The published papers and communicated manuscripts related to the dissertation are listed below:


2. Suddhasil Mookherjee, Moulinath Acharya, Deblina Banerjee, Ashima Bhattacharjee, Kunal Ray. Molecular basis for involvement of CYP1B1 in Myocilin mediated glaucoma pathogenesis (*Under Revision*).


8. EGLN1 involvement in high-altitude adaptation revealed through genetic analysis of extreme constitution types defined in Ayurveda. *Proc Natl Acad Sci*; 2010, 107(44):18961-6. [Contributed in the study as a member of Indian Genome Variation Consortium and as mentioned in the paper] (*Open Access*).
**WDR36 variants in East Indian primary open-angle glaucoma patients**

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**Purpose:** Glaucoma is a heterogeneous group of optic neuropathies with a complex genetic basis. To date, only the following four genes have been identified: viz. myocilin (MYOC), opticin (OPTN), WD repeat domain 36 (WDR36), and neurotrophin 4 (NTF4). However, there are conflicting reports regarding the involvement of WDR36 in the pathogenesis of primary open-angle glaucoma (POAG). In the Asian population, mutations in WDR36 appear to play a minor role in POAG pathogenesis but polymorphic variants have been found to be associated with POAG, especially in patients with high tension glaucoma (HTG). The purpose of this study is to determine the role of WDR36 in East Indian POAG patients. To date, no other studies have yet examined this role.

**Methods:** Ten single nucleotide polymorphisms (SNPs; rs1971050, rs1993465, rs13153937, rs10038177, rs11241095, rs10043631, rs10038058, rs10491424, rs17533936, and rs13186912) spanning almost the entire WDR36 gene were selected and their association with eastern Indian POAG patients was evaluated. Our study pool consisted of 323 POAG patients. Of these 116 were patients who had HTG with intraocular pressure (IOP) >21mmHg and 207 were found to be non-HTG patients (presenting IOP<21mmHg). The study also included 303 participants as controls. The polymorphisms were genotyped in both the patients and the controls using the PCR-RFLP method. Moreover, the SNP that showed significant association was validated by DNA sequencing. The haplotypes were obtained using Haploview 4.1 software. The allele and haplotype frequencies were compared between the patient group and the control group using Pearson’s X^2 test.

**Results:** First, we genotyped the selected SNPs in the 323 POAG patients and 119 of the participants in the control group, in which only rs10038177 (c.710+3001) was found to be strongly associated with the HTG cases (OR=2.186; 95% CI=1.458–3.277; p=1.4x10^-4). To increase the significance of the study, the SNP was genotyped in an additional 184 of the participants in the control group and it was observed that the SNP retained the association (OR=1.216; 95% CI=1.064–2.306; p=0.002). However, no haplotype was found to have any sustainable association with POAG. Based on the LD pattern and location of rs10038177, exon 5 of WDR36 was sequenced but no suspected disease-causing variant was detected.

**Conclusions:** Our study suggests a possible association between WDR36 SNP in a cohort of eastern Indian POAG patients who also have high intraocular pressure (IOP). This study needs to be further validated in a larger patient cohort.

Glucoma is a heterogeneous group of optic neuropathies with a complex genetic basis [1]. It diminishes vision, often without any symptoms or warning. After cataract, glaucoma is the second largest blinding disorder [2], The latest reports estimate that, in 2010, 60.5 million people had primary open-angle glaucoma (POAG). By 2020, this number is estimated to increase to 79.6 million. This could result in bilateral blindness in 8.4 million people in 2010 and 11.2 million people by 2020 [3]. Among the three principle subtypes of glaucoma [4], primary angle closure glaucoma (PACG) is reported to occur most frequently in the Asian and African population [5,6] while primary open-angle glaucoma (POAG) is the most frequently occurring subtype in the Western population [7,8]. However, Raychaudhuri et al. [9] reported that the incidence of POAG is more frequent than the incident of PACG (10:1) in the inhabitants of West Bengal (an eastern state of India); this region is the area in which this current study was conducted. The complexity of POAG has recently been reviewed in detail [1,10]. To date, 27 loci has been reported to be linked with POAG, but only four genes have been identified: viz. Myocilin (MYOC) [11], Optineurin (OPTN) [12], WD repeat domain 36 (WDR36) [13], and neurotrophin 4 (NTF4) [14, 15].

However, the articles that have been published following the discovery of WDR36 as a candidate for POAG suggested variable levels of involvement of the gene in the pathogenesis of this disease [16-24].

Most of the studies regarding the involvement of WDR36 in POAG have been conducted on Caucasian populations.
Association of \textit{IL1A} and \textit{IL1B} loci with primary open angle glaucoma

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\textbf{Abstract}

\textbf{Background:} Recent studies suggest that glaucoma is a neurodegenerative disease in which secondary degenerative losses occur after primary insult by raised Intraocular pressure (IOP) or by other associated factors. It has been reported that polymorphisms in the \textit{IL1A} and \textit{IL1B} genes are associated with Primary Open Angle Glaucoma (POAG). The purpose of our study was to investigate the role of these polymorphisms in eastern Indian POAG patients.

\textbf{Methods:} The study involved 315 unrelated POAG patients, consisting of 116 High Tension Glaucoma (HTG) patients with intra ocular pressure (IOP) > 21 mmHg and 199 non-HTG patients (presenting IOP < 20 mmHg), and 301 healthy controls from eastern India. Genotypes were determined by polymerase chain reaction and restriction digestion for three single nucleotide polymorphisms (SNPs): \textit{IL1A} (-889C/T; rs1800587), \textit{IL1B} (-511C/T; rs16944) and \textit{IL1B} (+3953C/T; rs1143634). Haplotype frequency was determined by Haploview 4.1 software. The association of individual SNPs and major haplotypes was evaluated using chi-square statistics. The p-value was corrected for multiple tests by Bonferroni method.

\textbf{Results:} No significant difference was observed in the allele and genotype frequencies for \textit{IL1A} and \textit{IL1B} SNPs between total pool of POAG patients and controls. However, on segregating the patient pool to HTG and non-HTG groups, weak association was observed for \textit{IL1A} polymorphism (-889C/T) where -889C allele was found to portray risk (OR = 1.380; 95% CI = 1.041-1.830; p = 0.025) for non-HTG patients. Similarly, 3953T allele of \textit{IL1B} polymorphism (+3953GT) was observed to confer risk to HTG group (OR = 1.361; 95% CI = 1.022-2.385; p = 0.039). On haplotype analysis it was observed that TTC was significantly underrepresented in non-HTG patients (OR = 0.538; 95% CI = 0.356-0.815; p = 0.003) while TCT haplotype was overrepresented in HTG patients (OR = 1.784; 95% CI = 1.084-2.937; p = 0.022) compared to control pool. However, after correction for multiple tests by Bonferroni method, an association of only TTC haplotype with non-HTG cases sustained (p\textsubscript{corrected} = 0.015) and expected to confer protection.

\textbf{Conclusion:} The study suggests that the genomic region containing the \textit{IL1} gene cluster influences the POAG pathogenesis mostly in non-HTG patients in eastern India. A similar study in additional and larger cohorts of patients in other population groups is necessary to further substantiate the observation.

\textbf{Background}

Glaucoma is a heterogeneous group of optic neuropathy, characterized by typical visual field loss, often associated with elevated intra ocular pressure. It affects over 60 million people worldwide and is the second largest cause of blindness after cataract [1]. Among different subtypes of glaucoma, Primary Open Angle Glaucoma (POAG) is the most frequently occurring subtype. Till date 25 loci have been implicated in the pathogenesis of POAG with three underlying genes, i.e. \textit{Myocilin} [2], \textit{Optineurin} [3] and \textit{WDR36} [4]. Recent studies suggest that POAG is caused mainly by genetic predisposition and interaction with other risk factors. It is estimated that 72% of all POAG cases represent the inherited and familial form of the disease that does not show a clear pattern of Mendelian inheritance [5].

POAG results from progressive excavation of the optic disc with corresponding loss of vision by raised Intraocular pressure (IOP) or by other associated factors. How-
Leu432Val polymorphism in CYP1B1 as a susceptible factor towards predisposition to primary open-angle glaucoma

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Purpose: Defects in cytochrome P450 1BI (CYP1B1) cause primary congenital glaucoma. However, defects in the gene have also been reported in primary open-angle glaucoma (POAG). Since POAG is primarily a complex disease, we examined the potential of coding single nucleotide polymorphisms (cSNPs) in the gene for association with the disease.

Methods: Five coding SNPs - c.514 C>G (Arg48Gly), c.727 G>T (Ala119Ser), c.1666 C>G (Leu432Val), c.1719 C>T (Asp449Asp), and c.1730 A>G (Asn453Ser) – were genotyped in 264 unrelated POAG patients and 95 controls. In addition, 542 normal individuals selected from various ethnic groups representing the Indian population were also genotyped for these cSNPs. The patterns of linkage disequilibrium between the SNPs and haplotype variations for comparison between POAG patients and controls as well as different ethnic groups of the Indian population were determined using Haploview. Allelic variants of Leu432Val were cloned by site-directed mutagenesis of normal cDNA, which were used for transfection of retinal pigment epithelium (RPE) cells. The generation of reactive oxygen species (ROS) was quantified by measuring fluorescence emission by degradation of CM-H2DCFDA using a fluorometer.

Results: The c.1666G allele of the Leu432Val in CYP1B1 showed a statistically significant higher representation among POAG patients compared to controls (p=0.0001; Odds ratio=6.027; 95% CI: 3.863-9.401) suggesting it to be a potential risk allele toward disease predisposition. Analysis of genotype frequencies of the polymorphism between the two groups demonstrated GG as a potential risk genotype (p=0.0001; Odds ratio=15.505; 95% CI: 5.529-43.474) for the disease. CYP1B1 Val432 was estimated to generate higher ROS in RPE cells compared to its allelic variant (Leu432; p=0.0245 for 15 min and p=0.0197 for 30 min). Comparison of haplotype diversities revealed CGGTA as the risk haplotype for the disease (p=0.0001, by Fisher's exact test).

Conclusions: We report CYP1B1 c.1666G (Val432) as a susceptible allele for POAG and CGGTA as the risk haplotype for the disease. Higher ROS generation by Val432 in CYP1B1 might lead to apoptotic change that leads to glaucoma. Remarkable variation of the cSNPs observed among ethnic groups of India could provide insight for future epidemiological studies on POAG in these population groups.

Primary open-angle glaucoma (POAG) is the most common form of glaucoma. Among 14 implicated chromosomal loci (GLC1A – GLC1N) [1-12], three underlying candidate genes have been identified – myocilin (MYOC), optineurin (OPTN), and WD40-repeat 36 (WDRL36) [2,13,14]. Recent studies suggest that POAG is caused mainly by genetic predisposition and interaction with other risk factors [15]. The study, which is based on published literature, estimated that 72% of all POAG cases represent the inherited or familial form of the disease that does not show a clear pattern of Mendelian inheritance.

Among genes implicated to have a potential role in POAG causation, cytochrome P450 1BI (CYP1B1) poses as an interesting candidate for investigation. CYP1B1, a member of the cytochrome p450 family of monooxygenases, is also widely known for its role in steroid metabolism and xenometabolic detoxification [16]. Defects in CYP1B1 cause an autosomal recessive form of primary congenital glaucoma (PCG) [17,18]. In addition, recent studies indicate the gene’s role in anterior segment dysgenesis like Peters’ anomaly [18]. The gene has been found to be involved in expediting disease onset in a familial case of open-angle glaucoma when present alongside a heterozygous mutation in MYOC. Therefore, the gene acts as a modifier locus [19]. Moreover, CYP1B1 has recently been shown to have primary involvement in a familial case of juvenile onset POAG [20,
Myocilin Variants in Indian Patients With Open-angle Glaucoma

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Objective: To identify and evaluate MYOC variant alleles among patients with primary open-angle glaucoma (POAG) and age-matched control subjects in an Indian population.

Methods: Three hundred fifteen patients with POAG and 100 unrelated control subjects from the same ethnic background were enrolled in the study. The coding sequence of MYOC was amplified by polymerase chain reaction using genomic DNA, followed by sequencing of the polymerase chain reaction products. Four single nucleotide polymorphisms were genotyped in different Indian subpopulations comprising 1,466 individuals using SEQUENOM's homogeneous MassEXTEND assay.

Results: One novel mutation (Gly399Asp), 6 reported mutations (Gln48His, Thr256Met, Thr353Ile, Gln368Stop, Pro370Leu, and Ala427Thr), and 6 single nucleotide polymorphisms were identified in MYOC. Ala427Thr was identified in a patient with POAG and Parkinson disease. Four single nucleotide polymorphisms genotyped in control subjects were highly heterozygous and displayed a similar pattern of linkage disequilibrium among all linguistic groups.

Conclusions: MYOC mutations account for 2.2% of POAG cases. The Gln368Stop mutation (common among persons of the white race) found in 2 families does not seem to be of white race origin. Identification of a MYOC mutation (Ala427Thr) in a patient with POAG and Parkinson disease is interesting with respect to reported interaction of myocilin with synucleins.

Clinical Relevance: Studying the genetics of POAG is helpful for preclinical identification and for better disease management.

Arch Ophthalmol. 2007;125:823-829

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Glaucoma is a heterogeneous group of optic neuropathies with a complex genetic basis. It is a multifactorial optic disc neuropathy in which there is a characteristic acquired loss of retinal ganglion cells and atrophy of the optic nerve.1 Primary open-angle glaucoma (POAG) (Online Mendelian Inheritance in Man [OMIM] 137760) is the most prevalent of the glaucoma subtypes. The disease is known to be transmitted as a monogenic disease and as a complex disease. Adult-onset POAG is inherited as a non mendelian trait, whereas juvenile-onset POAG exhibits autosomal dominant inheritance.2 Among 11 implicated loci and 3 identified candidate genes, mutations in the myocilin gene (MYOC) have been most widely studied. Although the pathophysiology is unknown, it has been suggested that mutant MYOC obstructs the outflow of the aqueous humor through the trabecular meshwork, resulting in increased intraocular pressure, which is frequently associated with glaucoma.1

MYOC, located on chromosome 1 (at 1q24.3) and spanning a genomic DNA region of approximately 17 kilobases (kb), contains 3 exons and is expressed as a 2.3-kb transcript with the translated product of 304 amino acids.2 Most of the identified MYOC mutations are located in exon 3.1 About 1.3 million people are reported to have blindness because of glaucoma in India.4 Previous studies5 among Indian populations were based on small samples of patients with POAG. In this study, we evaluated a larger subset of patients to investigate the molecular basis of POAG among eastern Indian patients by screening MYOC for causal variants. Because POAG is a complex disease, it is likely that it may be caused by an interplay of multiple genes and environmental factors.
RESEARCH ARTICLE

Genetic landscape of the people of India: a canvas for disease gene exploration

INDIAN GENOME VARIATION CONSORTIUM*

Abstract

Analyses of frequency profiles of markers on disease or drug-response related genes in diverse populations are important for the dissection of common diseases. We report the results of analyses of data on 405 SNPs from 75 such genes and a 5.2 Mb chromosome, 22 genomic region in 1871 individuals from diverse 55 endogamous Indian populations. These include 32 large (>10 million individuals) and 23 isolated populations, representing a large fraction of the people of India. We observe high levels of genetic divergence between groups of populations that cluster largely on the basis of ethnicity and language. Indian populations not only overlap with the diversity of HapMap populations, but also contain population groups that are genetically distinct. These data and results are useful for addressing stratification and study design issues in complex traits especially for heterogeneous populations.

Introduction

Genetically isolated populations are considered to be important in dissecting complex diseases and mapping underlying genes (Wright et al. 1999; Peltonen 2000; Heutink and Oostra 2002; Abecasis et al. 2005). However, the validation of results across populations has met with limited success. Population stratification, a consequence of differences in allele frequencies across populations arising mainly due to natural selection and genetic drift, is a major problem in association studies. It is, therefore, important to assess the nature and extent of population stratification in contemporary endogamous populations especially in the context of established or candidate disease genes. Indians, comprising about one-sixth of the world population, with large family sizes and high levels of endogamy, provide a unique resource for dissecting complex disease etiology and pathogenesis. Further, India provides a large patient pool with the majority being drug-naive. Historically, the Indian population is a conglomeration of multiple culture and evolutionary histories. Anatomically modern man is estimated to have reached the north-western periphery of the Indian subcontinent around 70,000 ybp and moved southward into Sri Lanka in the next 20,000 years (Habib 2001, 2002; Singh 2002). Modern human communities may also have migrated into eastern India from Myanmar around 4500 to 11,000 ybp (Habib 2001, 2002; Singh 2002). The evolutionary antiquity of Indian ethnic groups and subsequent migration from central Asia, west Asia and southern China has resulted in a rich tapestry of socio-cultural, linguistic and biological diversity. Broadly, Indians belong to Austro-Asiatic (AA), Tibeto-Burman (TB), Indo-European (IE) and Dravidian (DR) language families. Distinct religious communities, hierarchical castes and subcastes, and isolated tribal groups that comprise the people of India remain largely endogamous. Most of these groups have strict social rules governing mating patterns. Earlier studies using mitochondrial, Y-chromosomal and limited autosomal markers, that primarily addressed issues of origin and migrations, have demonstrated extensive genetic diversity in India (Bamshad et al. 2001; Roychoudhury et al. 2001; Basu et al. 2003; Kivisild et al. 2003; Cordaux et al. 2004; Kashyap et al. 2006; Sahoo et al. 2006; Sengupta et al. 2006; Thanseem et al. 2006). In contrast, a recent study based on autosomal microsatellite markers has inferred that Indian populations show low levels of genetic differentiation (Rosenberg et al. 2006). This inference was possibly due to biased recruitment

Keywords. Indian genome variation; polymorphism; SNP; Asia; HapMap; complex disease; pharmacogenomics.
**EGLN1 involvement in high-altitude adaptation revealed through genetic analysis of extreme constitution types defined in Ayurveda**


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Edited* by Charles R. Cantor, Sequenom, San Diego, CA, and approved September 20, 2010 (received for review May 20, 2010)

It is being realized that identification of subgroups within normal controls corresponding to contrasting disease susceptibility is likely to lead to more effective predictive marker discovery. We have previously used the Ayurvedic concept of Prakriti, which relates to phenotypic differences in normal individuals, including responses to external environment as well as susceptibility to diseases, to explore molecular differences between three contrasting Prakriti types: Vata, Pitta, and Kapha. EGLN1 was one among 251 differentially expressed genes between the Prakriti types. In the present study, we report a link between high-altitude adaptation and common variations rs479200 (C/T) and rs480902 (T/C) in the EGLN1 gene. Furthermore, the TT genotype of rs479200, which was more frequent in Kapha types and correlated with higher expression of EGLN1, was associated with patients suffering from high-altitude pulmonary edema, whereas it was present at a significantly lower frequency in Pitta and nearly absent in natives of high altitude. Analysis of Human Genome Diversity Panel-Centre d'Etude du Polymorphisme Humain (HGDP-CEPH) and Indian Genome Variation Consortium panels showed that disparate genetic lineages at high altitudes share the same ancestral allele (T) of rs480902 that is overrepresented in Pitta and positively correlated with altitude globally (P < 0.001), including in India. Thus, EGLN1 polymorphisms are associated with high-altitude adaptation, and a genotype rare in highlanders but overrepresented in a subgroup of normal lowlanders discernable by Ayurveda may confer increased risk for high-altitude pulmonary edema.

**Results**

Distribution of Common Variations in Extreme Constitution Types. We studied the distribution of 141 tag SNPs encompassing 30 genes (Dataset S1) selected from the 251 differentially expressed genes between the V, P, and K from our earlier study in the same cohort (2). The details of recruitment and assessment of Prakriti types are provided in SI Materials and Methods. Ninety-two individuals who were not phenotyped for their constitution types but were from the same ethnogenetic background, namely Indo-European (IE), and large populations (IE-LP) were used as

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Conflict of interest statement: S.A, M.A.Q.P, B.P, and M.M. are the inventors and have filed patent application no. 13365121016 in India. There are no implications of this patent application on the publication of the manuscript, because the provisional patent application has already been filed. S.N, P.J, P.K.S, S.G, A.A, T.S, and The Indian Genome Variation Consortium have been acknowledged for contributing to the invention but do not fulfill the criteria of authorship.

*This Direct Submission article had a prearranged editor.

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