LV Prasad Eye Institute

Translational Research Approach: Primary Congenital Glaucoma: Prevalence, genetics, and collaboration between scientists and clinicians for successful treatment

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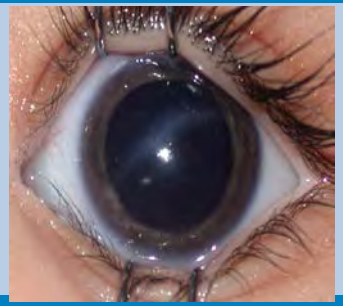
PRIMARY CONGENITAL GLAUCOMA

A severe form of childhood blindness

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

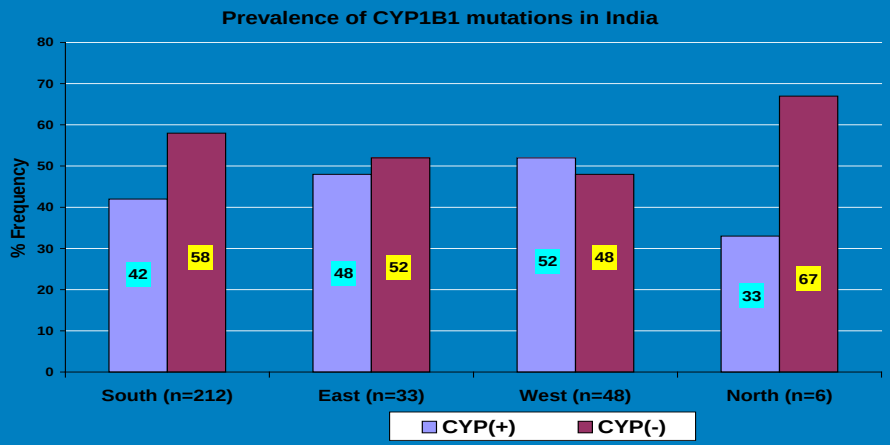
Western countries - 1: 10,000-30,000
Middle East - 1: 2,500
Slovak gypsies - 1: 1,250
India (Andhra Pradesh) ~ 1: 3,300

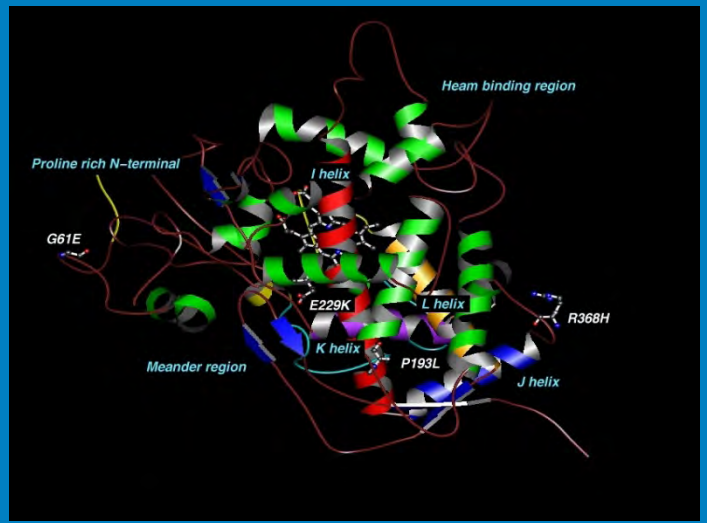


GLC3A(2p21) - *CYP1B1*
GLC3B(1p36)
GLC3C (14q23)
GLC3D(14q22) – *LTBP2*
GLC3E (9p21.2) – *TEK*

PCG results in 4.2% blindness in India

- ~43.0% patients showed *CYP1B1* mutations with variable penetrance
- 35 mutations were observed ←
- ~42.0% were homozygous ←
- R368Hwas the most common mutant allele

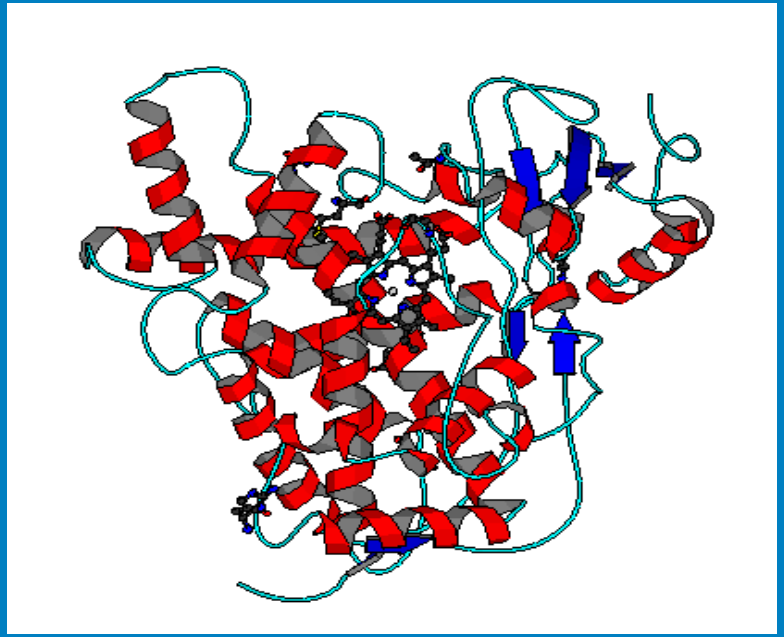




Mol Vis 2004; 10: 696-702

Biophys J 2006; 91: 4329-39

What are the structural properties of disease mutant forms (molecular phenotypes) of proteins as compared to their wild-type counterpart?



Human CYP1b1:

-A cell membrane anchored protein

- Involved in the development of trabacular mesh work in the eyes

-Certain mutants have been found in patients suffering from a genetic disorder called primary congenital glaucoma (PCG)

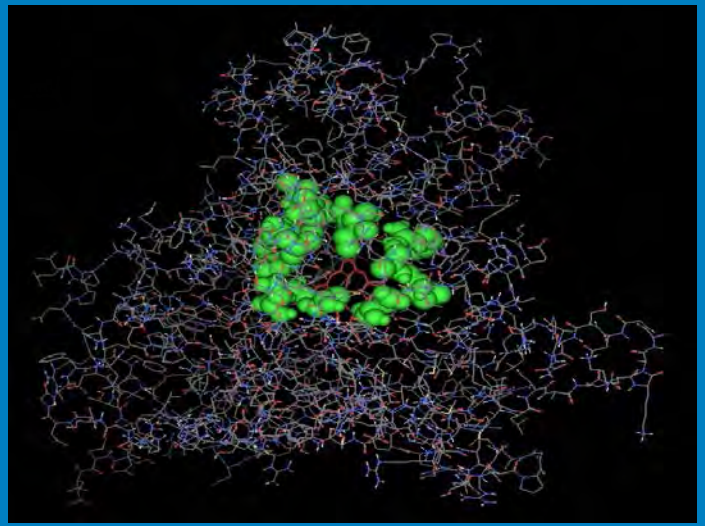
- The mutants which we studied are:

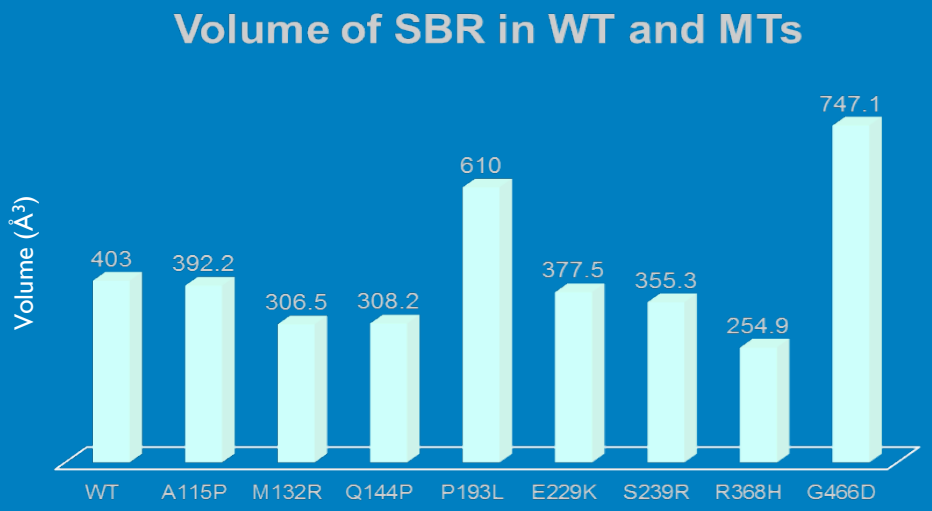
A155P ; M132R; Q144P; P193L; E229K; S239R; R368H; G466D

Achary et al (2006*) Biophysical J*.
Achary and Nagarajaram (2008) *J.Biosci*
Achary and Nagarajaram (2009) *J. Biomol. Struct. Dyn*.

Properties of Functionally Important Regions

Substrate Binding Region (SBR)

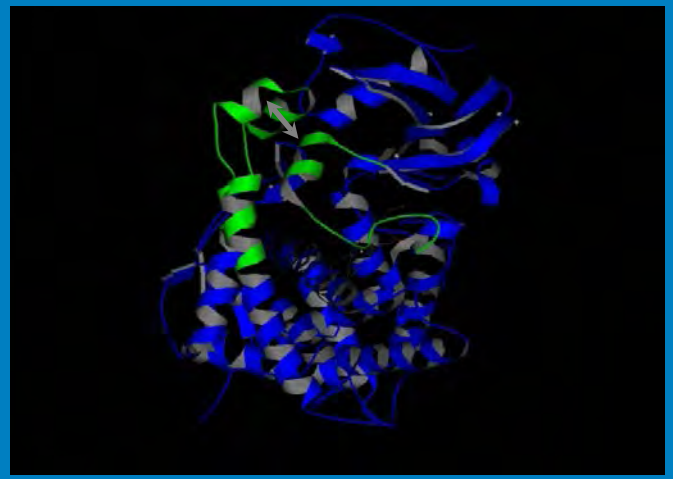


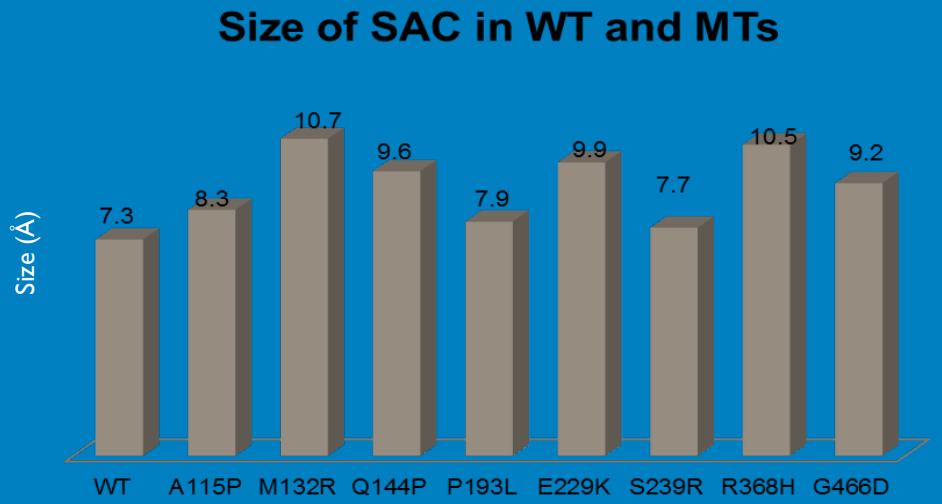


Note that the substrate binding region s in the mutants are too narrow or too wide

Properties of Functionally Important Regions

Substrate Access Channel (SAC)





Here again, the substrate access varies widely: no tight fit in the mutants

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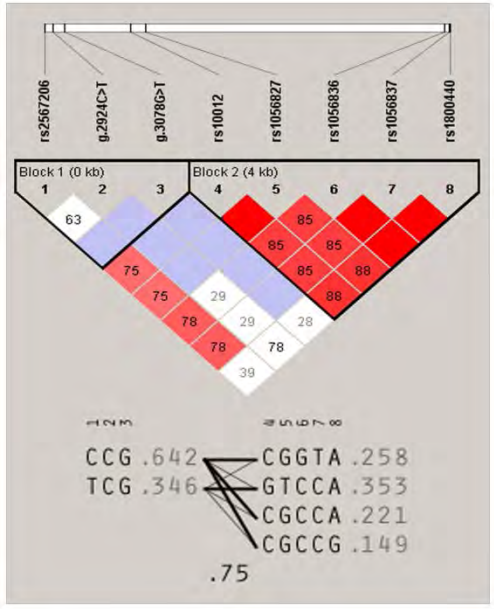
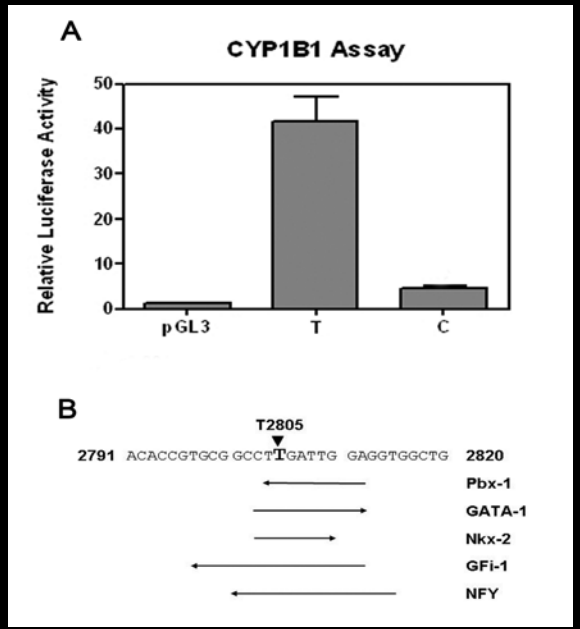
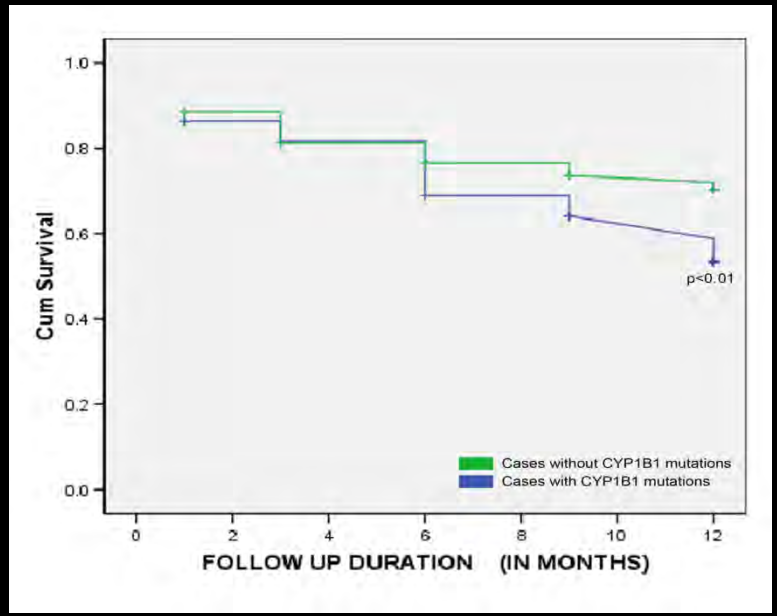
A polymorphism in the *CYP1B1* promoter is functionally associated with primary congenital glaucoma

Subhabrata Chakrabarti[1,*], Yashoda Ghanekar[1,†], Kiranpreet Kaur[1], Inderjeet Kaur[1], Anil K. Mandal[2], Kollu N. Rao[1], Rajul S. Parikh[2], Ravi Thomas[2,3] and Partha P. Majumder[4]







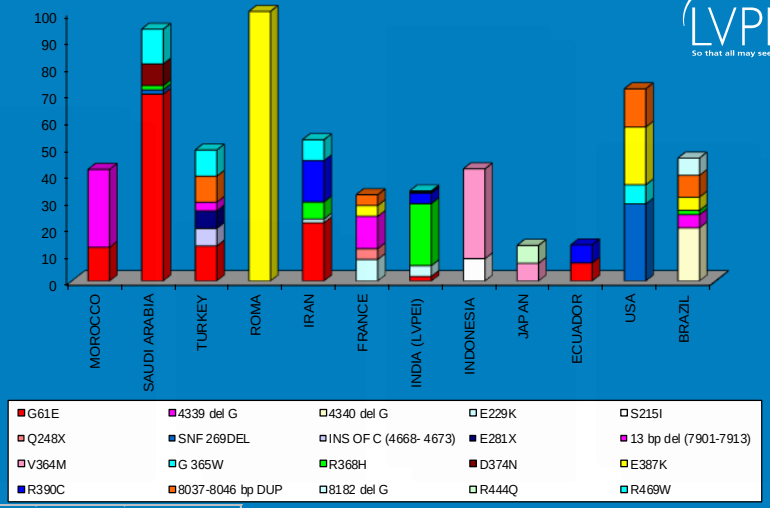
FREQUENCIES OF *CYP1B1* MUTATIONS

Population	PCG Cases (n)	Cases with *CYP1B1* mutation	Homo	Hetero	Compound hetero-zygous	Cases without *CYP1B1* mutation
Australia	37	8 (21.6%)	1 (2.7%)	2 (5.4%)	5 (13.5%)	29 (78.4%)
Brazil	52	26 (50%)	15 (29.0%)	4 (7.7%)	7 (13.5%)	26 (50.0%)
Ecuador	15	2 (13.3%)	1 (6.7%)	0	1 (6.7%)	13 (86.7%)
Egypt	11	5 (45.5%)	4 (36.4%)	1 (9.0%)	0	6 (54.5%)
France	31	15 (48.4%)	6 (19.4%)	2 (3.2%)	7 (22.6%)	16 (51.6%)
India	301	132 (43.8%)	73 (24.3%)	41 (13.6%)	18 (6.0%)	169 (56.1%)
Indonesian	21	6 (28.6%)	3 (14.3%)	2 (14.3%)	1 (4.8%)	15 (71.4%)
Japan	65	13 (20%)	1 (1.5%)	3 (4.6%)	8 (12.3%)	52 (80%)
Kuwait	17	12 (70.6%)	9 (52.9%)	1 (5.9%)	2 (11.8%)	5 (29.4%)
Mexico	12	4 (33.3%)	2 (16.7%)	0	2 (16.7%)	8 (66.7%)
Morocco	32	11 (34.4%)	9 (28.1%)	0	2 (6.3%)	21 (65.6%)
Romania	20	20 (100%)	20 (100%)	0	0	0
S. Arabia	62	57 (91.2%)	53 (85.5%)	0	4 (6.5%)	5 (8.0%)
Turkey	35	15 (42.9%)	8 (22.9%)	6 (17.1%)	1 (2.9%)	20 (57.1%)
US/Brazil	21	3 (4.8%)	1 (4.8%)	0	2 (9.5%)	18 (85.7%)

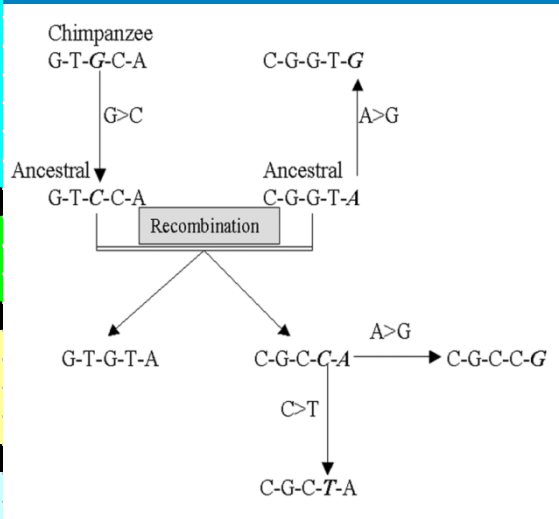
PCG is widely prevalent across the globe

HAPLOTYPE ANALYSIS

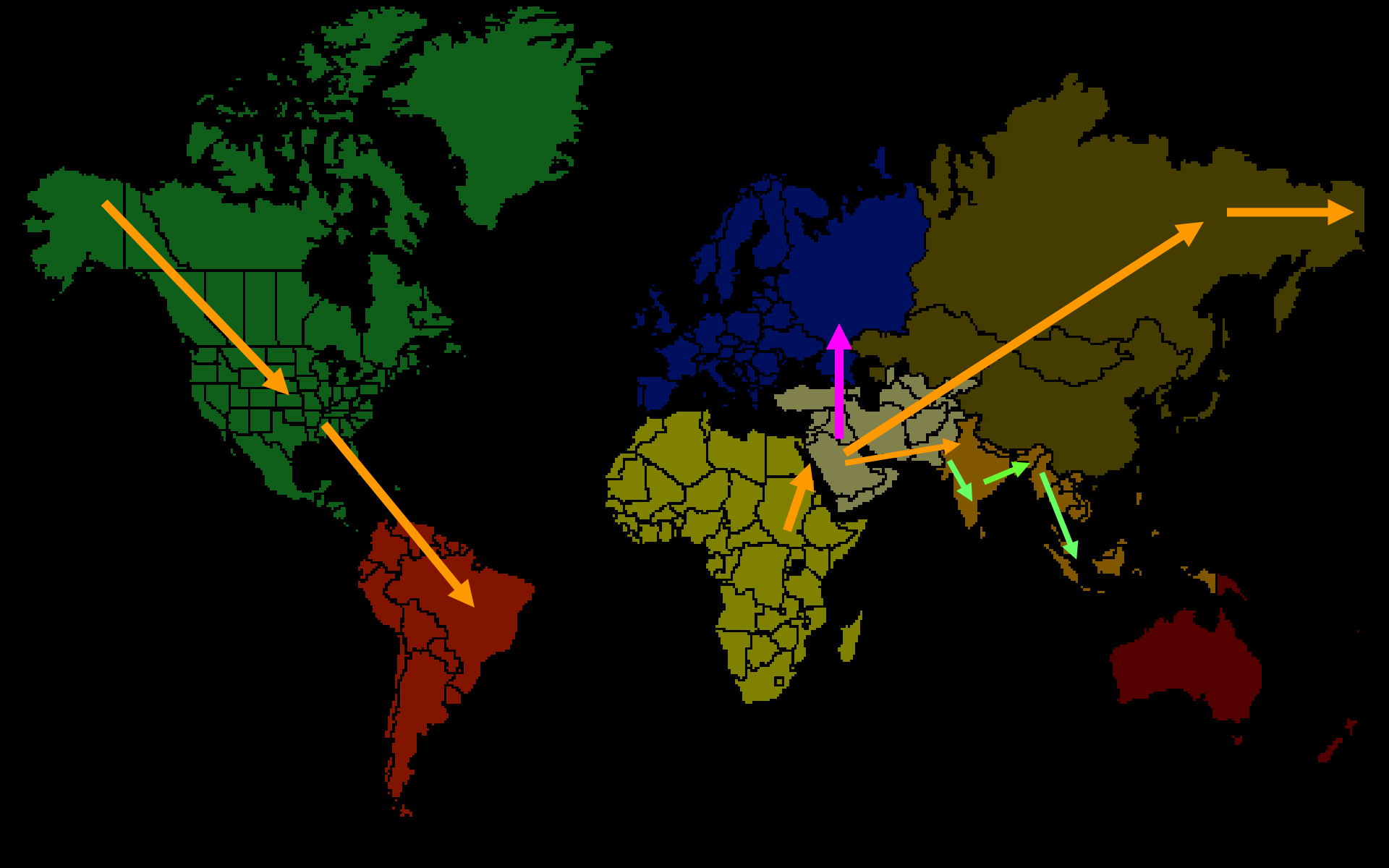
Haplotype (group of sequence variations and repeat sequences that occur in tandem) analysis was done on Indian patients first , vis-à-vis normal individuals; then these were compared with those found elsewhere in the world (from available data), in order to gain evolutionary insights. Note the occurrence of the haplotype s CGGTA and CGCCG across parts of the world, suggesting common ancestry. Indeed, compare this with chimpanzee, and see how variations could have occurred!! (Monkeys have glaucoma; Burgoyne 2015) Notice too the mutations G61E, R368H, R$69W occur across continents



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 C280X
JAPAN	C	G	C	C	ND	C280X
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



Globally, *CYP1B1* Mutations in Primary Congenital Glaucoma Are Strongly Structured by Geographic and Haplotype Backgrounds

Subhabrata Chakrabarti,[1] Kiranpreet Kaur,[1] Inderjeet Kaur,[1] Anil K. Mandal,[2,3] Rajul S. Parikh,[3] Ravi Thomas,[2,3] and Partha P. Majumder[4]

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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
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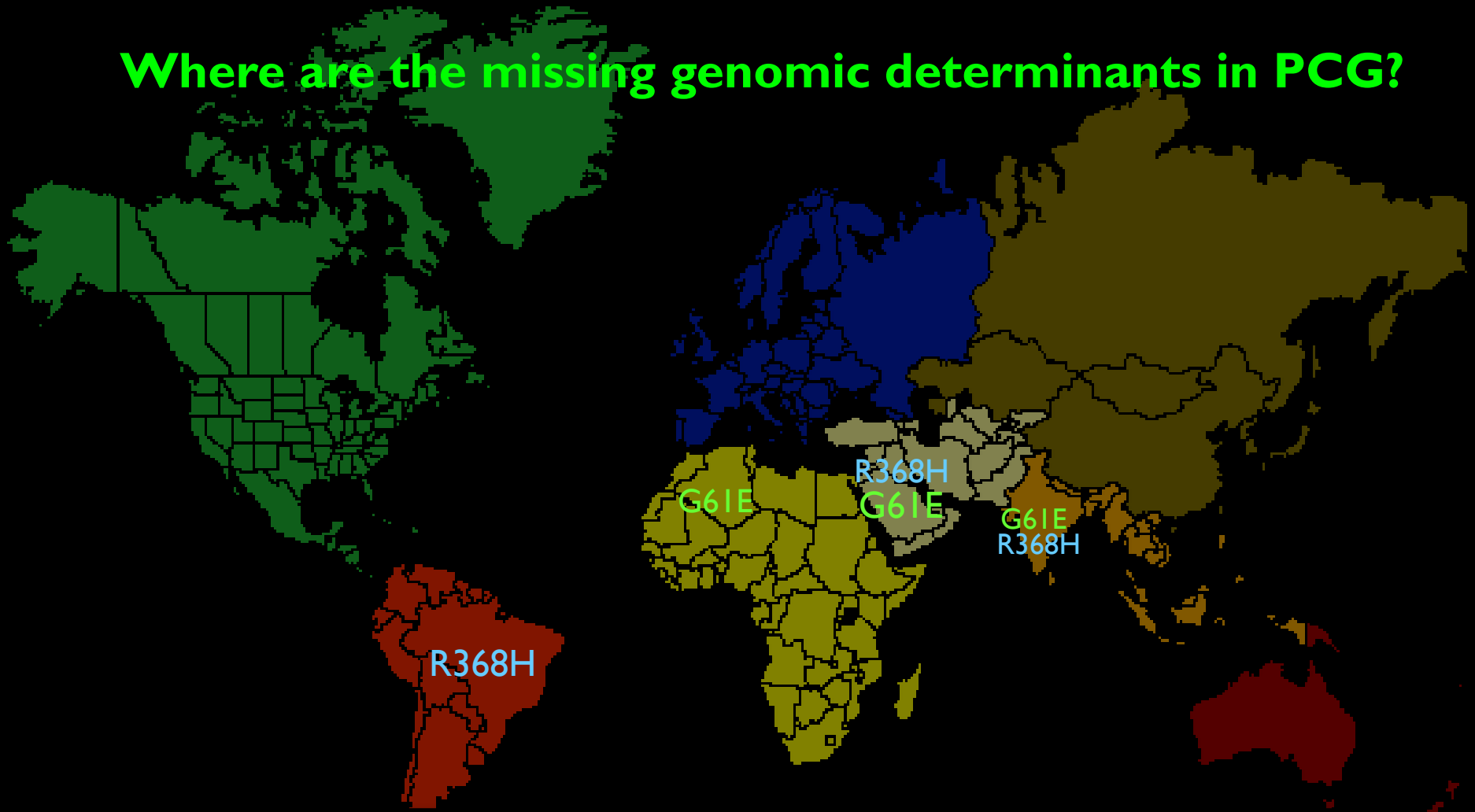
INDIA	C	G	C	C	G	L77P
PORTUGAL	C	G	C	C	G	8182 del G
BRAZIL	C	G	C	C	G	8182 del G
ROME	G	T	C	C	A	E387K
USA	G	T	C	C	A	E387K
BRAZIL	G	T	C	C	A	E387K

Invest Ophthalmol Vis Sci 2006; 47: 43 - 47

Where are the missing genomic determinants in PCG?



Gypsy genes may tells us the history

Conceptual Framework

- Isolated founder populations drawn from large ancestral population
  - Bulgarian gypsies // Indians
- Recent founding in evolutionary time scale
  - Ancestral disease causing allele on a same haplotype
- Haplotype sharing due to less founders
- Further validation in outbred populations
  - Indian
  - Australian
  - African
  - Brazilian

European Journal of Human Genetics (2010), 1–8

www.nature.com/ejhg

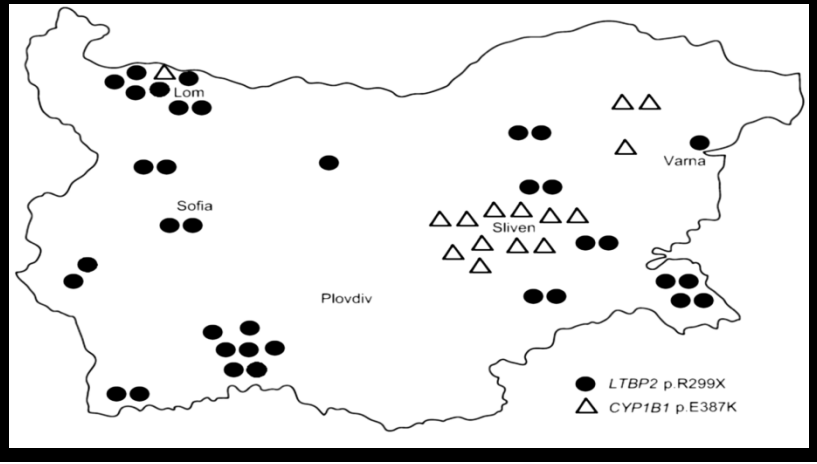
ARTICLE

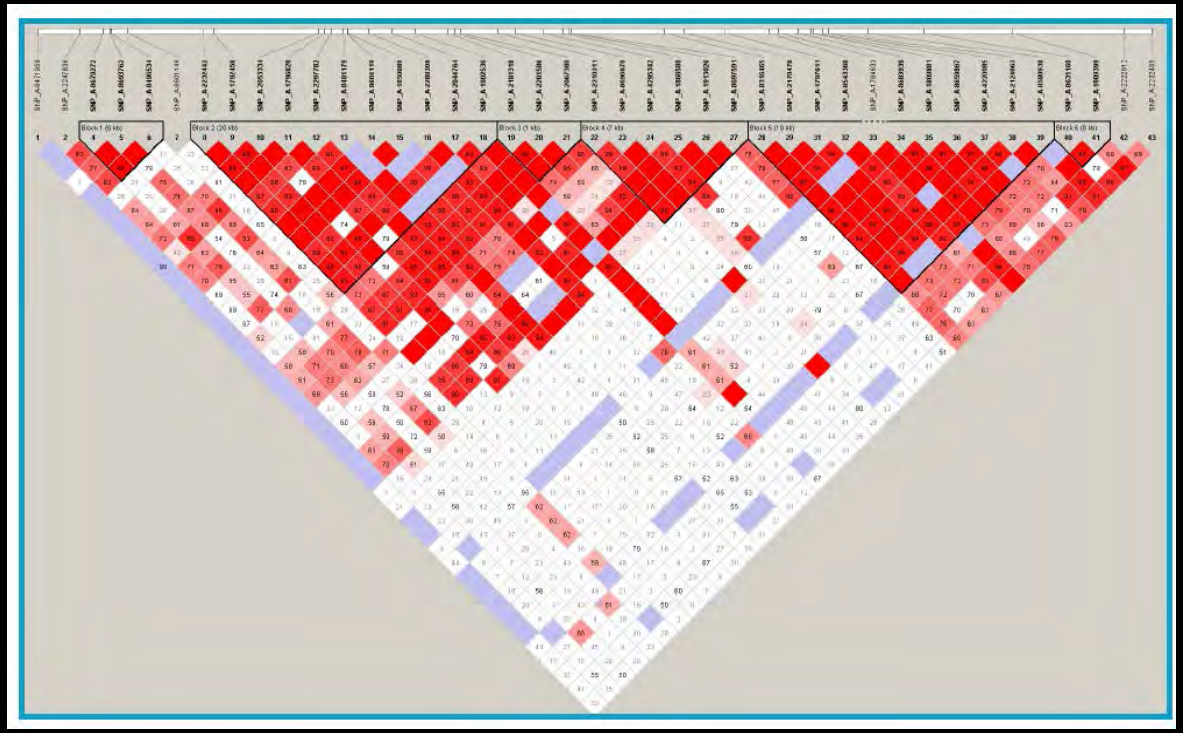
LTBP2 and *CYP1B1* mutations and associated ocular phenotypes in the Roma/Gypsy founder population

Dimitar N Azmanov[1], Stanislava Dimitrova[2,16], Laura Florez[1,16], Sylvia Cherninkova[3], Dragomir Draganov[4], Bharti Morar[1], Rosmawati Saat[1], Manel Juan[5], Juan I Arostegui[5], Sriparna Ganguly[6], Himla Soodyall[7], Subhabrata Chakrabarti[8], Harish Padh[9], Miguel A López-Nevot[10], Violeta Chernodrinska[11], Botio Anguelov[11], Partha Majumder[6,12], Lyudmila Angelova[13], Radka Kaneva[2], David A Mackey[14], Ivailo Tournev[3,15] and Luba Kalaydjieva*[,1]

CYP1B1 mutation	Haplotype	Indian PCG patients				Gypsy PCG patients
		South/ Dravidian	North	East Indo-European	West Indo-European	
R368H	C-G-G-T-A	13%	-	3%	15%	2.7%
	C-G-C-C-A	0.5%	-	-	-	-
E229K	G-T-C-C-A	0.7%	-	3%	1%	1.3%
	C-G-G-T-A	0.5%	-	-	-	-
	G-T-G-T-A	0.2%	-	-	-	-
R390C	C-G-C-C-A	3%	-	3%	-	-
	C-G-G-T-A	-	-	-	-	9.4%
E387K	G-T-C-C-A	-	-	-	-	17.6%

Population	Mutation	%
South INDIA (n=250)	Q329X	0.80%
	P1219T	0.40%
	V1543I	0.40%
	G1660W	0.80%
North INDIA (n=54)	-	-
Turkey (n=1)	-	-
British (n=90)	R538W	1.11%
US (n=57)	-	-
Iran (n=3)	S472fsX3	33.30%
	P989R	33.30%
	C1438Y	33.30%
Pakistan (n=4)	Q111X	25%
	R299X	25%
	E415RfsX596	25%
Gypsy (n=15)	R299X	53.30%





CONCLUSIONS, Thus far……

- The ROH harboring *KLHL26* and *TSHZ2* could be potential PCG-associated regions that needs further replications and functional validations
- Balkan gypsies and Indians may not share extended ROH, as thought hitherto
- Founder *LTBP2* mutations in gypsies and absence in Jats and PCG patients in Southern India, indicate rapid admixture and population stratification among them

PCG Cases with heterozygous for the candidate genes or with no variation (n=337)

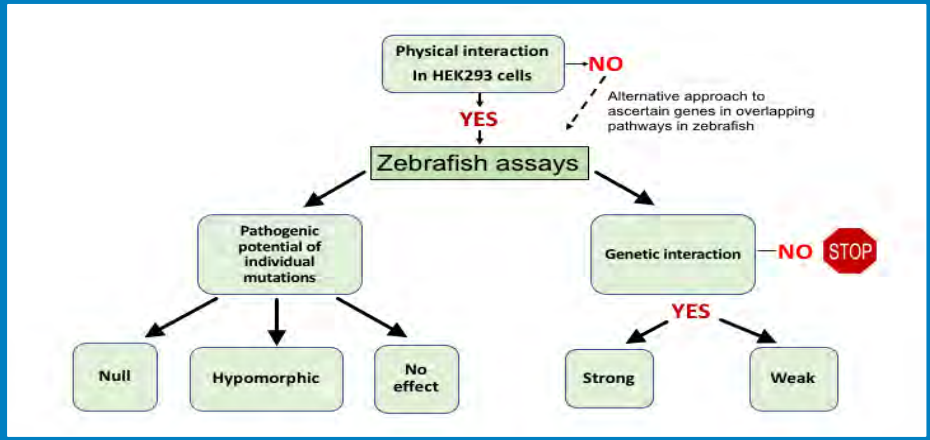
Targeted sequencing (Custom gene panel)

Homozygous mutations or heterozygous mutations in additional alleles detected (digenic inheritance)

No Mutations detected

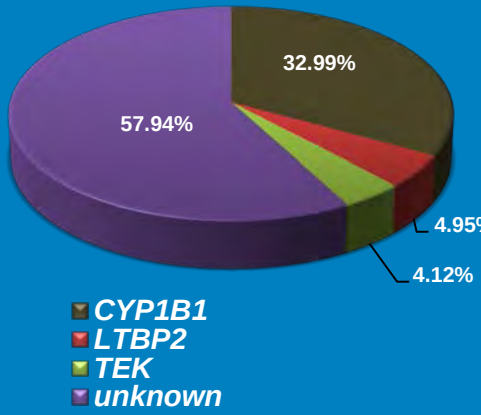
Mutation validation in additional PCG cases (n=300) and proceed to Aim 2

Whole exome sequencing to detect new mutations in a select set of trios (n=25)

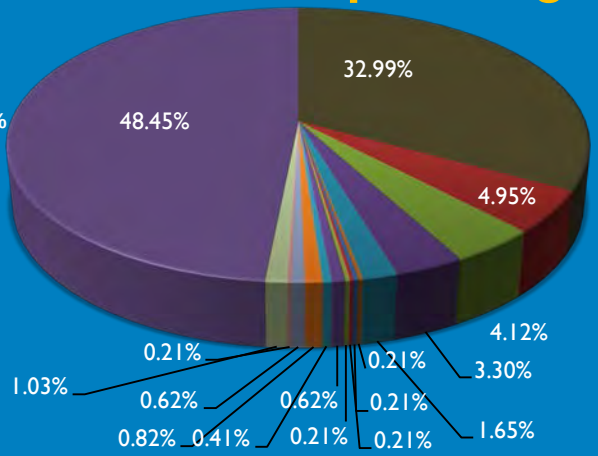


PCG (n=745)

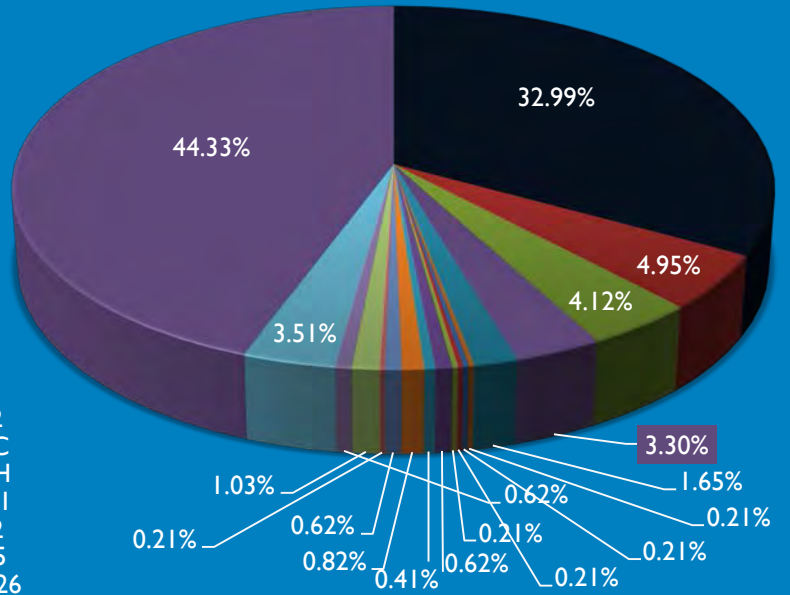
CYP1B1, LTBP2, TIE2



Targeted sequencing



Whole Exome Sequencing



CONCLUSIONS

- Exome sequencing indicated some novel genes that may be involved in PCG
- Functionally, these genes may interact with *CYP1B1* and other glaucoma-associated genes through a common biochemical pathway as they affect similar transcription factors involved in development
- The synergistic effects of these genes may hold key to PCG pathogenesis

INTEGRATED MANAGEMENT

6 months

25 years

LVPEI
So that all may see

Thank you!