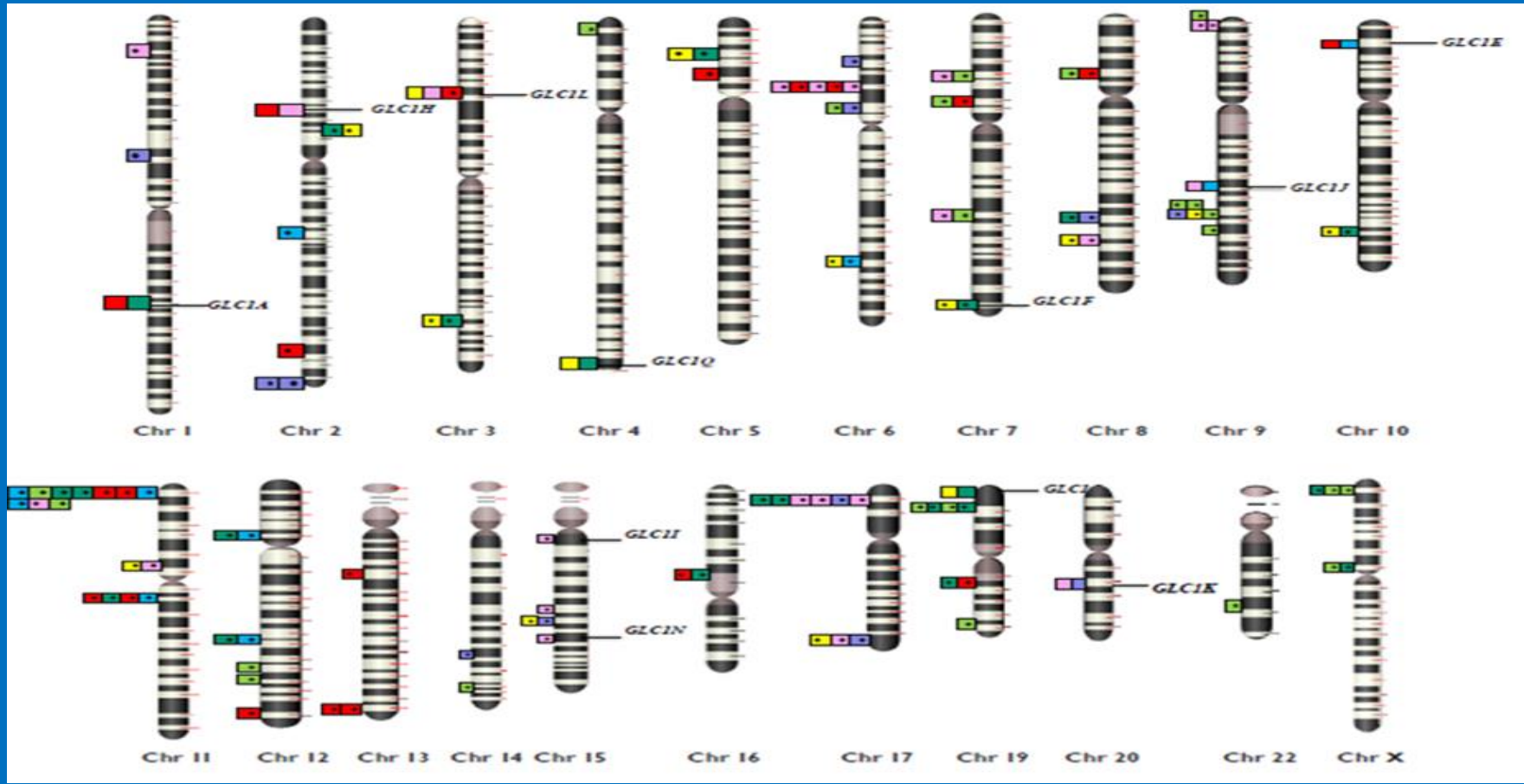


Co-segregation of the affected allele

Functional validation



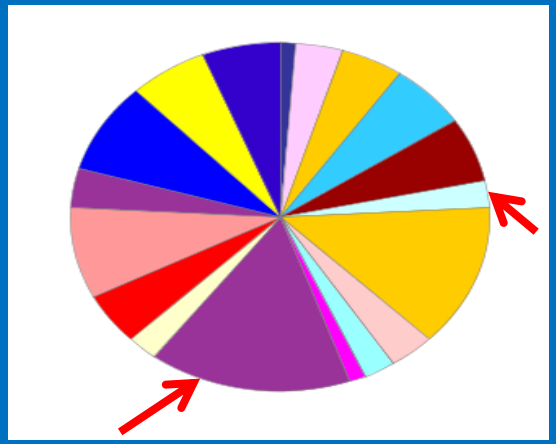
Novel genes across chromosomes



Many of the novel genes did not harbor in the linked glaucoma loci

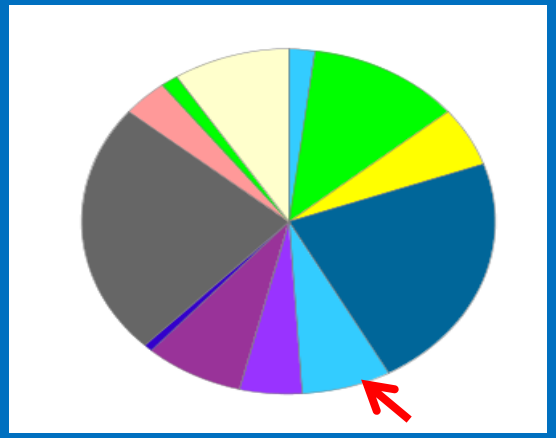
PANTHER analysis of identified genes

Common functions and interactions among genes



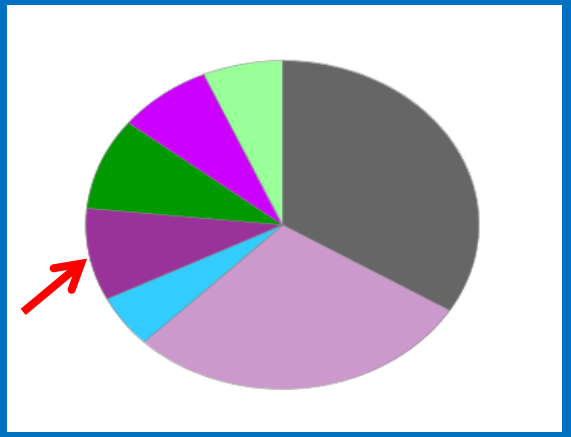
Protein class

Extracellular matrix protein and signaling molecules



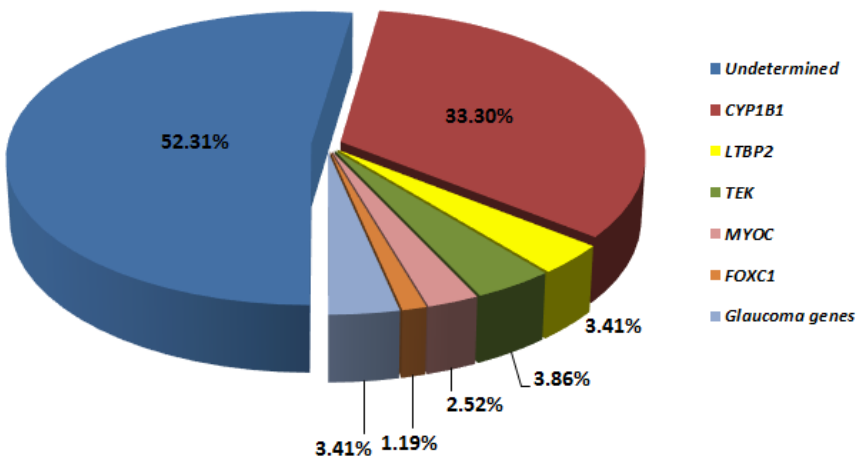
Biological processes

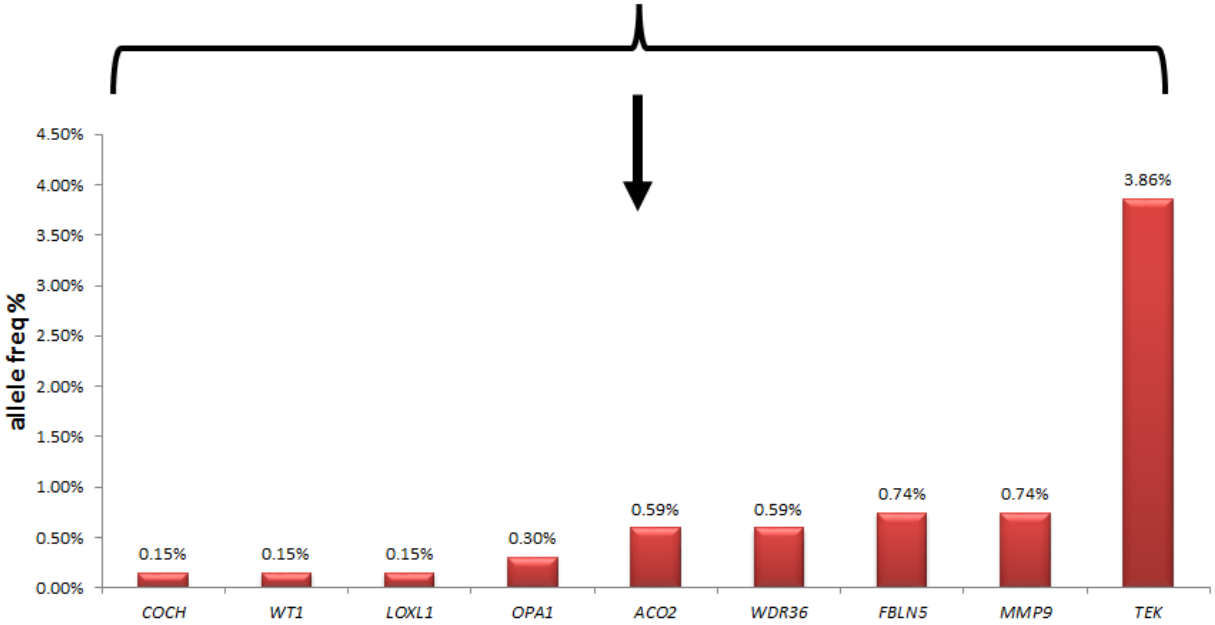
Developmental processes

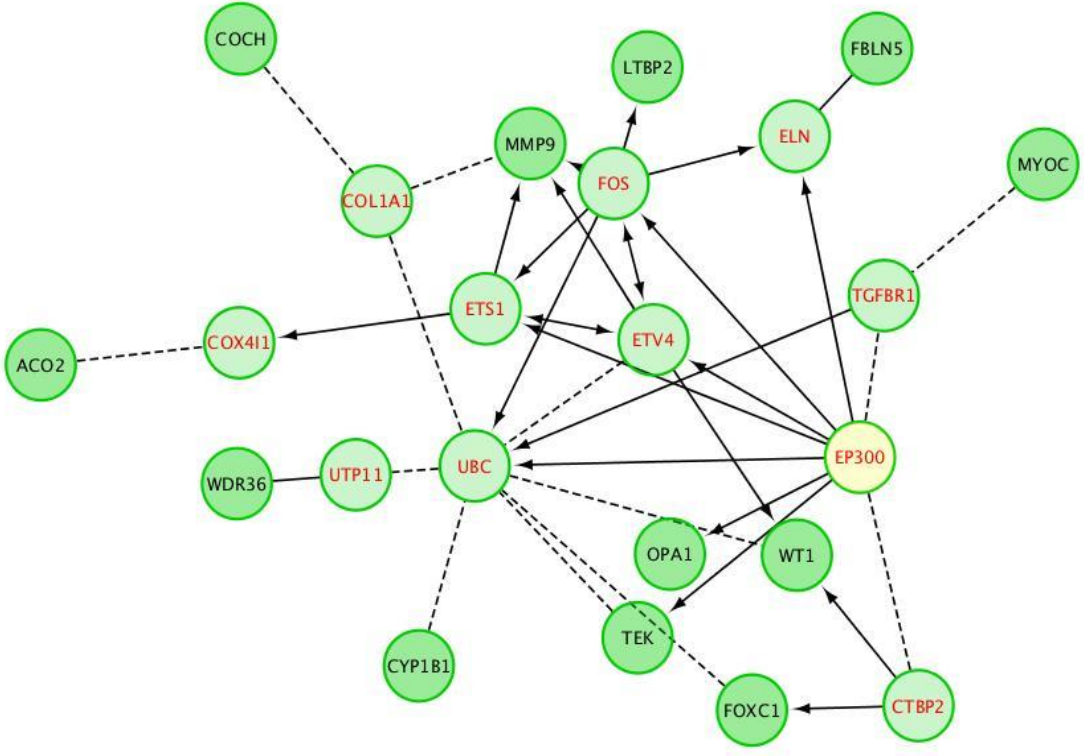


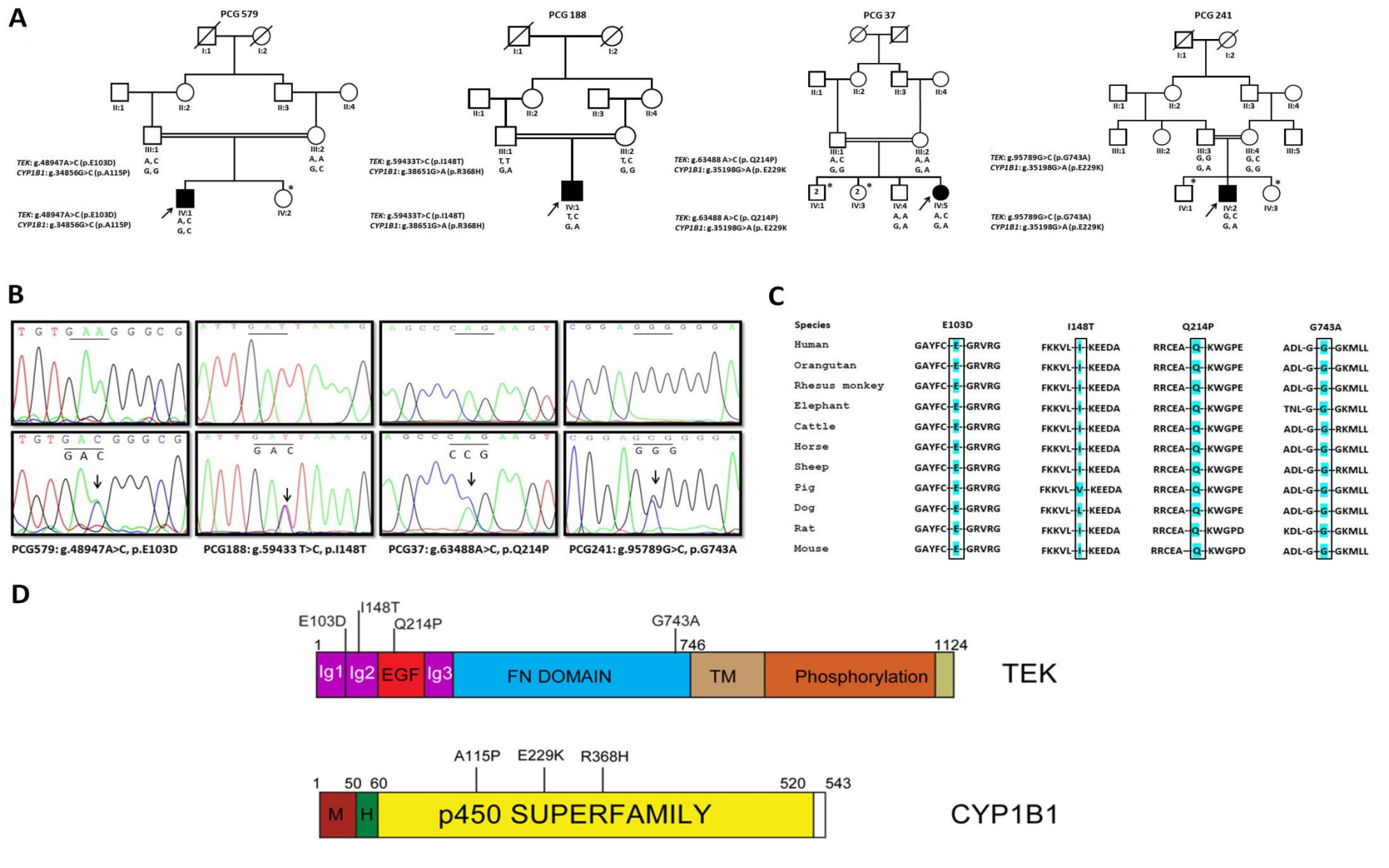
Molecular functions

Transcriptional factor genes

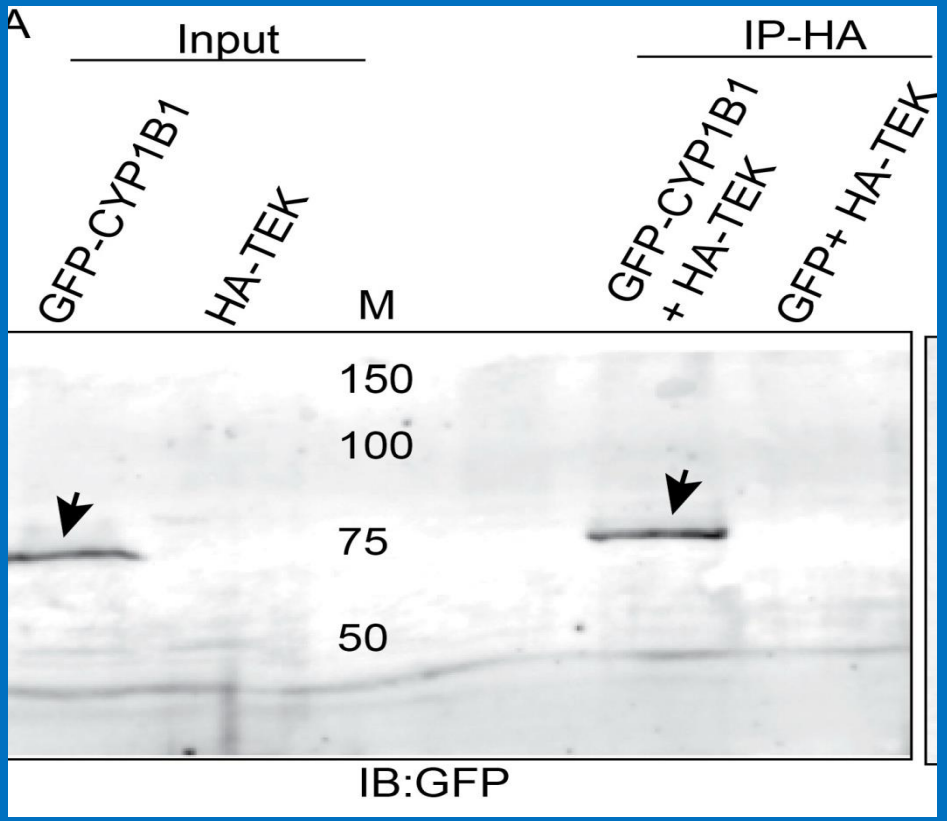

52.31%
33.30%
3.41%
3.86%
2.52%
1.19%
3.41%
Undetermined
CYP1B1
LTBP2
TEK
MYOC
FOXC1
Glaucoma genes

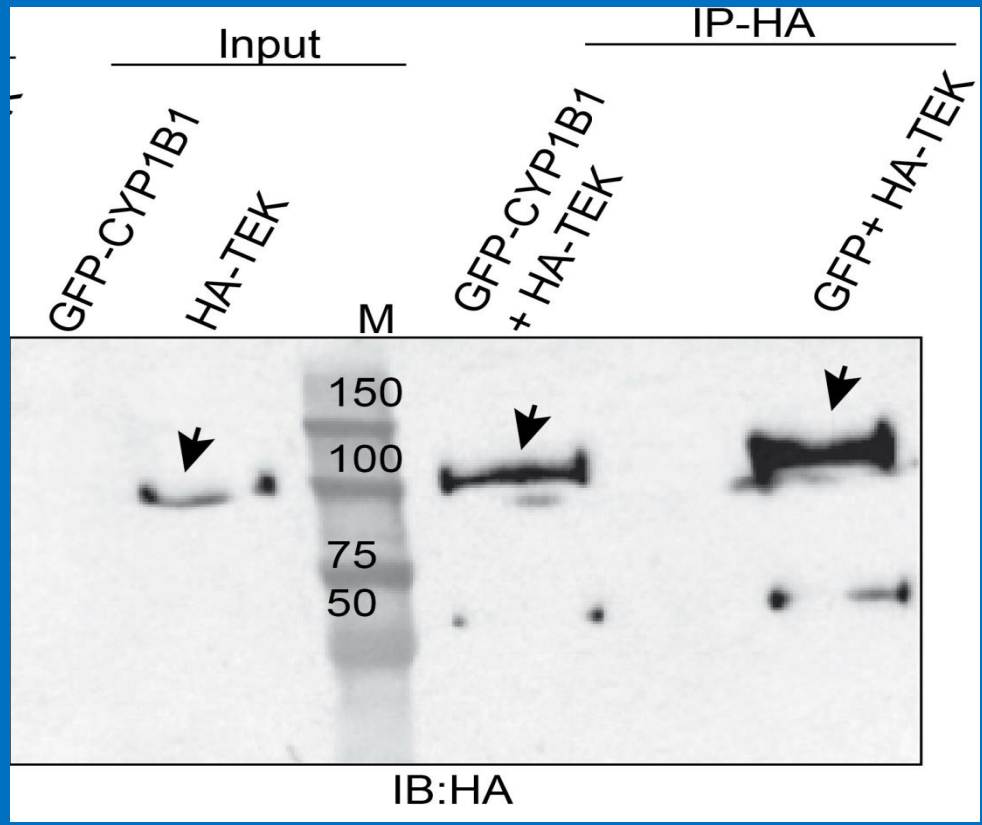

allele freq %
4.50%
4.00%
3.50%
3.00%
2.50%
2.00%
1.50%
1.00%
0.50%
0.00%
0.15%
0.15%
0.15%
0.30%
0.59%
0.59%
0.74%
0.74%
3.86%
COCH
WT1
LOXL1
OPA1
ACO2
WDR36
FBLN5
MMP9
TEK


COCH
LTBP2
FBLN5
MMP9
ELN
MYOC
COL1A1
FOS
TGFBR1
ETS1
COX4I1
ETV4
ACO2
EP300
UBC
WDR36
UTP11
OPA1
WT1
TEK
CYP1B1
FOXC1
CTBP2

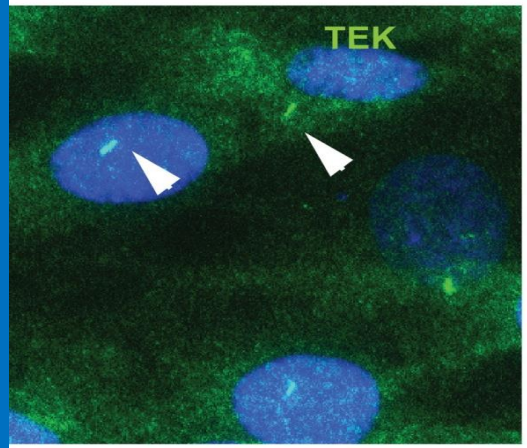

A
PCG 579
I:1
I:2
II:1
II:2
II:3
II:4
III:1
A, C
G, G
III:2
A, A
G, C
IV:1
A, C
G, C
IV:2
TEK: g.48947A>C (p.E103D)
CYP1B1: g.34856G>C (p.A115P)
TEK: g.48947A>C (p.E103D)
CYP1B1: g.34856G>C (p.A115P)
PCG 188
I:1
I:2
II:1
II:2
II:3
II:4
III:1
T, T
G, A
III:2
T, C
G, G
IV:1
T, C
G, A
TEK: g.59433T>C (p.I148T)
CYP1B1: g.38651G>A (p.R368H)
TEK: g.59433T>C (p.I148T)
CYP1B1: g.38651G>A (p.R368H)
PCG 37
II:1
II:2
II:3
II:4
III:1
A, C
G, G
III:2
A, A
G, A
IV:1
IV:3
IV:4
A, A
G, A
IV:5
A, C
G, A
TEK: g.63488 A>C (p. Q214P)
CYP1B1: g.35198G>A (p. E229K
TEK: g.63488 A>C (p. Q214P)
CYP1B1: g.35198G>A (p. E229K
PCG 241
I:1
I:2
II:1
II:2
II:3
II:4
III:1
III:2
III:3
G, G
G, A
III:4
G, C
G, G
III:5
IV:1
IV:2
G, C
G, A
IV:3
TEK: g.95789G>C (p.G743A)
CYP1B1: g.35198G>A (p.E229K)
TEK: g.95789G>C (p.G743A)
CYP1B1: g.35198G>A (p.E229K)
B
PCG579: g.48947A>C, p.E103D
PCG188: g.59433 T>C, p.I148T
PCG37: g.63488A>C, p.Q214P
PCG241: g.95789G>C, p.G743A
C
Species
E103D
I148T
Q214P
G743A
Human
GAYFC-E-GRVRG
FKKVL-I-KEEDA
RRCEA-Q-KWGPE
ADL-G-G-GKMLL
Orangutan
GAYFC-E-GRVRG
FKKVL-I-KEEDA
RRCEA-Q-KWGPE
ADL-G-G-GKMLL
Rhesus monkey
GAYFC-E-GRVRG
FKKVL-I-KEEDA
RRCEA-Q-KWGPE
ADL-G-G-GKMLL
Elephant
GAYFC-E-GRVRG
FKKVL-I-KEEDA
RRCEA-Q-KWGPE
TNL-G-G-GKMLL
Cattle
GAYFC-E-GRVRG
FKKVL-I-KEEDA
RRCEA-Q-KWGPE
ADL-G-G-RKMLL
Horse
GAYFC-E-GRVRG
FKKVL-I-KEEDA
RRCEA-Q-KWGPE
ADL-G-G-GKMLL
Sheep
GAYFC-E-GRVRG
FKKVL-I-KEEDA
RRCEA-Q-KWGPE
ADL-G-G-RKMLL
Pig
GAYFC-E-GRVRG
FKKVL-V-KEEDA
RRCEA-Q-KWGPE
ADL-G-G-GKMLL
Dog
GAYFC-E-GRVRG
FKKVL-L-KEEDA
RRCEA-Q-KWGPE
ADL-G-G-GKMLL
Rat
GAYFC-E-GRVRG
FKKVL-I-KEEDA
RRCEA-Q-KWGPD
KDL-G-G-GKMLL
Mouse
GAYFC-E-GRVRG
FKKVL-I-KEEDA
RRCEA-Q-KWGPD
ADL-G-G-GKMLL
D
E103D
I148T
Q214P
G743A
1
746
1124
Ig1
Ig2
EGF
Ig3
FN DOMAIN
TM
Phosphorylation
TEK
A115P
E229K
R368H
1
50
60
520
543
M
H
p450 SUPERFAMILY
CYP1B1

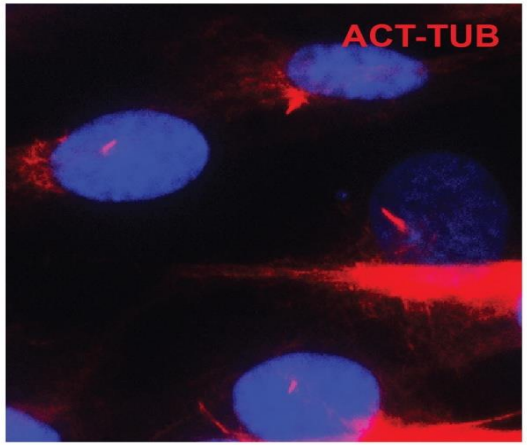
In vitro analysis

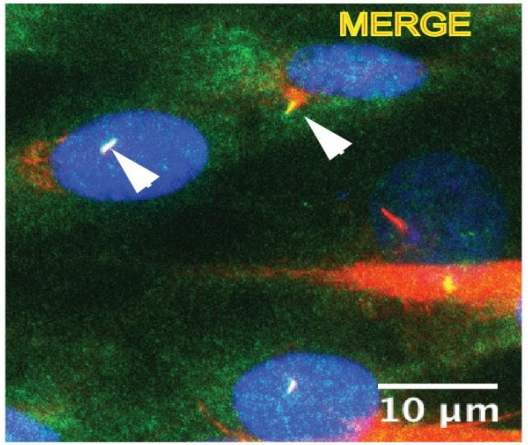




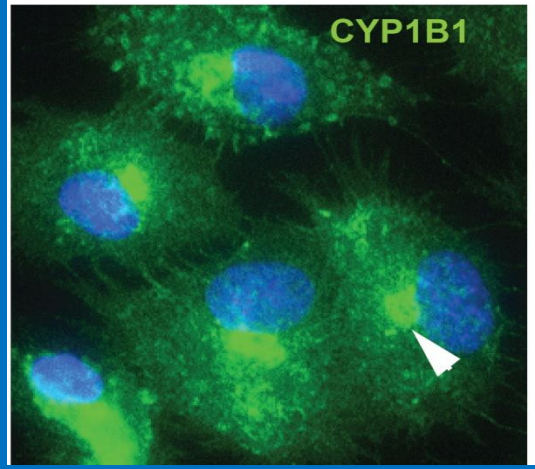
The physical interactions of these two genes are evident through the association of GFP-*CYP1B1* and HA-*TEK* with each other, that are absent in the negative controls.

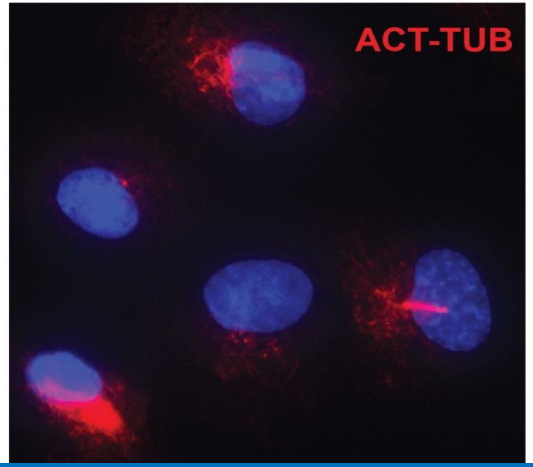


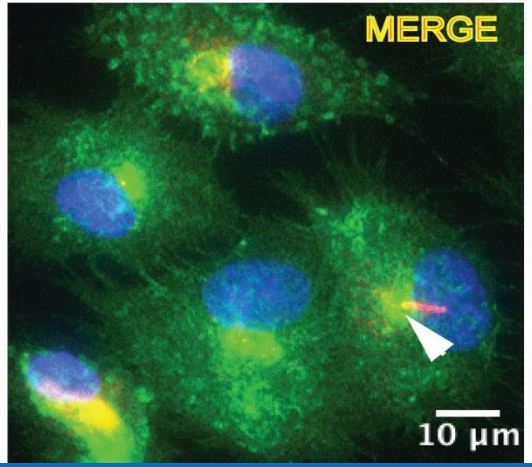




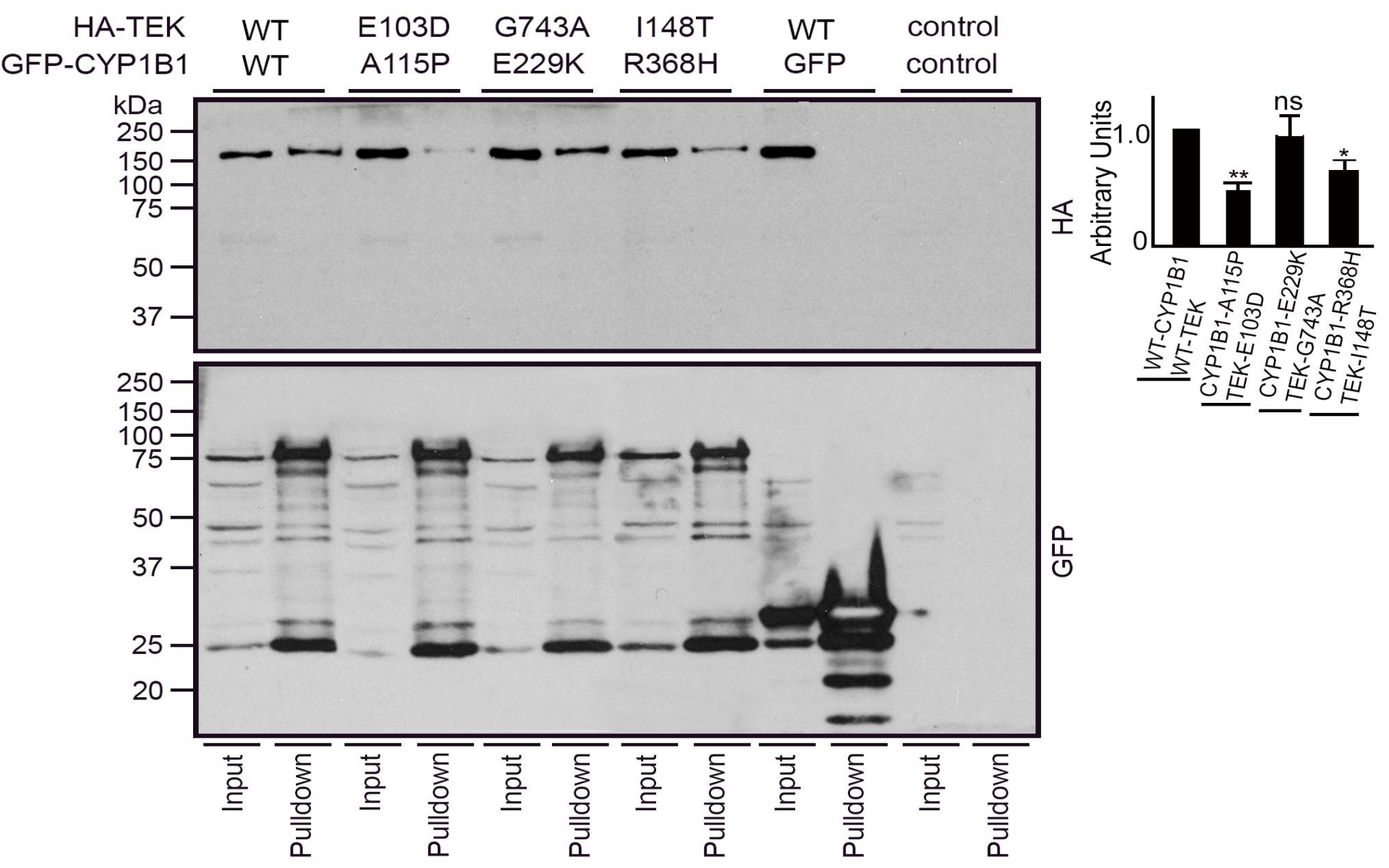
TEK /
ACT-TUB

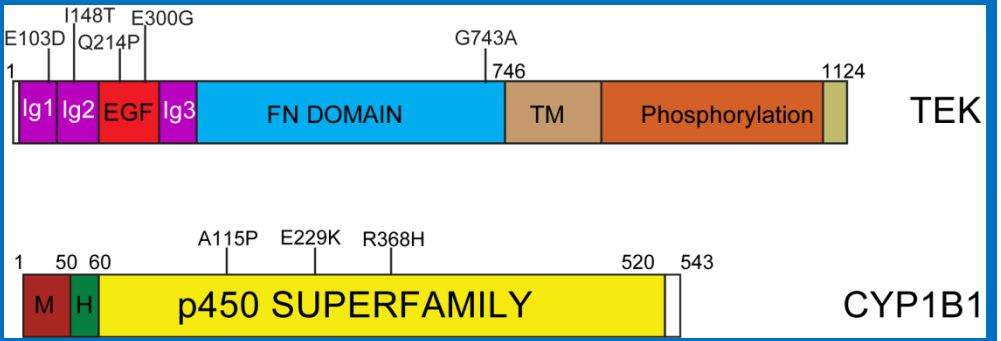


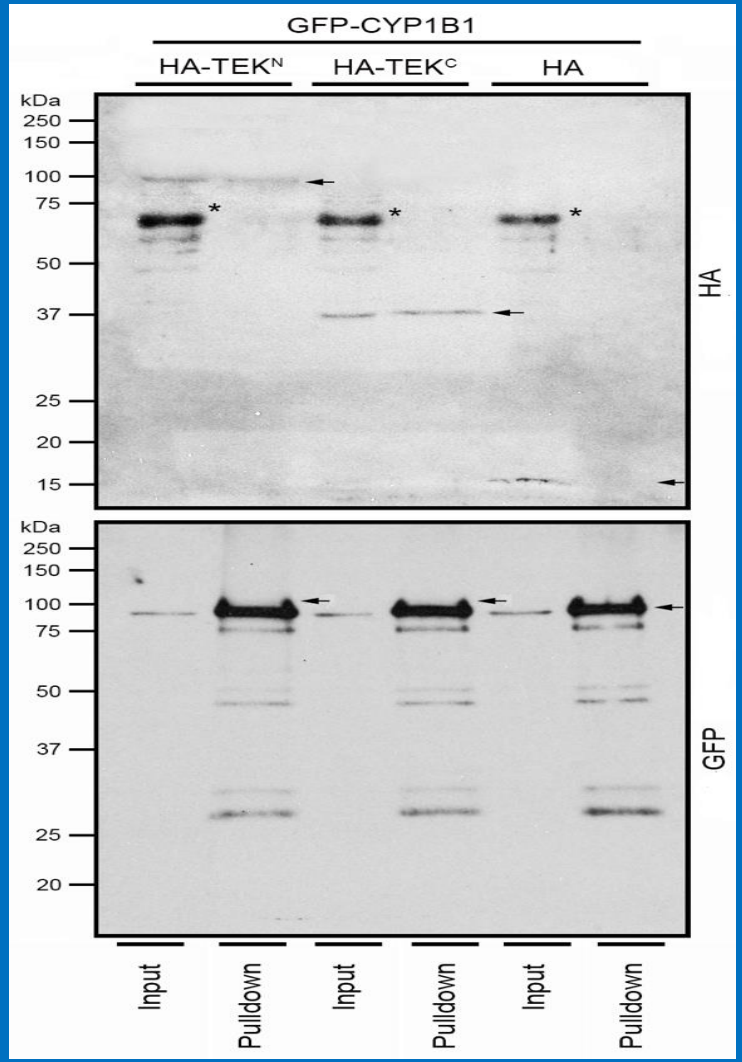


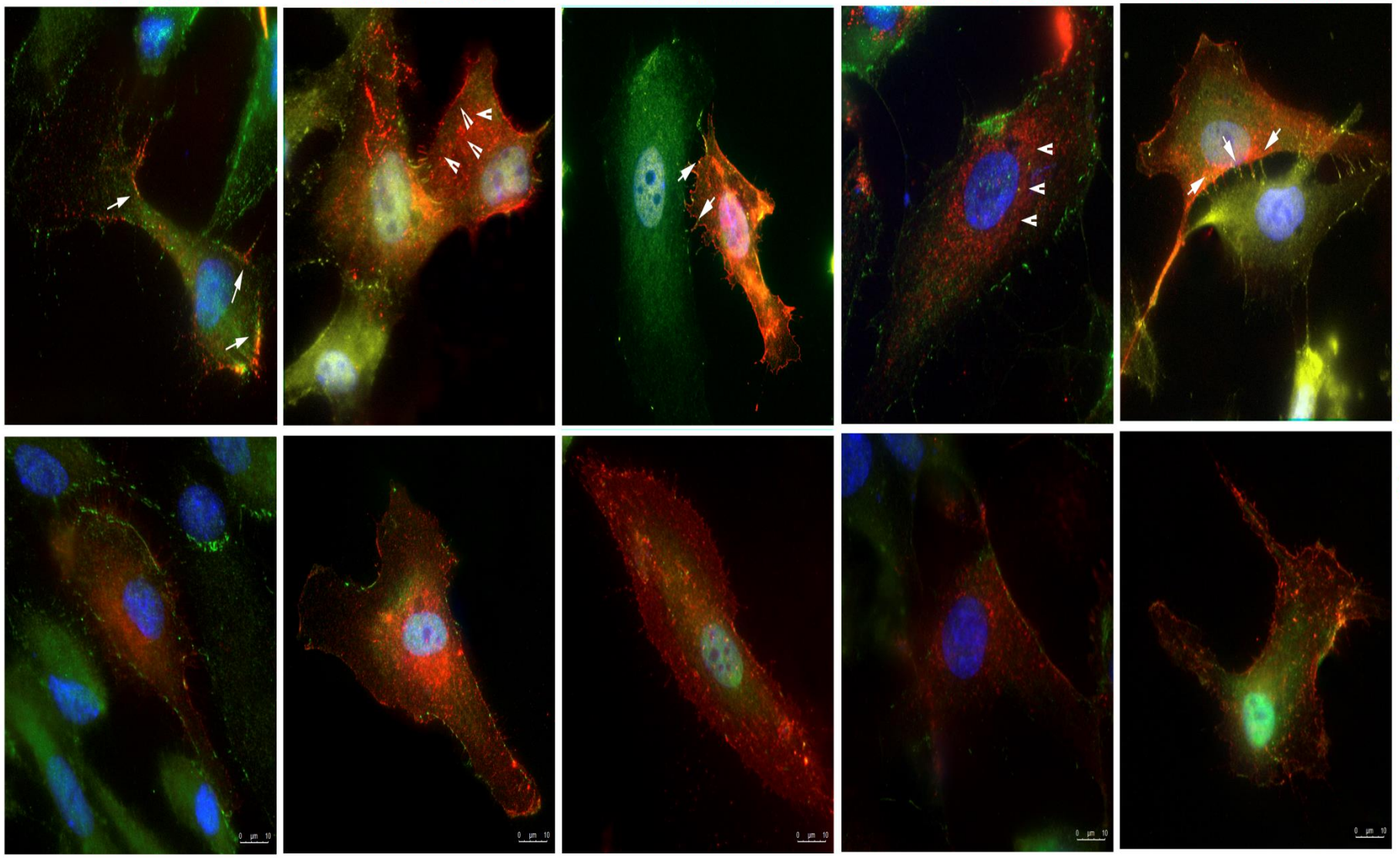


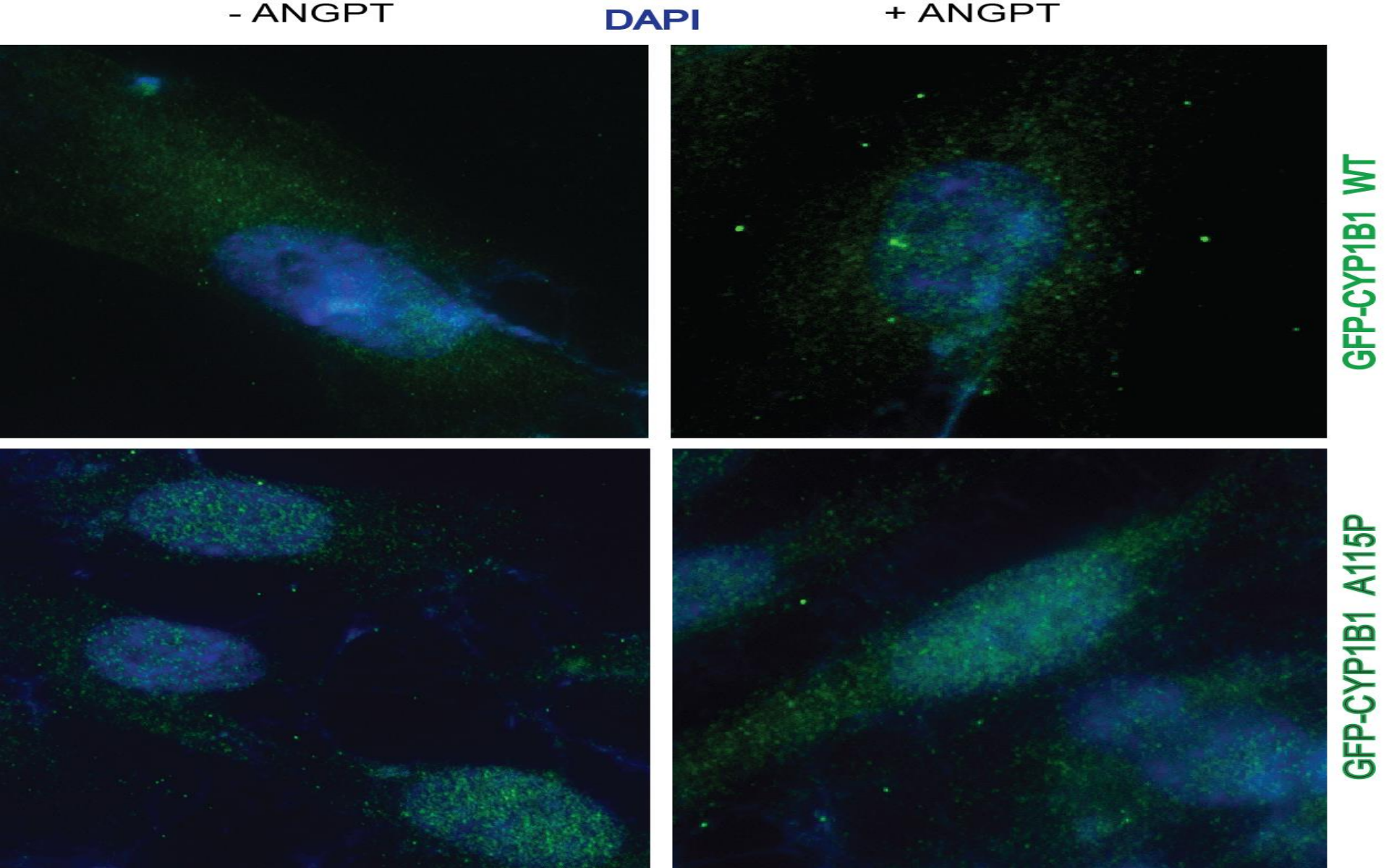
CYP1B1 /
ACT-TUB

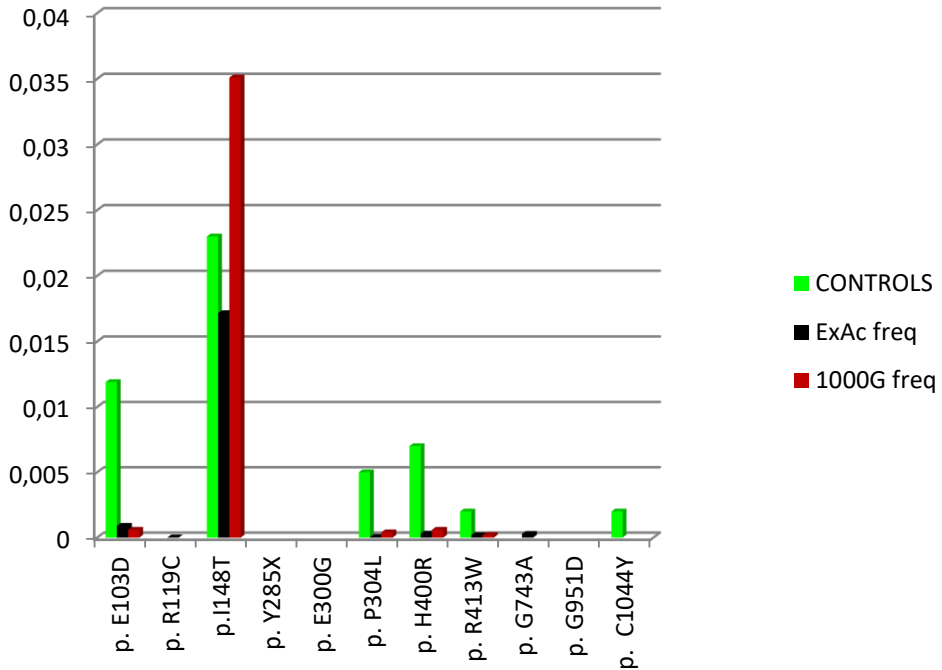

HA-TEK
GFP-CYP1B1
WT
WT
E103D
A115P
G743A
E229K
I148T
R368H
WT
GFP
control
control
kDa
250
150
100
75
50
37
HA
250
150
100
75
50
37
25
20
GFP
Input
Pulldown
Input
Pulldown
Input
Pulldown
Input
Pulldown
Input
Pulldown
Input
Pulldown
Arbitrary Units
1.0
0
ns
**
*
WT-CYP1B1
WT-TEK
CYP1B1-A115P
TEK-E103D
CYP1B1-E229K
TEK-G743A
CYP1B1-R368H
TEK-I148T

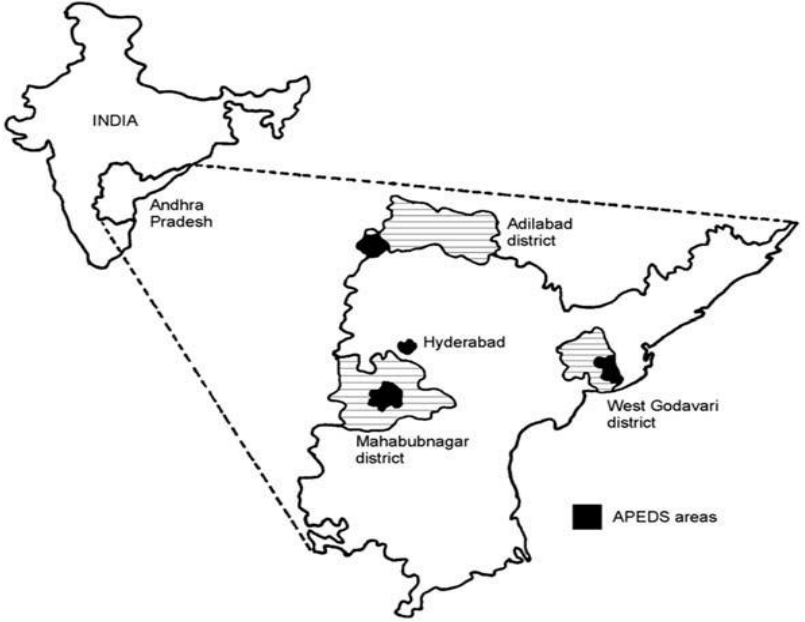

E103D
I148T
Q214P
E300G
G743A
1
746
1124
Ig1
Ig2
EGF
Ig3
FN DOMAIN
TM
Phosphorylation
TEK
A115P
E229K
R368H
1
50
60
520
543
M
H
p450 SUPERFAMILY
CYP1B1

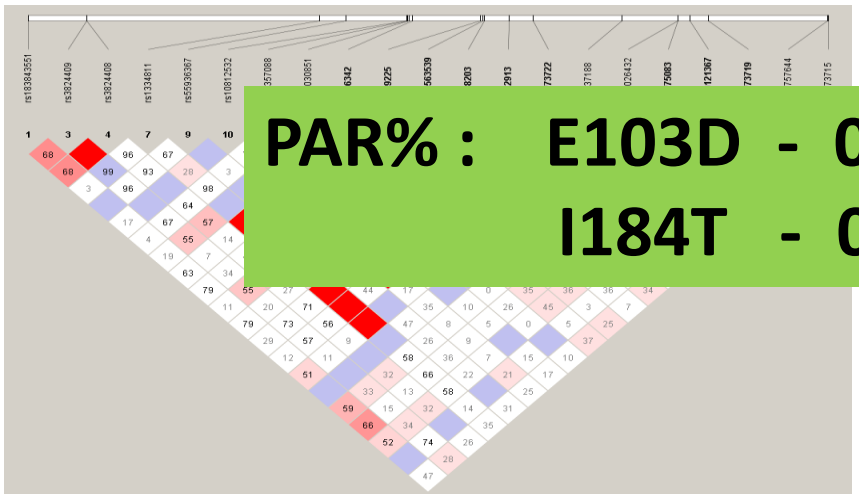

GFP-CYP1B1
HA-TEK^N
HA-TEK^C
HA
kDa
250
150
100
75
50
37
25
20
15
HA
kDa
250
150
100
75
50
37
25
20
GFP
Input
Pulldown
Input
Pulldown
Input
Pulldown


HA-TEK WT
HA-TEK E103D
HA-TEK I148T
HA-TEK-Q214P
HA-TEK G743A
ANGPT1
CONTROL
ZO-1/DAPI


- ANGPT
DAPI
+ ANGPT
GFP-CYP1B1 WT
GFP-CYP1B1 A115P

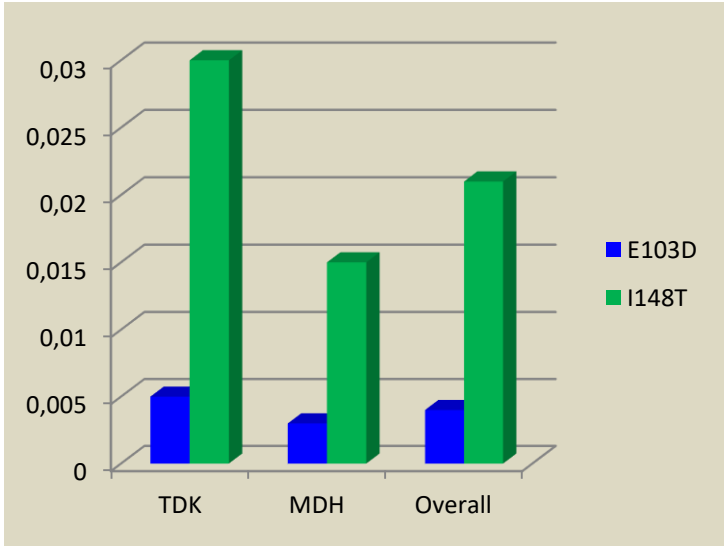






PAR% : E103D - 0.303

I184T - 0.548

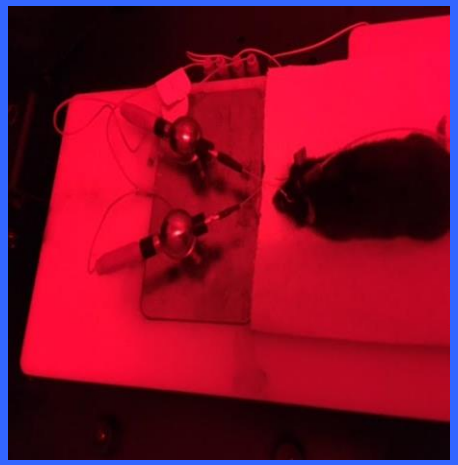


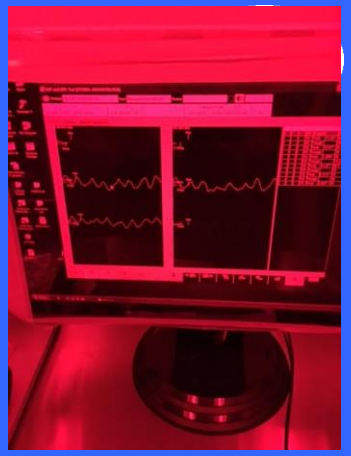
RECENT INSIGHTS.....

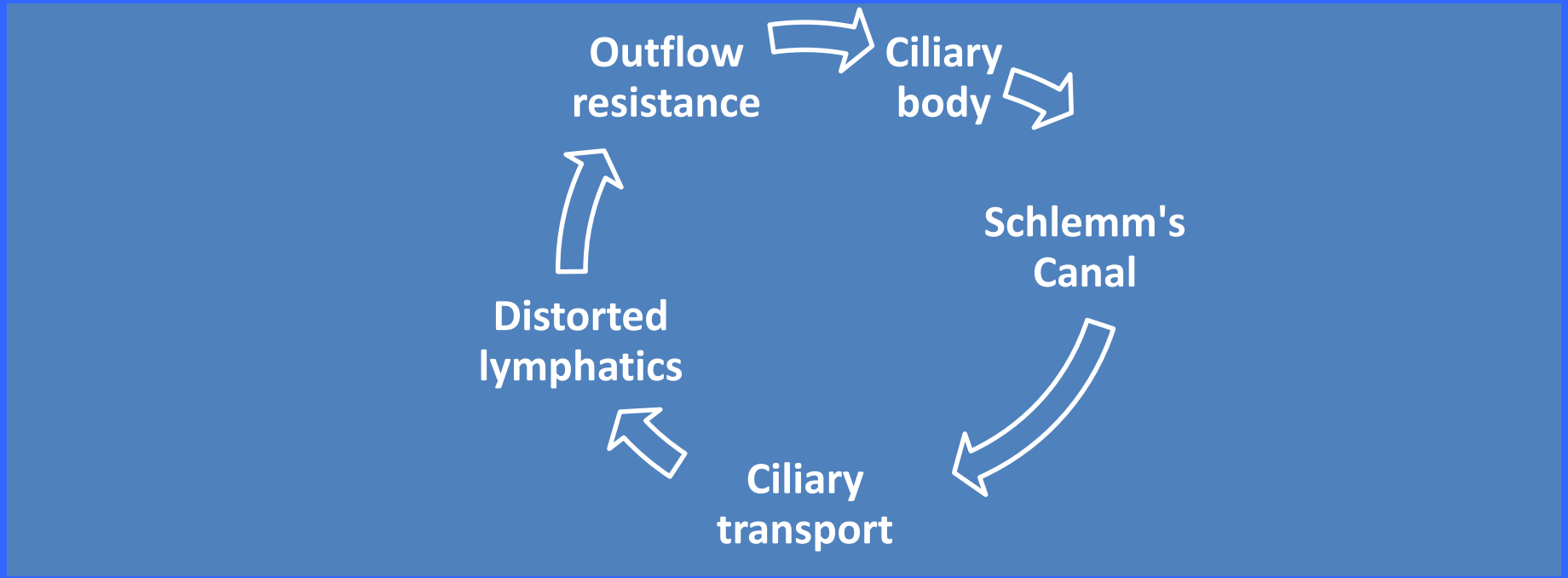
a) The *CYP1B1* and other glaucoma-associated genes may act as modifiers under different clinical conditions.

b) Exome and targeted sequencing revealed that genetic and physical interactions of *CYP1B1* and *TEK* could be an important disease target in PCG.

c) Multiple levels of interactions occur in the genome that need to be functionally characterized to dissect the underlying disease mechanisms.

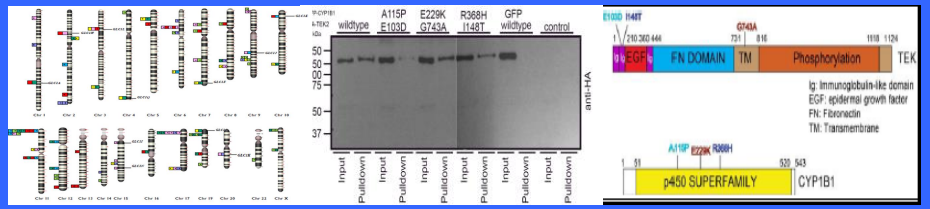




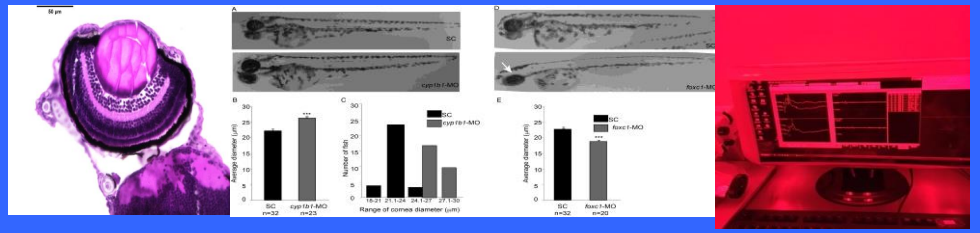

Outflow resistance
Ciliary body
Schlemm's Canal
Ciliary transport
Distorted lymphatics

Translational Research

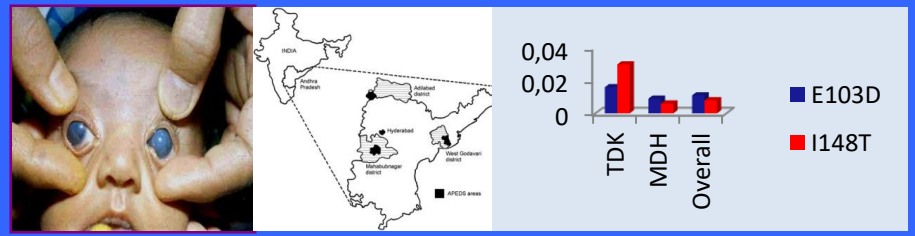
Bench



Bedside



Community



ACKNOWLEDGEMENTS

All patients and normal volunteers

- **Team LVPEI**
  - **Meha Kabra, Sonika Rathi, Gautham Pyatla, Seema Banerjee, Konegari Sekhar,** Sriparna Ganguly, Kiran Kaur
  - **Anil Mandal, Sirisha Senthil, Muralidhar Ramappa, Inderjeet Kaur, Srinivas Marmamula, Ashalatha Metla, Rohit Khanna**
- **Collaborators (India)**
  - Partha P Majumder, Yashoda Ghanekar
- **Collaborators (Abroad)**
  - Luba Kalaydjieva, Jamie Craig (Australia)
  - Monica Mello, Vital Costa, Ivan Taveres, Rubens Belfort (Brazil)
  - Guimera Fethi , Douik Hayet (Tunisia)
  - **Wei Zhang, Manisha Anand, Kollu Nageswara Rao, Hemant Khanna,** Deepak Edwards, Richard Libby (USA)

Champalimaud Foundation, Portugal, PS & CoE grant, Dept of Biotechnology (DBT), Bilateral grants, (DST, DBT), National Eye Institute, NIH, USA

Thank you!

L V Prasad Eye Institute

www.lvpei.org

Excellence Equity Efficiency