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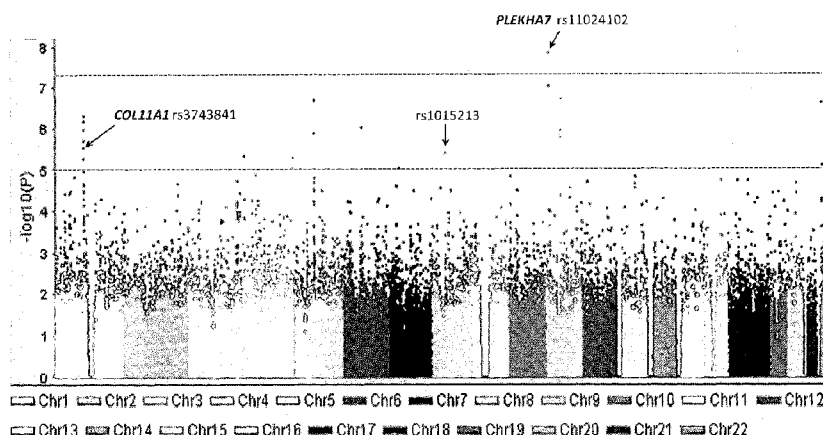
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(54) **Title:** METHOD AND/OR PROBE FOR DETERMINING GLAUCOMA SUSCEPTIBILITY

(57) **Abstract:** The present invention provides a method and/or probe for predicting and/or determining susceptibility to primary angle closure glaucoma in a subject. The method comprises and/or the probe is for determining the genotype of the subject for at least one single nucleotide polymorphism associated with primary angle closure glaucoma; wherein the single nucleotide polymorphism is selected from the group consisting of PLEKHA7 rs11024102, COL11A1 rs3753941 and rs1015213.

Method and/or probe for determining glaucoma susceptibility

Field of the invention

The present invention relates to the field of molecular biology, in particular in prediction and/or diagnosis of a condition and/or disease and/or determining
5 susceptibility to the condition and/or disease.

Background of the invention

Glaucoma is known to be a leading cause of irreversible blindness worldwide, characterised by progressive loss of axons in the optic nerve and corresponding visual field damage. Categorized according to the anatomy of the anterior
10 chamber angle, there are two main forms of glaucoma, primary open angle glaucoma (POAG) and primary angle closure glaucoma (PACG). PACG results from elevated intraocular pressure as a consequence of (or due to) iris-trabecular meshwork contact in the angle of the eye hindering aqueous outflow. While POAG is the more predominant form of glaucoma amongst Europeans
15 and Africans, 80% of the estimated 15 million people afflicted with PACG live in Asia. PACG is responsible for substantial blindness in many Asian countries, and in fact it has been estimated that PACG blinds proportionately more people than POAG globally. The pathogenesis of PACG likely involves multiple anatomical and physiological factors, and thus shows many indications of being
20 a complex disease, with both genetic and environmental etiological factors.

Summary of the invention

According to a first aspect, the present invention provides a method for predicting and/or determining susceptibility to primary angle closure glaucoma in a subject, comprising determining the genotype of the subject for at least one
25 variation in at least one single nucleotide polymorphism associated with primary angle closure glaucoma; wherein the single nucleotide polymorphism is

selected from the group consisting of *PLEKHA7* rs11024102, *COL11A1* rs3753941 and rs1015213.

The present invention also provides a probe for use in predicting and/or determining susceptibility to primary angle closure glaucoma in a subject, wherein the probe is for use in determining the genotype of the subject for at least one single nucleotide polymorphism associated with primary angle closure glaucoma, wherein the single nucleotide polymorphism is selected from the group consisting of *PLEKHA7* rs11024102, *COL11A1* rs3753941 and rs1015213.

10 Brief description of the figures

Figure 1 shows the principal component analysis between PACG cases and healthy controls for: (A) the Singaporean Chinese PACG collection, (B) the Hong Kong collection; (C) the Indian PACG collection; (D) the Malay PACG collection and (E), the Vietnam PACG collection; where the vast majority of cases have genetically matched controls and additional statistical correction for the top principal components using logistic regression was conducted to minimise residual population stratification.

Figure 2 shows the Manhattan plot of all Stage 1 data involving 1854 PACG cases and 9609 controls. Single nucleotide polymorphism (SNP) markers are plotted according to chromosomal location on the horizontal axis, with $-\log_{10}P$ -values on the vertical axis derived from the 1-degree-of-freedom score test; the lower horizontal line ($P < 1 \times 10^{-5}$) denotes the threshold used for bringing genetic loci forward for further testing in Stage 2. The upper horizontal line shows the formal threshold for genome-wide significance ($P < 5 \times 10^{-8}$).

PLEKHA7 rs11024102 showed evidence of association with PACG surpassing genome-wide significance on Stage 1 data alone.

Figure 3 pertains to Forest plots showing evidence of association between genome-wide significant SNPs; (a) *PLEKHA7* rs11024102, (b) *COL11A1* rs3753841, and (c) *PCMTD1* - *ST18* rs1015213. The vertical line represents a per-allele odds ratio of 1.00. The oblongs represent point estimates (referring to the per-allele odds ratio), with the height of the oblongs inversely proportional to the standard error of the point estimates. Horizontal lines indicate the 95% confidence interval for each point estimate. Meta-analysis of stages 1 and 2 are reflected by blue diamonds, and meta-analysis from all sample collections reflected by purple diamonds. The width of the diamonds indicates their 95% confidence intervals. The number in brackets before each sample collection denotes (minor allele frequency in PACG cases / minor allele frequency in controls).

Figure 4 shows Stage 1 data showing regional association (upper panel) and linkage disequilibrium (LD; lower panel) plots of *PLEKHA7* locus around rs11024102, (B) *COL11A1* locus around rs3753841, and (c) Chromosome 8q locus around rs1015213. Gene annotations are superimposed. The vertical axis represents $-\text{Log}_{10}P$ -values for association with PACG, and the horizontal axis represents base-pair positions along the chromosome.

Figure 5 shows the expression analysis of *PLEKHA7*, *COL11A1*, *PCMTD1* and *ST18* in human ocular tissues. Gene specific RT-PCR products, confirmed to be so through re-sequencing, were observed differentially in anterior sclera (AS), posterior sclera (PS), cornea (corneal epithelium, CE; stroma, CS; and endothelium, CEn), iris (I), trabecular meshwork (TM), lens (L), lens capsule (LC), retina and retinal pigment epithelium (R), choroid (CRD), optic nerve head (ONH) and optic nerve (ON). The ubiquitously expressed gene, *ACTB* was used as the normalizing control. A no template sample acted as the negative control (NC) to ensure non-contamination of the RT-PCR reaction mix. M denotes molecular-weight marker.

Figure 6 shows the genotyping cluster plot for (A) rs11024102; (B) rs1015213 and (C) rs3753841.

Definitions

5 “Determine” or “determining” means to find out or come to a decision about by investigation, reasoning, or calculation, or to settle or decide from alternatives or possibilities; “determine” or “determining” encompasses the meaning of “predicting” or “assessing”.

10 “Determining the genotype of a subject for a nucleic acid variation” refers to determining the genetic constitution or makeup of the subject for that nucleic acid variation and includes determining the absence or presence of the nucleic acid variation and; where the nucleic acid variation comprises several alternatives, determining the alternative in the subject.

15 A “single nucleotide polymorphism (SNP)” refers to a DNA and/or RNA sequence variation occurring when a single nucleotide in an organism’s DNA sequence differs between members of the species (or between paired chromosomes in the organism).

A “primer” refers to an oligonucleotide to which deoxyribonucleotides may be added by a DNA polymerase. A single primer may be used to amplify a DNA or RNA region, for example, for sequencing.

20 A “primer pair” usually comprises a first primer complementary to one strand of a DNA or RNA molecule and a second primer complementary to a second strand of a DNA or RNA molecule, with both primers flanking a target DNA or RNA region, to be amplified by a DNA polymerase.

Detailed description of the invention

The present invention provides a method for predicting and/or determining susceptibility to primary angle closure glaucoma in a subject, comprising determining the genotype of the subject for at least one variation in at least one
5 single nucleotide polymorphism associated with primary angle closure glaucoma; wherein the single nucleotide polymorphism is selected from the group consisting of *PLEKHA7* rs11024102, *COL11A1* rs3753941 and rs1015213.

Further, the present invention also provides a probe for use in predicting and/or
10 determining susceptibility to primary angle closure glaucoma in a subject, wherein the probe is for use in determining the genotype of the subject for at least one single nucleotide polymorphism associated with primary angle closure glaucoma, wherein the single nucleotide polymorphism is selected from the group consisting of *PLEKHA7* rs11024102, *COL11A1* rs3753941 and
15 rs1015213.

The method comprises determining the genotype, nucleic acid variation and/or the allele present in the genomic sequence. The method may detect the nucleic acid variation from genomic DNA. In particular, the method is performed on an isolated sample from the subject. The method comprises determining at least
20 one nucleic acid variation and/or allele present for at least one of the single polynucleotide polymorphism in the subject.

The method may comprise determining the genotype, nucleic acid variation and/or allele present for one, two or all three of the single nucleotide polymorphisms. As a first example, the single nucleotide polymorphism
25 comprises *PLEKHA7* rs11024102. In another example, the single polymorphism comprises *COL11A1* rs3753941. In yet another example, the single polynucleotide polymorphism comprises rs1015213.

For examples, the probe is for use in detecting the *PLEKHA7* rs11024102, *COL11A1* rs3753941 or rs1015213 single nucleotide polymorphism. The nucleic acid variation may comprise different alleles of the single nucleotide polymorphism. Accordingly, the probe may be used to identify different alleles.

- 5 The probe is for use in determining the genotype, nucleic acid variation and/or the allele present in the genomic sequence. The probe may comprise at least one nucleic acid molecule. For example, different nucleic acid molecules hybridizing to different variants of the single nucleotide polymorphism may be used to identify the nucleic acid variation. Different hybridisation probes may be
- 10 differentially labelled. Detection of hybridized probes may be by any conventional method, including but not limited to fluorescence-based hybridization assays; chemiluminescence-based hybridization assays; and capture hybridization microtitre assays.

- The nucleic acid molecules comprising the probes may also be used as
- 15 polymerase chain reaction (PCR) primers. PCR may be performed on genomic DNA with PCR primer pairs. Reverse-transcriptase PCR may also be performed. Conventional PCR may be performed or real-time PCR may be performed. Detection of PCR products may be by conventional methods, including but not limited to agarose gel electrophoresis, polyacrylamide gel
- 20 electrophoresis and capillary electrophoresis. The PCR primers may also be labelled for detection.

- A combination of PCR and hybridisation techniques may also be used to identify the nucleic acid variation. Genotyping methods as described in Ragoussis (2009) may also be used for identifying the nucleic acid variation.
- 25 Alternatively, DNA sequencing may also be used to identify the nucleic acid variation.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

EXAMPLES

- 5 Standard molecular biology techniques known in the art and not specifically described were generally followed as described in Sambrook and Russel, Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (2001).

Example 1

- 10 A two-staged genome-wide association analysis (GWAS) was conducted with replication involving 3771 cases and 18551 controls to identify sequence variants that confer susceptibility to PACG. The discovery staged (Stage 1) included 1854 PACG cases and 9608 controls recruited across 5 independent collections (Singapore, Hong Kong, India, Malaysia and Vietnam; Table 1). The
15 replication stage (Stage 2) included a further 1917 PACG cases and 8943 controls across 6 independent collections (two sites in China, and one site each in Singapore, India, Saudi Arabia and the UK; Table 1).

Table 1: Sample collections of PACG cases and controls for Stages 1 (GWAS discovery) and 2 (replication).

Stage 1		
Collection	PACG cases (N)	Controls (N)
Singapore (Chinese)	984	943
Hong Kong (Chinese)	297	1,044
Malaysia (Malays)	83	3,065
India (Indians)	337	2,538
Vietnam (Vietnamese)	153	2,018
All Stage 1	1,854	9,608

Stage 2		
Collection	PACG cases (N)	Controls (N)
Singapore (Chinese)	309	1,479
Beijing (Chinese)	992	1,672
Saudi (Middle Eastern descent)	165	175
UK (European descent)	127	4,703
India (Indians)	80	309
Shantou (Chinese)	244	605
All Stage 2	1917	8943
All samples	3771	18551

Uniform quality control (QC) filters were applied for both individual samples and SNP markers across all 5 PACG case-control collections for Stage 1. From a starting number of 1925 PACG cases and 9630 controls, genotype data on 493501 SNPs were available for 1,854 PACG cases and 9608 controls after stringent QC were applied on SNPs and samples. Within each sample collection, it was ensured that each PACG case had genetically matched controls as visualized spatially using principal component analysis (Figure 1). The genotypes between PACG cases and healthy controls were contrasted via single-SNP analysis using unconditional logistic regression fitted for genotype trend effects (a 1-degree-of-freedom score test). As the genetic matching between cases and controls was not perfect, the association tests were subjected to adjustments on significant axes of population ancestry to remove any residual population stratification which may be present within each collection following procedures as described in The Australia and New Zealand Multiple Sclerosis Genetics Consortium (2009), Hoglinger *et al.*, (2011), Khor *et al.*, (2011a), Kooner *et al.*, (2011) and Reveille *et al.*, (2010). This was followed by random-effects meta-analysis using inverse-variance weights (Nalls *et al.*, 2011). No evidence of genomic inflation ($\lambda_{gc} \leq 1.00$) was observed, thereby excluding the likelihood of significant cryptic population substructure between cases and controls. From Stage 1 data alone, genome-wide significance at

PLEKHA7 rs11024102 on Chromosome 11 was observed (Table 2, Figures 2 and 3; per-allele OR = 1.27, $P = 1.43 \times 10^{-8}$).

A total of 15 SNPs at 13 independent loci showing evidence of association with PACG exceeding $P < 1 \times 10^{-5}$ with no evidence of heterogeneity (I^2 index = 0.0%) across the 5 sample collections in Stage 1 were brought forward for replication genotyping in Stage 2, where a further 1,973 PACG cases and 9,066 controls were enrolled. Similar QC filters were applied to SNPs and samples in this stage, and a total of 1,917 PACG cases and 8,943 controls passing QC were brought forward for association analysis. Three SNP markers (*PLEKHA7* rs11024102, *COL11A1* rs3753841, and rs1015213) showed significant evidence of replication in Stage 2 ($3.72 \times 10^{-5} \leq P \leq 1.77 \times 10^{-4}$), and surpassed genome-wide significance on meta-analysis of all data from both stages ($5.33 \times 10^{-12} \leq P \leq 3.29 \times 10^{-9}$; Table 2 and Figure 3). Regional association analysis for these three sequence variants clearly identified *PLEKHA7* and *COL11A1* as the likeliest PACG candidate susceptibility genes at the Chromosome 11 and Chromosome 1 hit regions respectively, whilst rs1015213 was located in an intergenic region between *PCMTD1* and *ST18* (Figure 4). Evidence of association at loci previously associated with POAG was not observed (Table 3). Conditional logistic regression did not reveal secondary signals of association at each of the 3 genome-wide significant loci, suggesting that the most significant SNP reported largely accounts for the observed disease associations (Tables 4, 5, 6). As there is a paucity of information on these genes in the eye, the expression of *PLEKHA7*, *COL11A1*, *PCMTD1* and *ST18* was tested in several eye tissues. Significant expression of *PLEKHA7*, *COL11A1*, and *PCMTD1* was observed in the iris and trabecular meshwork, the ocular tissues intimately involved in the pathogenesis of PACG. Expression of *ST18* was not observed in either tissue (Figure 5).

Table 2 Hits from the GWAS on primary angle closure glaucoma

Chr	SNP	Gene	A1	GWAS (Stage 1)		Replication (Stage 2)		All data (Stages 1 + 2)			
				OR	P	OR	P	OR	P	Phet	I ²
11	rs11024102	PLEKHA7	G	1.27	1.43×10^{-8}	1.18	3.72×10^{-5}	1.22	5.33×10^{-12}	0.64	0%
1	rs3753841	COL11A1	G	1.22	2.82×10^{-6}	1.18	6.62×10^{-5}	1.20	9.22×10^{-10}	0.59	0%
8	rs1015213	PCMTD1 - ST18	A	1.56	3.90×10^{-6}	1.44	1.77×10^{-4}	1.50	3.29×10^{-9}	0.19	19.0%

Top tier SNP markers exceed $P < 5 \times 10^{-8}$ on meta-analysis.

TXNRD2 rs3788317 showed Stage 2 $P < 0.05$, and will need further studies for definite verification.

A1: effect allele

OR: per-allele odds ratio of the effect allele

P: P-value for association

Phet: P-value for heterogeneity

I²: I-squared index quantifying heterogeneity (see Methods)

Table 3 Stage 1 association tests (1,854 PACG cases vs 9,608 controls) looking up previously reported susceptibility loci in Primary Open Angle Glaucoma (POAG).

Chr.	SNP	Gene	A1	Collection	P	OR	P _{het}	I ²
7	rs4236601	CAV1-CAV2	A	Singapore	0.088	1.70	0.37	6.14%
				Hong Kong	0.31	0.60		
				India	0.16	1.16		
				Vietnam	0.63	0.77		
				Malays	0.73	0.87		
				All Stage 1	0.26	1.13		
7	rs1052990	CAV1-CAV2	C	Singapore	0.63	0.96	0.3875	3.37%
				Hong Kong	0.56	0.92		
				India	0.26	1.11		
				Vietnam	0.090	1.31		
				Malays	0.79	1.07		
				All Stage 1	0.42	1.05		
9	rs4977756	CDKN2BAS	G	Singapore	0.079	0.87	0.0253	64.03%
				Hong Kong	0.20	0.86		
				India	0.013	1.26		
				Vietnam	0.77	0.96		
				Malays	0.65	1.09		
				All Stage 1	0.93	0.99		
9	rs10120688	CDKN2BAS	G	Singapore	0.30	0.93	0.1624	38.82%
				Hong Kong	0.33	0.90		
				India	0.052	1.18		
				Vietnam	0.40	1.14		
				Malays	0.77	0.95		
				All Stage 1	0.86	1.01		
1	rs10800150	TMCO1	A	Singapore	0.53	0.96	0.0385	60.46%
				Hong Kong	0.017	1.26		
				India	0.19	1.18		
				Vietnam	0.017	1.35		
				Malays	0.11	1.31		
				All Stage 1	0.039	1.17		

1	rs2790051	TMCO1	A	Singapore	0.41	0.93	0.0225	64.87%
				Hong Kong	0.12	1.20		
				India	0.12	1.14		
				Vietnam	0.0011	1.56		
				Malays	0.97	1.01		
				All Stage 1	0.12	1.14		
14	rs10483727	SIX1	G	Singapore	0.12	1.13	0.0047	73.34%
				Hong Kong	0.076	1.23		
				India	0.042	0.84		
				Vietnam	0.034	0.72		
				Malays	0.36	0.82		
				All Stage 1	0.62	0.95		

P: *P*-value for association with PACG.

OR: per-allele odds ratio for the effect allele (A1).

*P*_{het}: *P*-value for heterogeneity in Stage 1 meta-analysis.

5 *I*²: I-squared index for heterogeneity.

Table 4 Conditional logistic regression analysis at the *PLEKHA7* locus on Chromosome 11; the conditioning SNP is rs11024102

SNP	BP	A1	Before conditioning		After conditioning	
			<i>P</i>	OR	<i>P</i>	OR
rs11024102	16965181	G	7.97 x 10 ⁻⁹	1.28	-	-
rs6486334	16972133	A	9.07 x 10 ⁻⁸	1.25	0.027	0.84
rs3950680	17095242	A	9.67 x 10 ⁻⁵	1.18	0.63	0.97
rs12577525	17216692	A	1.04 x 10 ⁻⁴	1.18	0.73	0.98
rs10832757	17292907	G	2.27 x 10 ⁻⁴	1.17	0.22	1.06
rs2354867	17327277	A	5.50 x 10 ⁻⁴	1.16	0.19	1.06
rs10766383	17286374	C	6.39 x 10 ⁻⁴	1.16	0.33	1.05
rs4756887	17351173	A	8.96 x 10 ⁻⁴	1.15	0.34	1.05
rs214105	17270097	G	9.10 x 10 ⁻⁴	1.15	0.96	1.00
rs7127347	17257420	C	0.0011	0.87	0.30	0.95
rs1330	17272605	A	0.0030	1.13	0.87	0.99
rs366590	16829016	A	0.0055	1.14	0.020	1.12
rs1557765	17360215	A	0.021	1.10	0.64	0.98
rs757110	17375053	C	0.035	1.09	0.89	0.99
rs2285676	17364601	A	0.036	1.09	0.95	1.00

Table 5 Conditional logistic regression analysis at the COL11A1 locus on Chromosome 1; the conditioning SNP is rs3753841.

SNP	BP	A1	Before conditioning		After conditioning	
			P	OR	P	OR
rs3753841	103152506	G	3.85×10^{-6}	1.22	-	-
rs1676486	103126726	A	1.09×10^{-5}	1.22	0.11	1.11
rs12138977	103166045	G	2.34×10^{-5}	1.21	0.76	1.03
rs7550513	102994366	A	3.04×10^{-5}	1.21	0.06	1.11
rs2229783	103125039	A	3.50×10^{-5}	1.19	0.95	1.01
rs12074523	103040347	A	4.99×10^{-5}	1.20	0.14	1.08
rs713162	102989469	A	6.89×10^{-5}	1.18	0.52	1.05
rs1020918	103103937	A	1.25×10^{-4}	1.18	0.65	1.03
rs1241163	103128353	A	1.47×10^{-4}	1.18	0.51	1.04
rs1085	103128756	A	1.86×10^{-4}	1.18	0.33	1.07
rs921416	103001167	G	2.76×10^{-4}	1.16	0.76	1.02
rs8179334	103139683	A	4.36×10^{-4}	1.18	0.79	1.02
rs12030173	102949180	C	9.44×10^{-4}	1.17	0.16	1.08
rs9659030	103114980	G	0.0011	1.16	0.59	1.03
rs1241202	103077609	C	0.0012	1.15	0.98	1.00
rs12022173	102874238	G	0.0016	1.17	0.17	1.08
rs11164556	102852775	A	0.0051	1.14	0.47	1.04
rs10874639	102906497	G	0.0083	1.13	0.43	1.04
rs11164585	102944531	A	0.010	1.13	0.34	1.05
rs1376359	102975695	A	0.012	1.11	0.88	1.01
rs4907975	102802807	G	0.021	1.10	0.99	1.00
rs7539441	102793412	G	0.033	1.09	0.92	1.00
rs2222748	103530366	G	0.036	1.16	0.22	1.09
rs967554	102836762	G	0.039	1.09	0.69	0.98
rs11164544	102785098	G	0.041	0.92	0.77	0.99
rs7517663	102781216	G	0.044	0.92	0.78	0.99

Table 6 Conditional logistic regression analysis at the *PCMTD1-ST18* locus on Chromosome 8; the conditioning SNP is rs1015213.

SNP	BP	A1	Before conditioning		After conditioning	
			P	OR	P	OR
rs1015213	53050094	A	9.38×10^{-6}	1.53	-	-
rs7846408	52523096	A	3.80×10^{-4}	0.84	0.0013	0.85
rs9792278	52593356	G	0.0016	0.88	0.0023	0.88
rs13271514	52561732	G	0.0040	0.88	0.0094	0.89
rs2017341	52516457	A	0.0040	1.23	0.0041	1.23
rs10111115	52778748	G	0.0054	1.15	0.13	1.08
rs16916394	52555316	A	0.0074	0.87	0.013	0.88
rs16916425	52582435	G	0.010	0.75	0.013	0.76
rs2304365	53292766	A	0.013	0.85	0.013	0.85
rs12155844	53292222	G	0.015	0.85	0.013	0.85
rs7830945	53295719	G	0.027	0.85	0.045	0.86
rs11786371	52598500	G	0.029	0.90	0.047	0.90
rs7000275	52602042	A	0.047	0.90	0.072	0.91

5

Methods

Detailed information on all PACG sample collections is provided below. All patients were enrolled into the study following informed consent and ethical approval from the relevant authorities. DNA was extracted from patient blood samples using standard laboratory procedures.

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Sample collections for Stage 1

15 Singapore: Cases of primary angle closure glaucoma (PACG) included patients with chronic PACG or those with acute PACG. Chronic PACG was defined as the presence of appositional angle closure for > 180 degrees with (a) peripheral anterior synechiae and/ or (b) raised intraocular pressure associated with glaucomatous optic neuropathy (defined as loss of neuroretinal rim with a vertical cup: disc ratio of > 0.7 or an inter-eye asymmetry of > 0.2, and/or notching attributable to glaucoma). Patients with acute PACG had a previous symptomatic episode defined as the presence of at least two of the following

20

symptoms: ocular or periocular pain, nausea or vomiting or both, and an antecedent history of intermittent blurring of vision with haloes; a presenting intraocular pressure of more than 30 mmHg on Goldmann applanation tonometry; and the presence of at least three of the following signs: conjunctival
5 injection, corneal epithelial edema, mid-dilated unreactive pupil, and shallow anterior chamber. Patients diagnosed with secondary angle closure (such as neovascular or uveitic glaucoma) were excluded. All Singapore PACG cases for Stage 1 were recruited between years 2005 – 2010. Controls were ascertained from an on-going population based study of Chinese persons aged 40 years
10 and older (the Singapore Chinese Eye Study [SCES]). The SCES is a population-based, cross-sectional study of Chinese adults residing in the south-western part of Singapore. The Ministry of Home Affairs of Singapore provided an initial computer-generated list ethnic Chinese names of adults aged 40-80+ years of age. A final sampling frame of 6,350 ethnic Chinese residents was
15 derived from this list using an age-stratified random sampling strategy. A control was defined as IOP $\leq$ 21 mm Hg with open angles, healthy optic nerves and normal visual fields, and no previous intraocular surgery.

Hong Kong: Patients with PACG were defined using the same criteria as that in the Singaporean GWAS collection. All subjects of the Hong Kong study
20 population were recruited from the Prince of Wales Hospital and the Hong Kong Eye Hospital, Hong Kong. A total of 303 PACG patients of Han Chinese ancestry were recruited. The controls comprised of 225 Hong Kong Chinese and 976 Guangdong Chinese of Han ancestry, of which 1,049 were genotyped (73 from Hong Kong and 976 from Guangdong) and 1,044 passed quality
25 checks (73 Hong Kong and 971 from Guangdong). The Hong Kong control subjects were recruited in a hospital-based manner. They were all given complete ocular examinations, and confirmed to have no sign of glaucoma, angle closure or narrow angle, or other major eye diseases except for mild cataract and mild refractive errors. Control subjects were recruited from elderly

people aged ≥ 60 years to ensure they were at least free of early-onset major eye diseases. They had IOP < 21 mmHg, and had no known family history of glaucoma. Additional healthy controls were recruited from local communities in the Guangdong province of Southern China (Bei *et al.*, 2010).

- 5 Vietnam: PACG cases were recruited from the Vietnam National Institute of Ophthalmology in Hanoi, Vietnam. They were defined using the same criteria as that in the Singaporean GWAS collection. The controls comprise 2,018 cord bloods, previously described (Khor *et al.*, 2011b).

- 10 Malaysia: PACG cases were recruited from the Department of Ophthalmology, School of Medical Sciences, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia. They were defined using the same criteria as that in the Singaporean GWAS collection. The controls were a population-based collection of healthy ethnic Malays recruited from Singapore (N = 3065), previously described (Vinthana *et al.*, 2011).

- 15 India: 337 PACG cases (as defined above) of South Indian ancestry were recruited from a large population based cross sectional study, the Chennai Glaucoma study, and from the outpatient department of the Medical Research Foundation, Sankara Nethralaya, Chennai, India. All PACG cases had peripheral anterior synechiae on gonioscopy, and were identified prior to laser
20 iridotomy. The Chennai Glaucoma study recruited 7851 persons from rural and urban cohorts in the Southern Indian state of Tamil Nadu (Arvind *et al.*, 2003). The controls were a population-based collection of healthy ethnic Indians recruited from Singapore (N = 2538), previously described (Cornes *et al.*, 2012).

Sample collections for Stage 2

- 25 Beijing: PACG patients were recruited from the Beijing Tongren Hospital, Xingtai Eye Hospital and Anyang Eye Hospital, China. The diagnostic criteria for PACG were as described above. Controls were recruited from the Handan Eye

Study (HES), a population-based study of eye disease in rural Chinese aged 30 years and over. The criteria for selecting controls are also as described above.

Singapore: All PACG cases and controls were recruited using the exact same criteria from Stage 1. They were recruited from year 2010 onwards.

- 5 India: 80 PACG cases (as defined above for Stage 1) were recruited from the outpatient department of the Medical Research Foundation, Sankara Nethralaya, Chennai, India and 309 unrelated healthy controls were recruited from the Chennai Glaucoma study. A control was defined as IOP $\leq$ 21 mm Hg with open angles, healthy optic nerves and normal visual fields, and no previous
10 intraocular surgery.

- UK: Cases of PACG in the UK were defined using the same criteria as that in the Singaporean GWAS collection. All subjects were of UK European descent and were recruited from Moorfields Eye Hospital, London UK. A total of 132 cases were genotyped and 127 passed quality checks. The controls comprised
15 of 4,703 healthy individuals of UK European descent, recruited and genotyped by the Wellcome Trust Case-Control Consortium 2. Their extensive use for genetics studies (such as ankylosing spondylitis, psoriasis, ulcerative colitis, ischaemic stroke, Meningococcal sepsis, and Kawasaki disease) has been described.

- 20 Saudi Arabia: PACG cases and controls were collected at the glaucoma clinic at King Abdulaziz University Hospital (KAUH), Department of Ophthalmology, College of Medicine, King Saud University, Riyadh, Saudi Arabia. They were defined using the same criteria as that in the Singaporean GWAS collection. All patients and controls were unrelated Saudi Arabs, all whose known ancestors
25 were of Saudi Arabian origin. As judged by the family names, PACG patients and controls were representative of the five provinces of the Kingdom of Saudi Arabia.

Shantou: PACG cases and controls were collected at the glaucoma clinic of the Chinese University of Hong Kong Joint Shantou International Eye Centre, Shantou, China. They were defined using the same criteria as that in the Singaporean GWAS collection, and were of Chinese descent.

5 Genotyping

For Stage 1, genome-wide genotyping was performed using the Illumina 610K Quad beadchips, following manufacturer's instructions. For Stage 2 (replication stage), genotyping was performed using the Sequenom MassArray platform, with the exception of the Shantou collection which was genotyped using
10 Taqman probes (Applied Biosystems).

Statistical analysis

Stringent quality control filters were used to remove poorly performing samples and SNP markers in both the GWAS discovery (Stage 1) and replication (Stage 2) phases. SNPs with a call rate of 95 percent, minor allele frequency of less
15 than 1 percent, and showing significant deviation from Hardy-Weinberg Equilibrium (P -value for deviation $< 10^{-6}$) were removed from further statistical analysis. Likewise, samples with an overall genotyping success rate of less than 95% were removed from further analysis. The remaining samples were then subjected to biological relationship verification by using the principle of
20 variability in allele sharing according to the degree of relationship. Identity-by-state (IBS) information was derived using PLINK (Purcell *et al.*, 2003). For those pairs of individuals who showed evidence of cryptic relatedness (possibly either due to duplicated or biologically related samples), we removed the sample with the lower call rate before performing principal component (PC) analysis. PC
25 analysis was undertaken to account for spurious associations resulting from ancestral differences of individual SNPs and PC plots were performed using the R statistical program package (www.r-project.org/). For Stage 1, all cases had

genetically matched controls as visualized spatially on PC analysis for each sample collection (Figure 1).

For both the GWAS and replication stages, analysis of association with PACG disease status was carried out using a 1 degree of freedom (d.f.) score-based test using logistic regression. This test models for a trend-per-copy effect of the minor allele on disease risk. It has the best statistical power to detect association for complex traits across a wide range of alternative hypotheses, with the exception of those involving rare recessive variants. The threshold for significant independent replication was set at $P < 0.05$ in the combined Stage 2 datasets. For Stage 1 (GWAS discovery), we incorporated the top four principal components of genetic stratification which were significant on uni-variate analysis into the logistic regression model whilst performing the analysis for association to minimize the effect of residual population stratification. As Stage 2 (Replication) only tested 15 SNP markers, we were unable to adjust for population stratification for the Stage 2 sample collections.

Meta-analysis was conducted using inverse variance weights for each sample collection, which calculates an overall Z-statistic, its corresponding P-value, and accompanying per-allele odds ratios for each SNP analyzed. Genotyping clusters were directly visualized for the 15 SNPs exceeding $P < 10^{-5}$ and confirmed to be of good quality before inclusion for statistical analysis. Figure 6 shows the cluster plots for the SNPs surpassing the formal threshold for genome-wide significance ($P < 5 \times 10^{-8}$): PLEKHA7 rs11024012, COL11A1 rs3753841, and Chr. 8q rs1015213. The I^2 (I-squared) index is calculated to quantify the extent of heterogeneity between sample collections in the meta-analysis. $I^2 < 25\%$ reflects low heterogeneity, $25\% < I^2 < 50\%$ reflects moderate heterogeneity, and $I^2 > 50\%$ reflects high heterogeneity. Analysis of linkage disequilibrium was performed using the R software package.

Power calculations

All statistical power calculations were performed as previously described . For the Stage 1 discovery analysis, power calculations indicated that there is 90 percent power of detecting loci at $P < 1.0 \times 10^{-5}$ (the threshold for following up sequence variants in Stage 2) at minor allele frequencies as low as 15 percent with per-allele odds ratios of 1.30.

The entire sample set of 3,771 PACG cases and 18,551 controls has 90 percent power to detect loci at the formal threshold for genome-wide significance ($P < 5.0 \times 10^{-8}$) at minor allele frequencies as low as 15 percent with per-allele odds ratios as low as 1.25, in line with the effect sizes we are reporting in this manuscript. Table 7 shows the formal power calculations in the context of the final meta-analysis.

Table 7 Study power as a function of disease allele frequency and per-allele odds ratio.

Risk allele frequency	Per-allele odds ratio						
	1.20	1.25	1.30	1.35	1.40	1.50	1.60
0.10	21.0%	62.2%	92.1%	>99.9%	>99.9%	>99.9%	>99.9%
0.15	51.4%	91.0%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%
0.20	74.0%	98.1%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%
0.25	86.3%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%
0.30	92.3%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%
0.35	95.0%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%

These power calculations are based on a GWAS and replication (double staged) study design whereby SNPs showing P -values $< 10^{-4}$ in the GWAS is brought forward for replication in 4 independent additional samples. The power calculation is based on final meta-analysis, as previously described (Gharavi *et al.*, 2011 and Purcell *et al.*, 2003). Bold areas represent > 90 percent statistical power for detection.

Gene expression analysis

The expression of *PLEKHA7*, *COL11A1*, *PCMTD1* and *ST18* genes was assessed by semi quantitative reverse transcription PCR (RT-PCR) using the following primers (*PLEKHA7*(F) 5'-TAAAGACAGCCGAGAAGAAG-3' [SEQ ID

NO: 1], *PLEKHA7*(R) 5'-TGTCGGCACTGAAGTAGTAG-3' [SEQ ID NO: 2];
COL11A1(F) 5'-GCAGGGAGAGATGGAGTTCAAGG-3' [SEQ ID NO: 3],
COL11A1(R) 5'-TGCCCAAACATCCCCTGCTG-3' [SEQ ID NO: 4]; *PCMTD1*(F)
 5'- ACACAGATTATGCGAACTGG-3' [SEQ ID NO: 5], *PCMTD1*(R) 5'-
 5 TGTAATACGAGCCAAGTCC-3' [SEQ ID NO: 6]; *ST18*(F) 5'-
 AGGGTAGAGAAGGCAGGACGG-3' [SEQ ID NO: 7, *ST18*(R) 5'-
 AGGTCTCTTCCACATCGCCCC-3' [SEQ ID NO: 8]). Primers were selected
 specifically to target the mRNA and not the genomic DNA of the said genes.
 Total RNA was extracted from a variety of ocular tissues (sclera, cornea, iris,
 10 trabecular meshwork, lens, lens capsule, retina and retinal pigment epithelium,
 choroid, optic nerve head and optic nerve) with TRIzol® Reagent (Invitrogen,
 Carlsbad, California) in accordance with the manufacturer's protocol. First-
 strand cDNA synthesis was performed with SuperScript First-Strand Synthesis
 System for RT-PCR (Invitrogen, Carlsbad, California) using random primers.
 15 Semi quantitative RT-PCR was performed according to manufacturer's protocol,
 with the SYBR® Green Master Mix (Invitrogen, Carlsbad, California) using the
 specified gene primers and equal amount of cDNA template. The resulting PCR
 products were separated on a 2% agarose gel and visualized by ethidium
 bromide staining. The ubiquitously expressed beta-actin (*ACTB*) gene, amplified
 20 with primers F 5'- CCAACCGCGAGAAGATGA -3' [SEQ ID NO: 9] and R 5'-
 CCAGAGGCGTACAGGGATAG-3' [SEQ ID NO: 10], was used as an
 amplification and normalizing control. All RT-PCR products were re-sequenced
 to confirm that the correct template was targeted by the primer pair selected for
 each gene.

25 Discussion

PLEKHA7 encodes for Pleckstrin-homology-domain-containing protein 7, which
 is critical for the maintenance and stability of adherens junctions (Pulimeno *et al.*, 2010; Meng *et al.*, 2008). In adult tissues, the adherens junctions maintain
 tissue homeostasis and along with tight junctions control epithelial and

endothelial para-cellular permeability (Harris and Tepass, 2010). In the eye, tight junctions and adherens junctions play an essential role in structures of particular relevance to glaucoma such as the ciliary body, iris, the aqueous humor outflow system and the choroid by providing a barrier to fluid leakage (Tian *et al.*, 2000). Factors, such as an attenuated reduction in iris volume with pupillary dilation, and exaggerated choroidal expansion have been proposed as having a significant role in the spectrum of angle closure pathogenesis. Given its role in maintaining a protein complex that regulates paracellular permeability, it is possible that *PLEKHA7* may be involved in the pathophysiology of angle closure related to aberrant fluid dynamics. Recently, a GWAS on blood pressure in excess of 60,000 individuals identified SNPs within this gene to be associated with systolic blood pressure (Levy *et al.*, 2009) a systemic risk factor for glaucoma. The SNP associated with PACG in our study is located 80 - 100 Kb upstream from the SNPs associated with blood pressure. No evidence of association between *PLEKHA7* rs11024074, the SNP reported to be strongly associated with systemic hypertension 35 and PACG status was observed in the present Stage 1 meta-analysis ($P = 0.13$, per-allele OR = 1.11). The pairwise measures of linkage disequilibrium (LD) between rs11024074 (SNP for systemic hypertension) and rs11024102 (SNP for PACG) was very weak ($D' = 0.154$, $r^2 = 0.009$), suggesting that different genetic polymorphisms could underlie the subtly different mechanisms influencing control for distinct phenotypes even though the underlying gene is a common denominator (Davila *et al.*, 2010; Gharavi *et al.*, 2011; Klein *et al.*, 2005).

COL11A1 encodes for one of the two alpha chains of type XI collagen. Pathogenic mutations in *COL11A1* cause Marshall (MIM:154780), Stickler type 2 (STL2) [MIM: 604841], or Stickler-like syndromes (Richards *et al.*, 1996). All are associated with ocular, orofacial, auditory and skeletal manifestations (Snead and Yates 1999). Interestingly, one of the ocular features of these diseases is non-progressive axial myopia likely caused by an aberrant fibrillar

collagen matrix within the sclera. The present data suggest that common variations in *COL11A1* are associated with PACG, and eyes predisposed to PACG are generally hyperopic having a shorter axial length and a crowded anterior segment (George *et al.*, 2003). Therefore the causal variants predisposing towards PACG within *COL11A1* may alter its gene expression such as to engineer a reverse effect to that observed in myopic eyes. *COL11A1* is also expressed in the human ocular trabecular meshwork cells (Michael *et al.*, 2008), which is pivotal for regulating the drainage of the aqueous humor from the eye. Therefore the aberrant action of this gene, albeit mild, could be at multiple sites within the PACG eye.

The third locus, rs1015213 on Chromosome 8q, is located within an intergenic region located 120Kb upstream of *PCMTD1* and 130K downstream of *ST18*. *PCMTD1* encodes for protein-L-isoaspartate O-methyltransferase domain-containing protein 1, whose function remains relatively unknown. *ST18* encodes for the protein named Suppression of Tumorigenicity 18, and has been shown to be significantly down-regulated in breast cancer cell lines (Jandrig *et al.*, 2004). More recent studies have also shown it to be a mediator for apoptosis and inflammation (Yang *et al.*, 2008). The LD block where rs1015213 was located extends into *PCMTD1* (Figure 4C), but not into *ST18*, suggesting that *PCMTD1* was the likelier candidate susceptibility gene for PACG at this locus, in keeping with the gene expression data (Figure 5). The minor allele frequency of rs1015213 appears to be rare (between 1 and 3 percent) in many of the sample collections (Figure 3C), particularly in individuals of Chinese and Vietnamese descent. It is possible that residual population stratification could confound the genetic association in these collections (Mathieson and McVean 2012). It was somewhat reassuring that ten out of the eleven PACG sample collections show the same direction of effect size for rs1015213. Furthermore, the overall meta-analysis for all sample collections revealed only mild heterogeneity (I^2 index = 19 percent), which was not statistically significant.

between the collections (P-value for heterogeneity = 0.19), thus arguing against population stratification as the cause of the observed association. In terms of the biological consequences of rs1015213, we are unable to exclude the possibility that this sequence variant could be tagging the presence of functional variants which are exerting long-range control on distant gene targets in a position- and orientation-independent manner (Pomerantz *et al.*, 2009; Tuupanen *et al.*, 2009).

Sequence information for the three SNPs are provided below. The SNP is indicated in bold.

10 **PLEKHA7** rs11024102 (SEQ ID NO: 11)

CCAGTCTTAC TGCCCAGCCC AACCCAGCCC AGGGAAGGCG GAGAGGAAGC AGTGAGCTGT
 TTTACAATCC ATCAAATTAA **Y** TGCCCCAAAT GGGAAATTTG CAGGCCTCCT ACAGCCTCCT
 GTGTATTCCA TAATCTCCCA TGGCCCTAAT CTGCTGAGAA AGAATGTGTG GGCTGTAATC
 15 TTTTCAGCCC ACTGTTTACA

Y is pyrimidine

COL11A1 rs3753941 (SEQ ID NO: 12)

20 GGCCTGGAGA AGAGCCACTG TCTACATTTT AACAACTTC ACAGAGGCTG TAGATAGAAT
 TGGTCTATGA TCACAATTCT **R** AGAACTTTA AGAACAATAC TGATGAGAAA TGGGAAATAC
 TATGTGCTTA AGAGATGCCC AAAGGTTTCA AAAAATTTGA AAAGCCAAAT CATCAACGGA
 TGCATGGAAT GGGTAATGAG

25 R is purine

rs1015213 (SEQ ID NO: 13)

30 TTTAAGCTC CTCAGCTTAT TCTATTCTC AGAGAGGGTT GAAGGTTGAC AATCCACAG **Y** GTTGTCTGGC
 ACTAATTGAT TTGACCTACT TTTTGAGTAT TCAGACAAAG AAGGAGGAAT

Y is pyrimidine

Information for these SNPs may also be obtained from the dbSNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP/>).

35 No evidence of association at previously reported POAG loci surpassing genome-wide significance was observed despite sufficient statistical power to

detect the previously-reported effect sizes (see Methods). This reinforces clinical and epidemiological data that PACG and POAG are distinct disease entities, with different molecular signatures underlying their pathogenic mechanisms. In summary, three novel loci for PACG, a major blinding disease with largely unresolved causal mechanisms were identified. These findings provide insight into the genetic mechanisms responsible for individual susceptibility to PACG. These observed associations have significant potential for developing novel therapies for the prevention or treatment of the condition. Further elucidation of the genetic architecture of PACG may eventually allow the development of a clinically useful genetic profile for the identification, risk stratification and thus treatment of PACG patients.

References

- Arvind *et al.*, (2003) Methods and design of the Chennai Glaucoma Study. Ophthalmic Epidemiol 10: 337-48
- The Australia and New Zealand Multiple Sclerosis Genetics Consortium (2009) Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20 Nat. Genet. 41: 824-8
- Bei *et al.*, (2010) A genome-wide association study of nasopharyngeal carcinoma identifies three new susceptibility loci. Nat Genet 42: 599-603.
- Cornes. *et al.* (2012) Identification of four novel variants that influence central corneal thickness in multi-ethnic Asian populations. Hum. Mol. Genet. 21: 437-45.
- Davila, S. *et al.* Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease. Nat. Genet. 42: 772-6 (2010).
- George *et al.*, (2003) Ocular biometry in occludable angles and angle closure glaucoma: a population based survey. Br. J. Ophthalmol. 87: 399-402.
- Gharavi. *et al.*, (2011) Genome-wide association study identifies susceptibility loci for IgA nephropathy. Nat. Genet. 4: 321-7.
- Harris, T. J. and Tepass, U. Adherens junctions: from molecules to morphogenesis. Nat rev Mol Cell Biol 11:502-514

Hoglinger *et al.*, (2011) Identification of common variants influencing risk of the tauopathy progressive supranuclear palsy Nat. Genet. 43: 699-705.

Jandrig *et al.*, (2004) ST18 is a breast cancer tumor suppressor gene at human chromosome 8q11.2. Oncogene 23: 9295-302.

- 5 Kooner *et al.*, (2011) Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci Nat. Genet. 43: 984-9 (2011).

Khor *et al.*, (2011a) Genome-wide association study identifies FCGR2A as a susceptibility locus for Kawasaki disease Nat. Genet. 43:1241-6.

- 10 Khor *et al.*, (2011b) genome-wide association study identifies susceptibility loci for dengue shock syndrome at MICB and PLCE1. Nat. Genet. 43: 1139-41.

Klein *et al.*, (2005) Complement factor H polymorphism in age-related macular degeneration. Science 308: 385-9.

- 15 Levy. *et al.*, (2009) Genome-wide association study of blood pressure and hypertension. Nat. Genet. 41: 677-87.

Mathieson and McVean (2012). Differential confounding of rare and common variants in spatially structured populations. Nat. Genet. 44: 243-6.

Meng *et al.*, (2008) Anchorage of microtubule minus ends to adherens junctions regulates epithelial cell-cell contacts. Cell 135: 948-59.

- 20 Michael *et al.*, (2008). Interactions between trabecular meshwork cells and lens epithelial cells: a possible mechanism in infantile aphakic glaucoma. Invest. Ophthalmol. Vis. Sci. 49: 3981-7.

- 25 Nalls *et al.*, (2011) Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet 377: 641-9.

Pomerantz *et al.*, (2009) The 8q24 cancer risk variant rs6983267 shows long-range interaction with MYC in colorectal cancer. Nat. Genet. 41: 882-4.

- 30 Pulimeno *et al.*, (2010) PLEKHA7 is an adherens junction protein with a tissue distribution and subcellular localization distinct from ZO-1 and E-cadherin. PLoS One 5: e12207.

Purcell *et al.*, (2003) Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19:149-50.

Purcell *et al.*, (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81: 559-75.

Ragoussis (2009) Genotyping technologies for genetic research. *Annu. Rev. Genomics Human Genet.* 10:117-33.

- 5 Reveille *et al.*, (2010) Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. *Nat. Genet.* 42: 123-7.

Richards *et al.*, (1996) A family with Stickler syndrome type 2 has a mutation in the COL11A1 gene resulting in the substitution of glycine 97 by valine in alpha 1 (XI) collagen. *Hum. Mol. Genet.* 5: 1339-43.

- 10 Snead and Yates (1999) Clinical and Molecular genetics of Stickler syndrome. *J. Med. Genet.* 36: 353-9.

Tian *et al.*, (2000) Cytoskeletal involvement in the regulation of aqueous humor outflow. *Invest. Ophthalmol. Vis. Sci.* 41: 619-23.

- 15 Tuupanen *et al.*, (2009) The common colorectal cancer predisposition SNP rs6983267 at chromosome 8q24 confers potential to enhanced Wnt signaling. *Nat. Genet.* 41: 885-90.

Vithana *et al.*, (2011) Collagen-related genes influence the glaucoma risk factor, central corneal thickness. *Hum. Mol. Genet.* 20: 649-58.

Claims

1. A method for predicting and/or determining susceptibility to primary angle closure glaucoma in a subject, comprising determining the genotype of the subject for at least one single nucleotide polymorphism associated with primary angle closure glaucoma; wherein the single nucleotide polymorphism is selected from the group consisting of *PLEKHA7* rs11024102, *COL11A1* rs3753941 and rs1015213.
5
2. The method according to claim 1, comprising determining at least one nucleic acid variation and/or allele present for said at least one single nucleotide polymorphism in the subject.
10
3. The method according to claim 1 or 2, comprising determining the genotype, nucleic acid variation and/or the allele present in the genomic sequence, its corresponding transcriptional and/or translational product.
4. The method according to any one of the preceding claims, wherein the method is performed on an isolated sample from the subject.
15
5. The method according to any one of the preceding claims, wherein the single nucleotide polymorphism comprises *PLEKHA7* rs11024102.
6. The method according to any one of claims 1 to 4, wherein the single nucleotide polymorphism comprises *COL11A1* rs3753941.
- 20 7. The method according to any one of claims 1 to 4, wherein the single nucleotide polymorphism comprises rs1015213.
8. A probe for use in predicting and/or determining susceptibility to primary angle closure glaucoma in a subject, wherein the probe is for use in determining the genotype of the subject for at least one single nucleotide polymorphism associated with primary angle closure glaucoma, wherein
25

the single nucleotide polymorphism is selected from the group consisting of *PLEKHA7* rs11024102, *COL11A1* rs3753941 and rs1015213.

9. The probe according to claim 8, for use in determining at least one allele present in said at least one single nucleotide polymorphism in the subject.
10. The probe according to claim 8 or 9, for use in detecting the *PLEKHA7* rs11024102 single nucleotide polymorphism.
11. The probe according to claim 8 or 9, for use in detecting the *COL11A1* rs3753941 single nucleotide polymorphism.
12. The probe according to claim 8 or 9, for use in detecting the rs1015213 single nucleotide polymorphism.
13. The probe according to any one of claims 8 to 12, for use in determining the genotype, nucleic acid variation and/or the allele present in the genomic sequence.
14. The probe according to any one of claims 8 to 13, wherein the probe comprises a nucleic acid molecule.
15. A kit comprising at least one probe according to any one of claims 8 to 14.

1/12

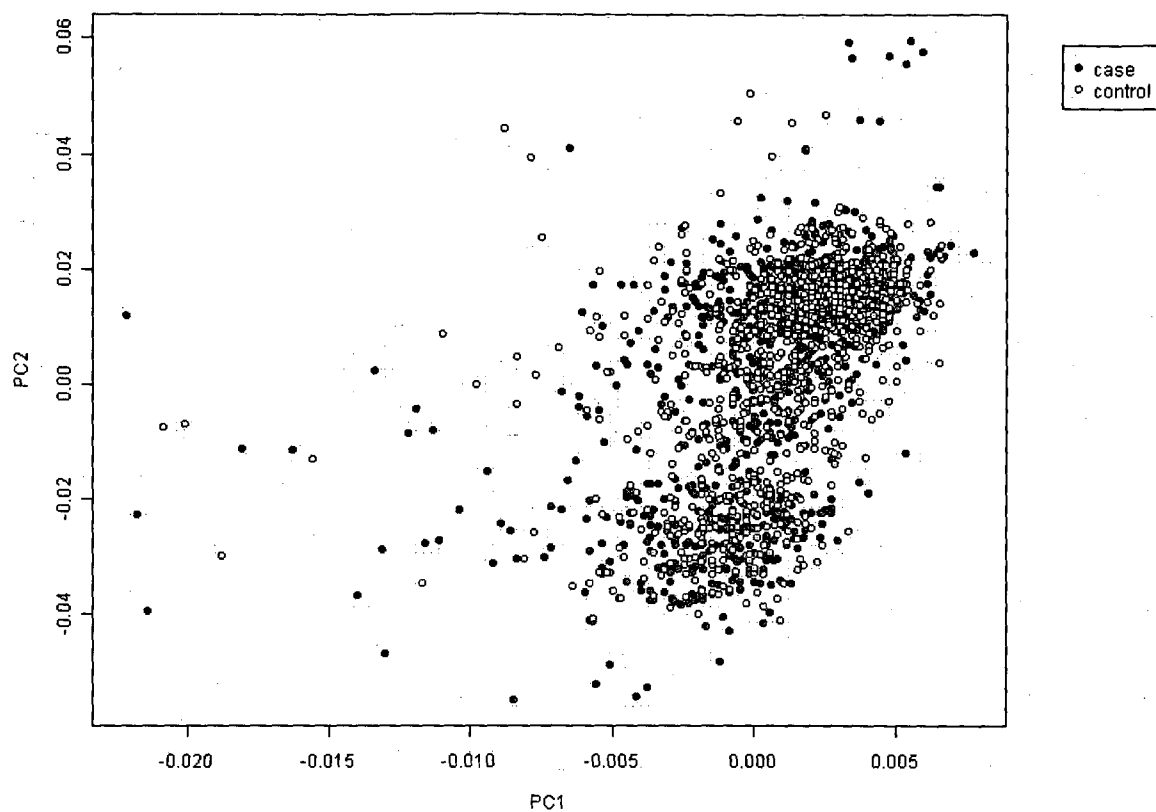


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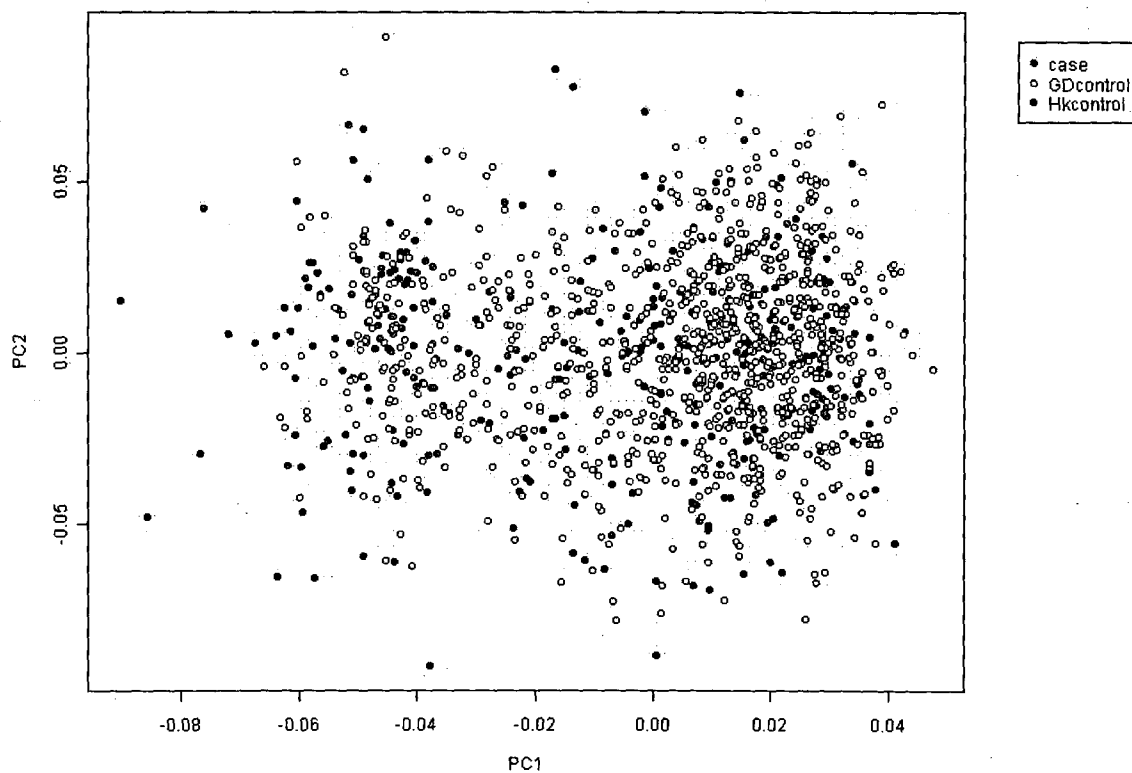


Figure 1(B)

2/12

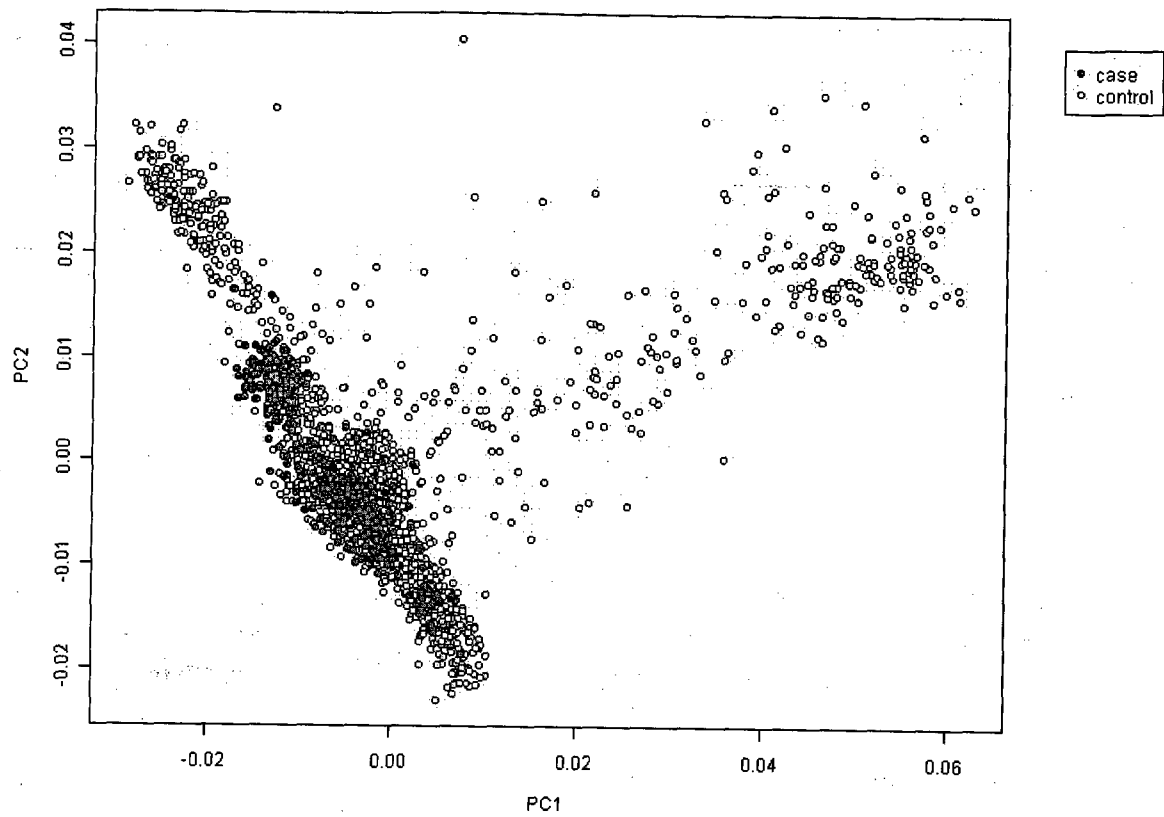


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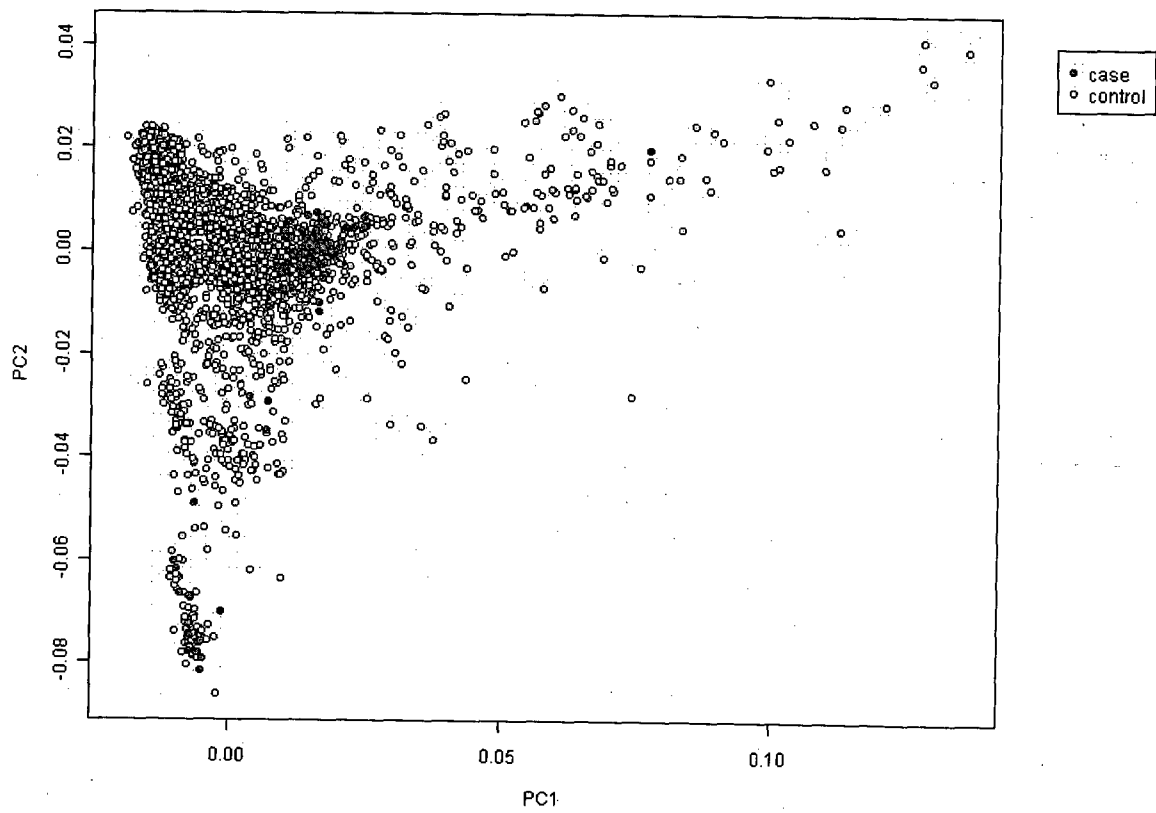


Figure 1(D)

3/12

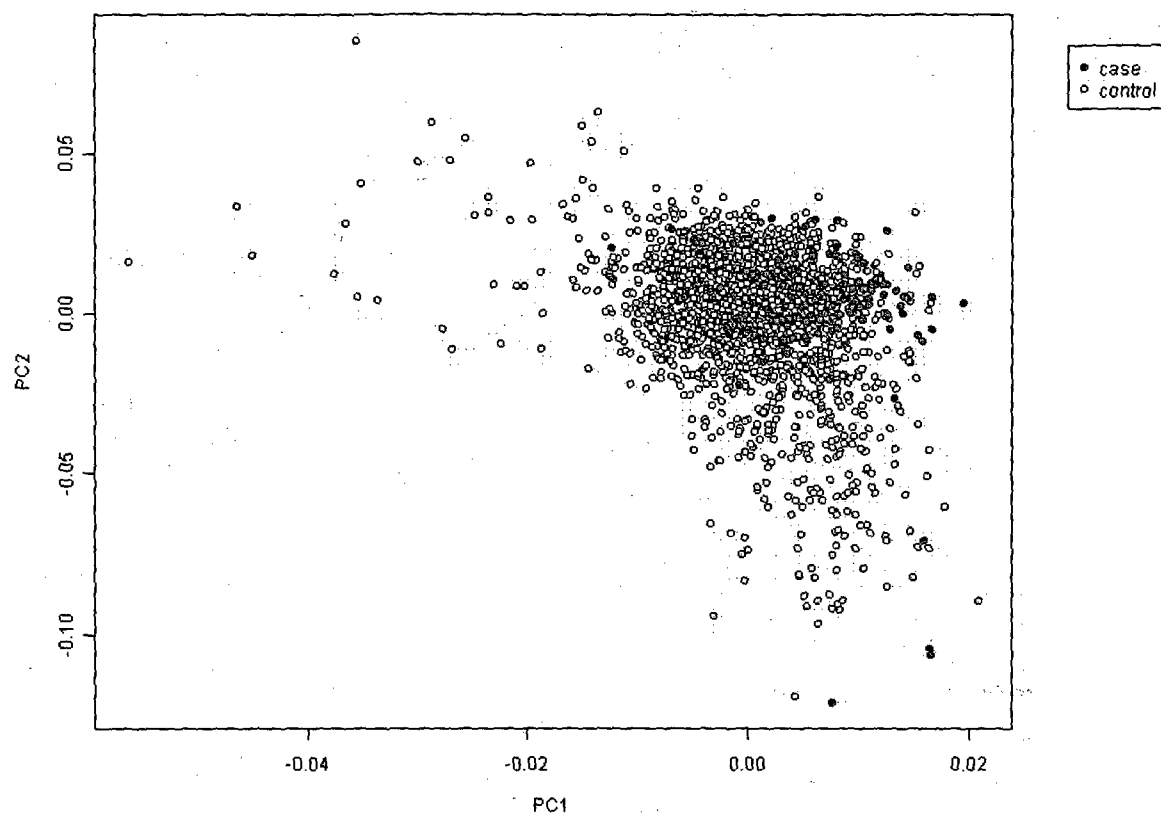


Figure 1(E)

4/12

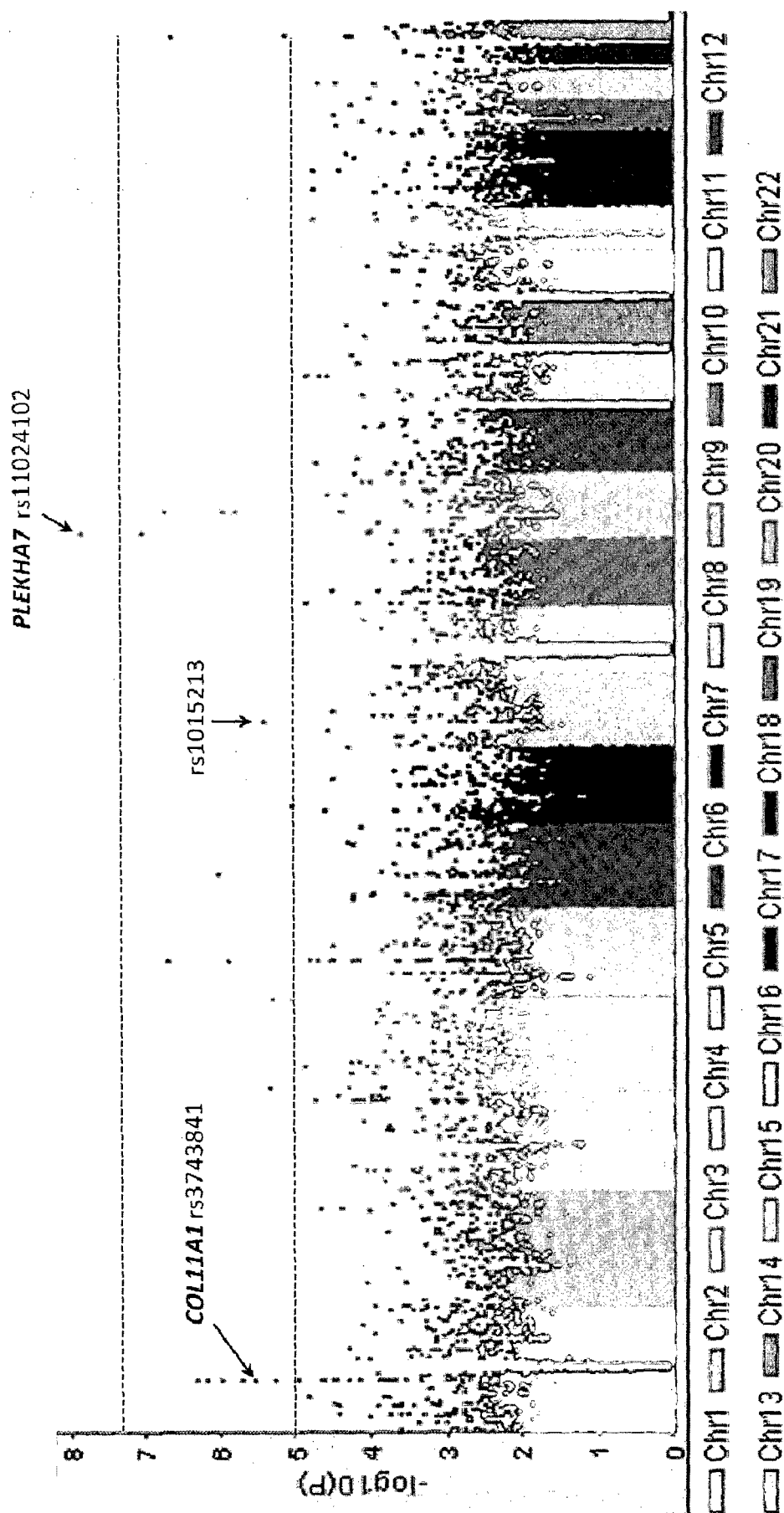


Figure 2

5/12

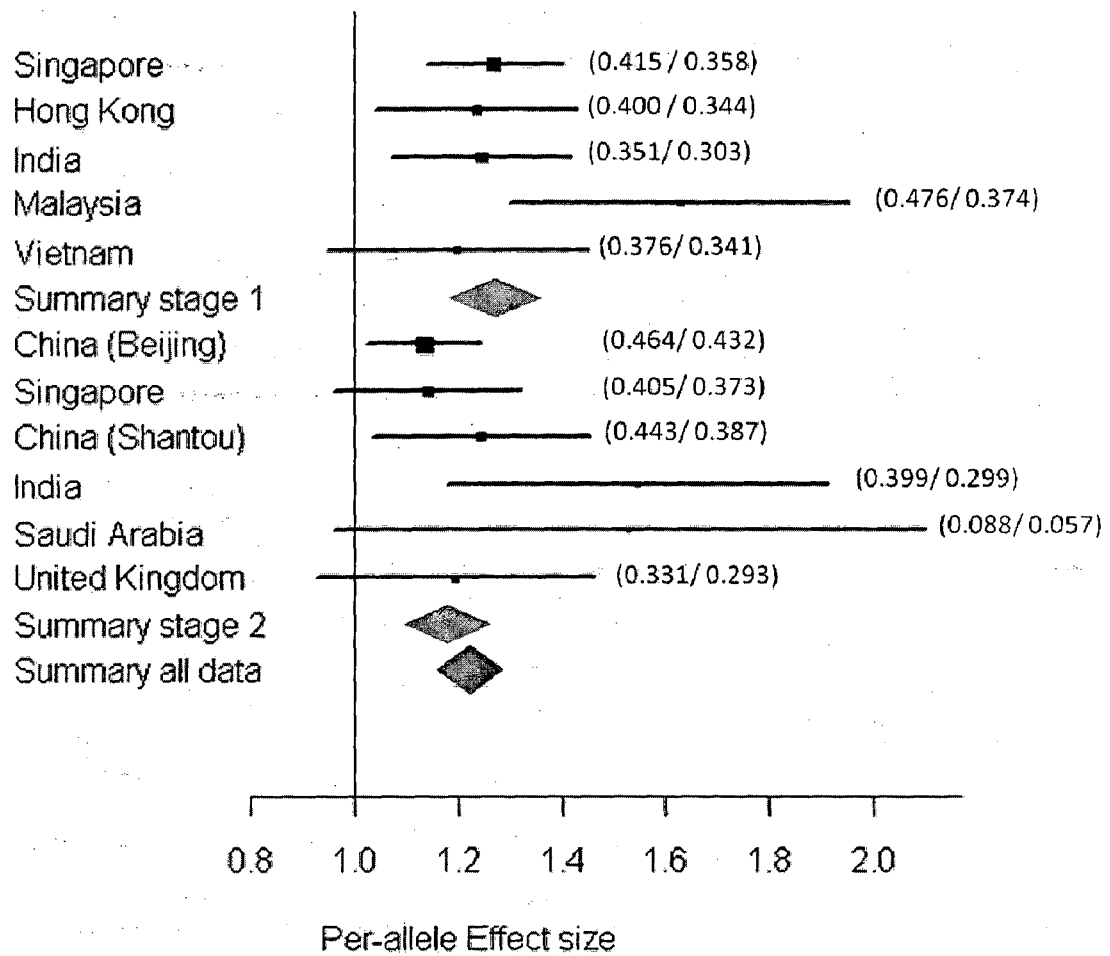


Figure 3(A)

6/12

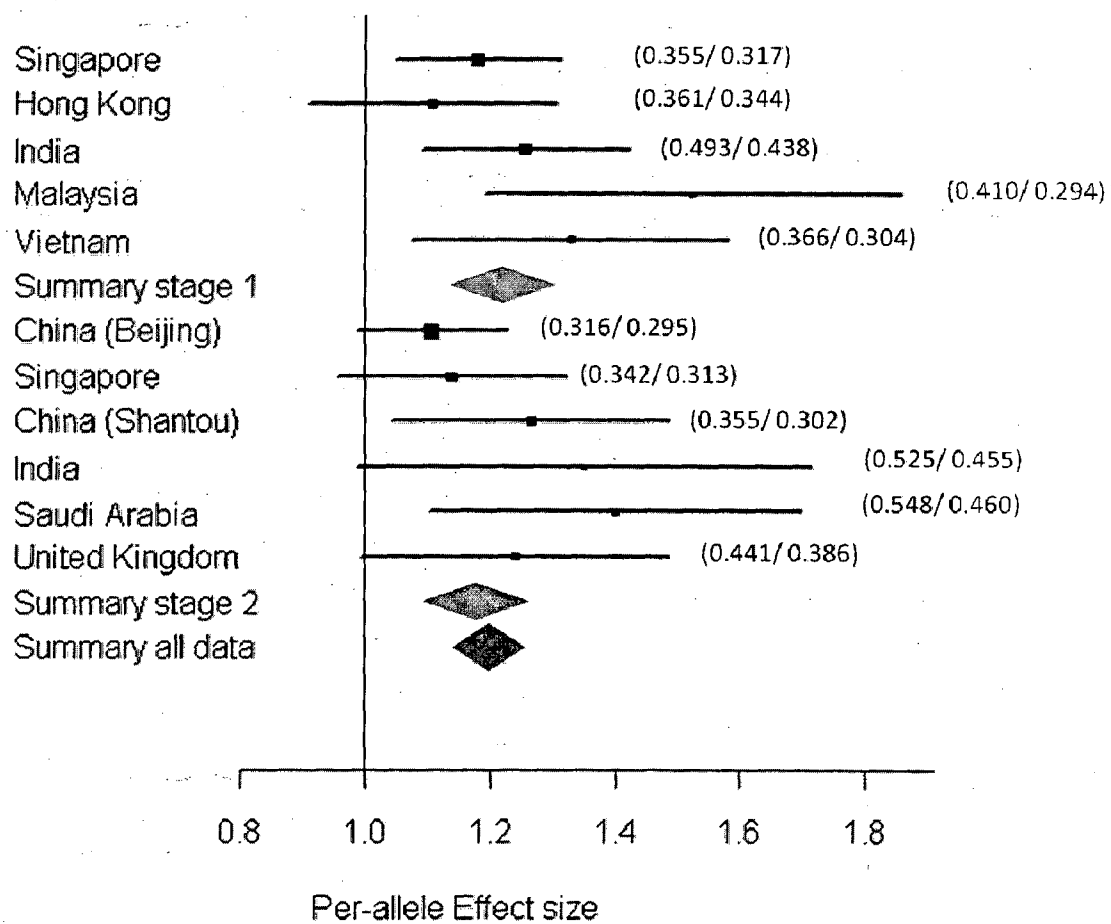


Figure 3(B)

7/12

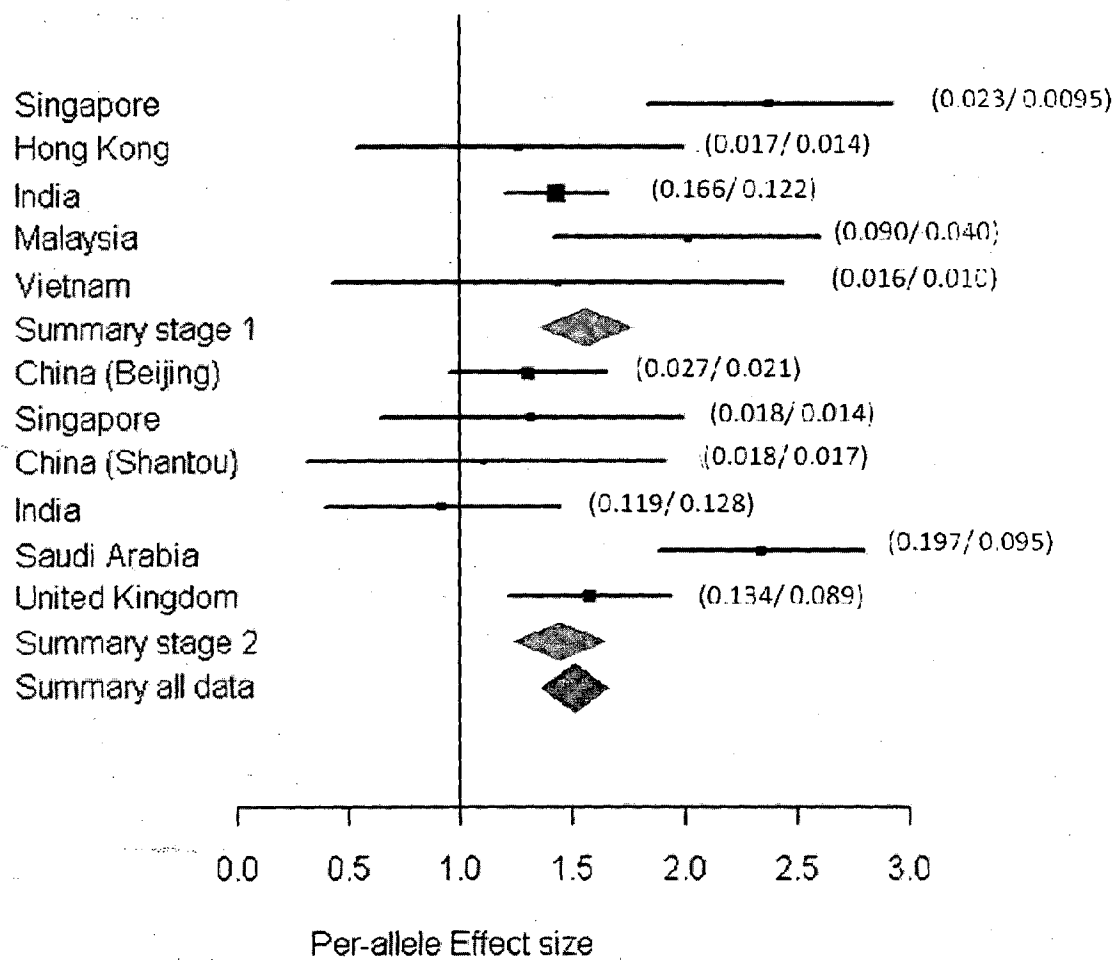


Figure 3(C)

8/12

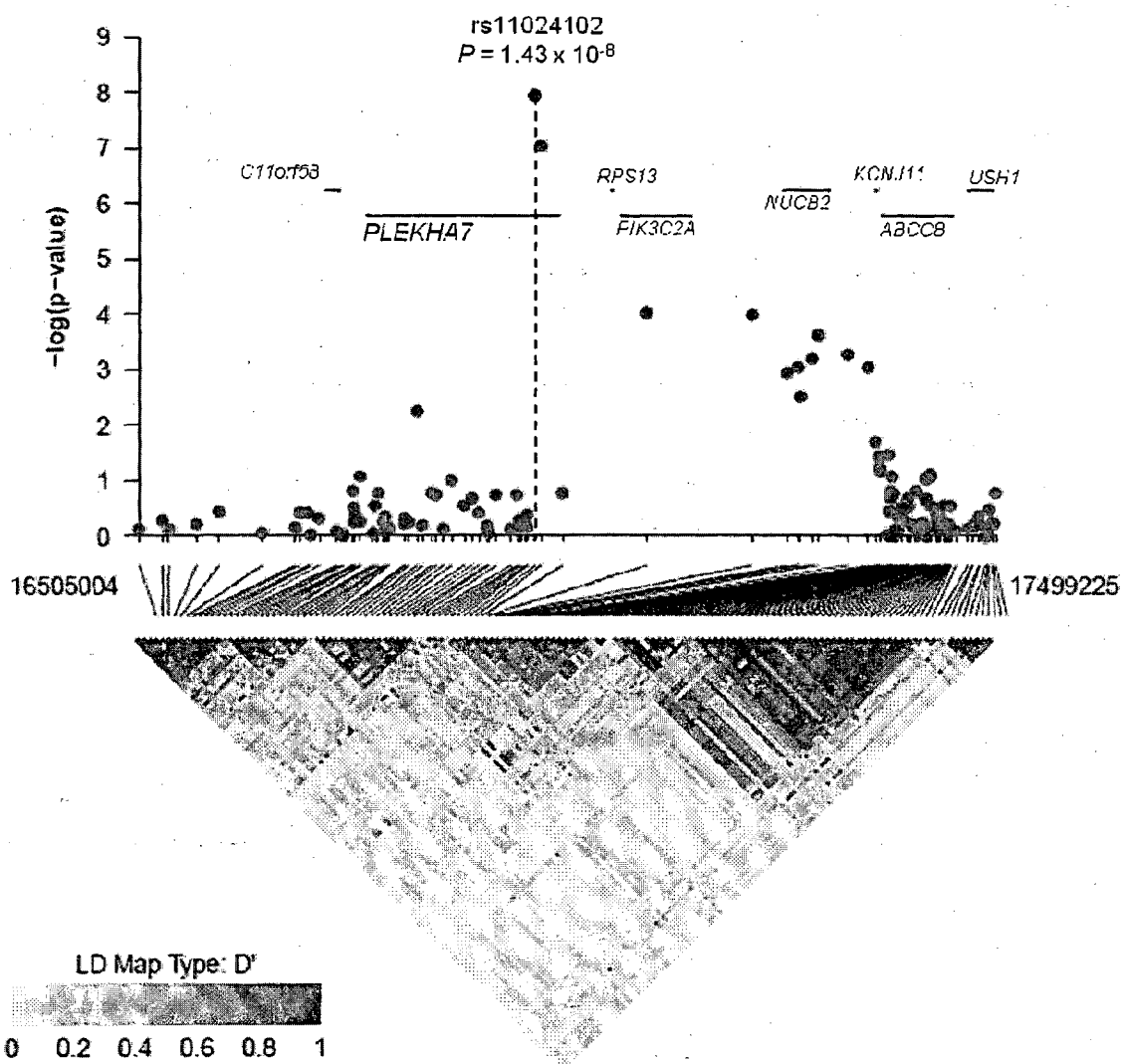


Figure 4(A)

9/12

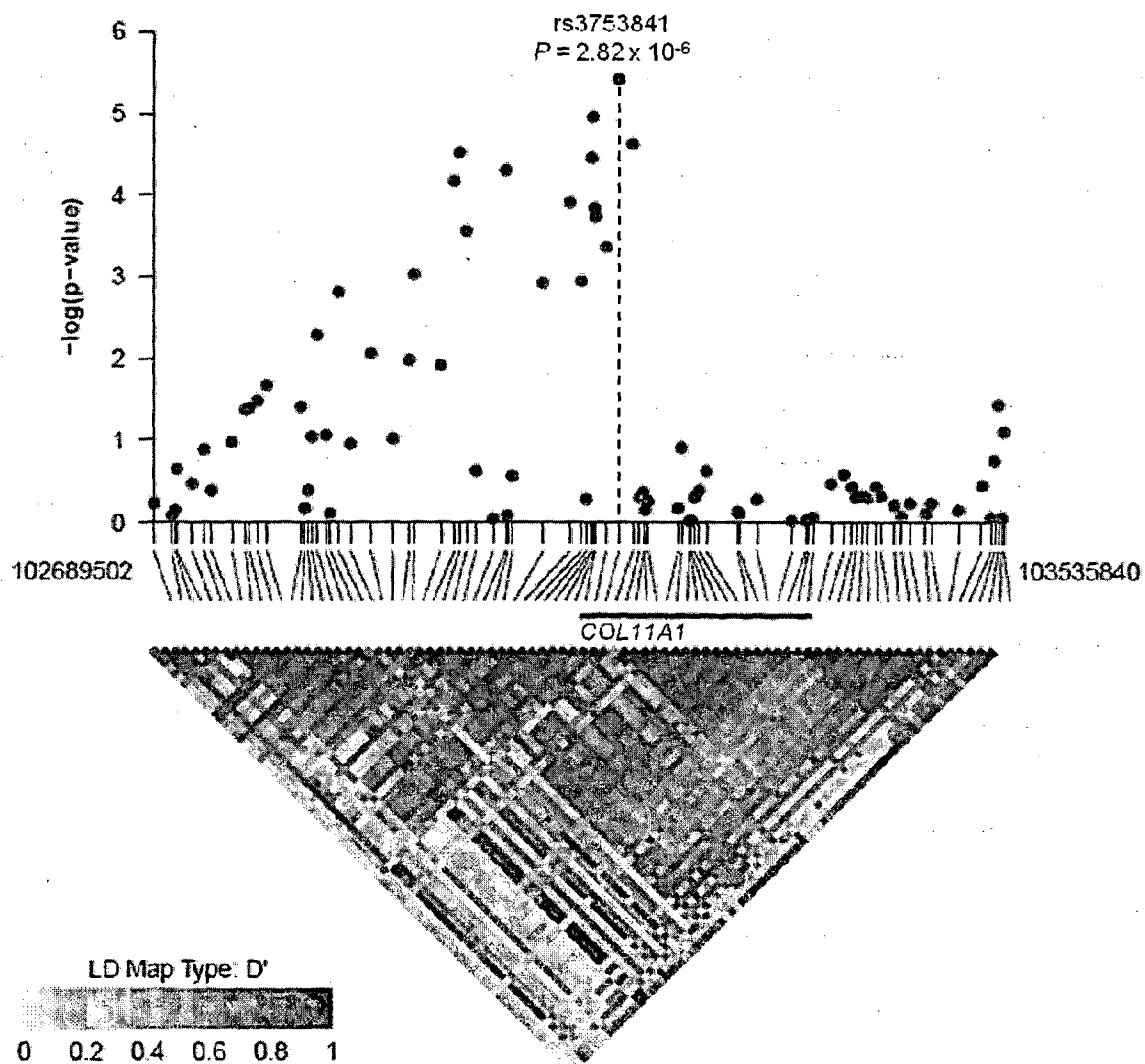


Figure 4(B)

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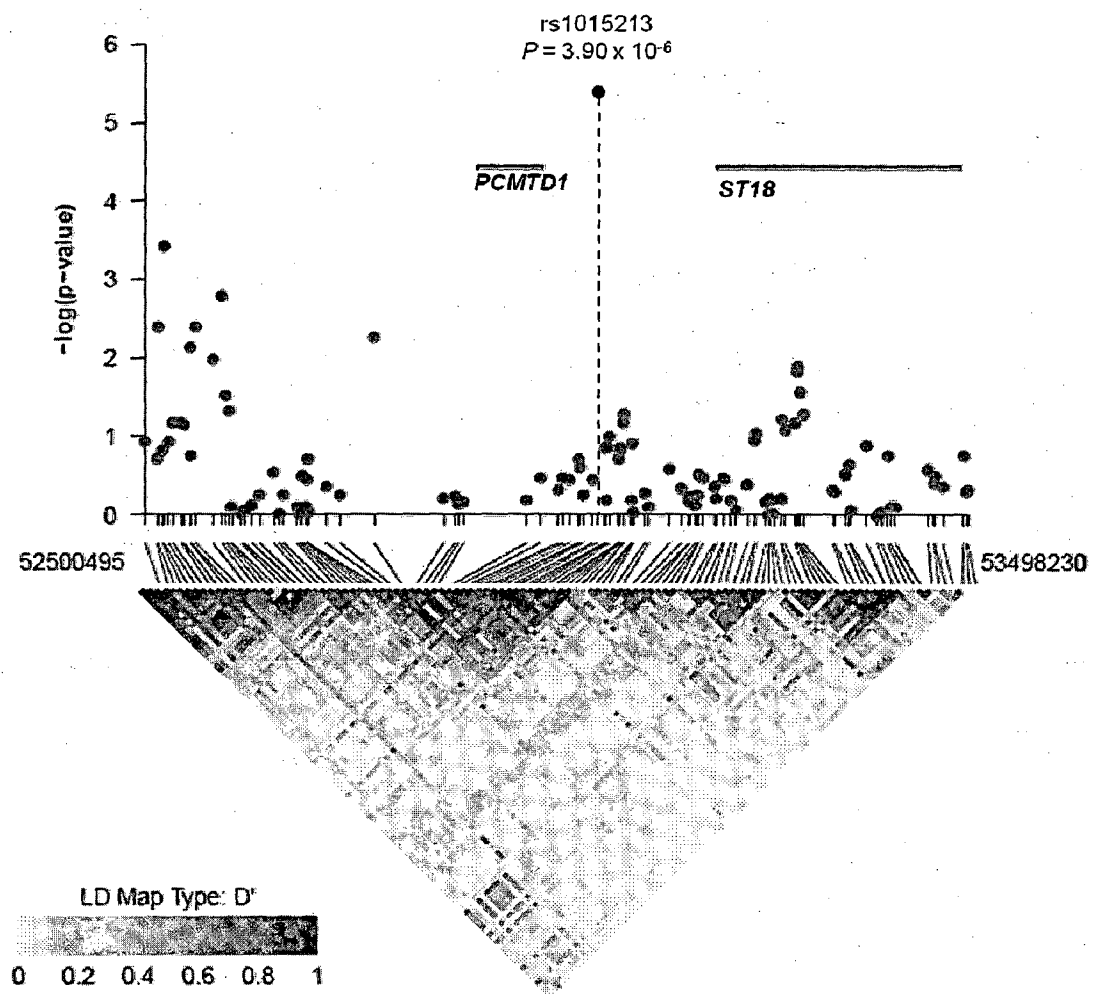


Figure 4(C)

11/12

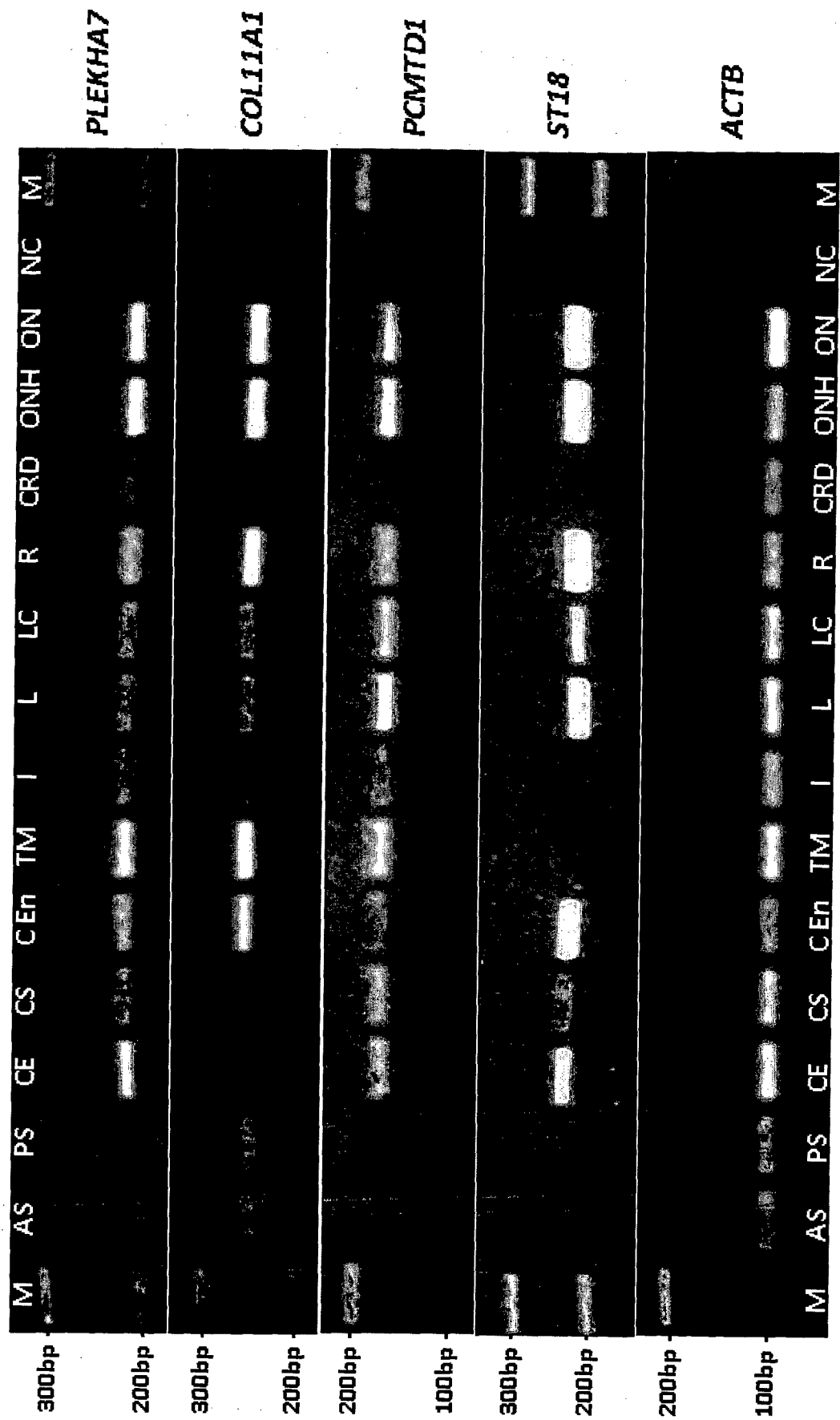
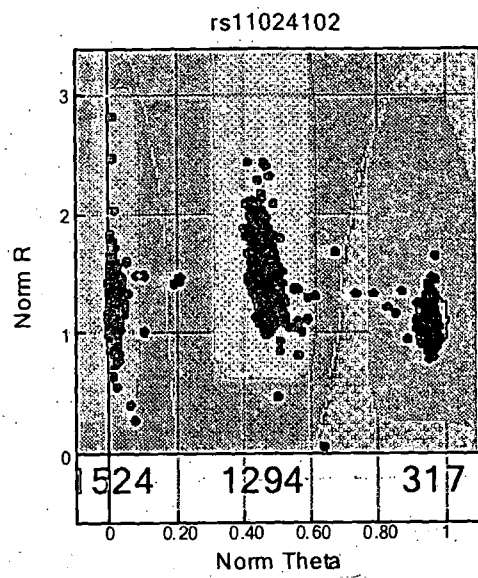
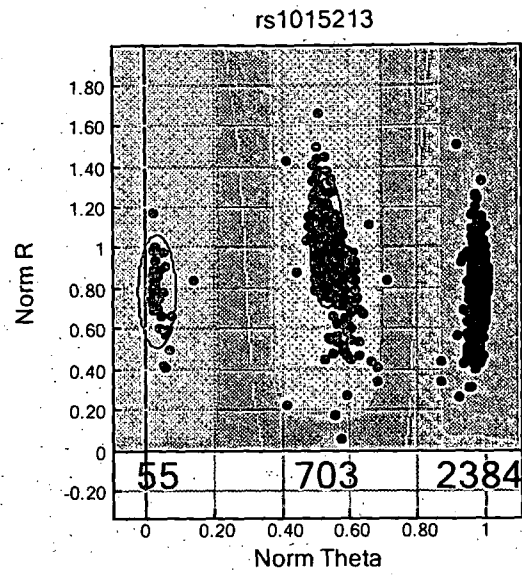


Figure 5

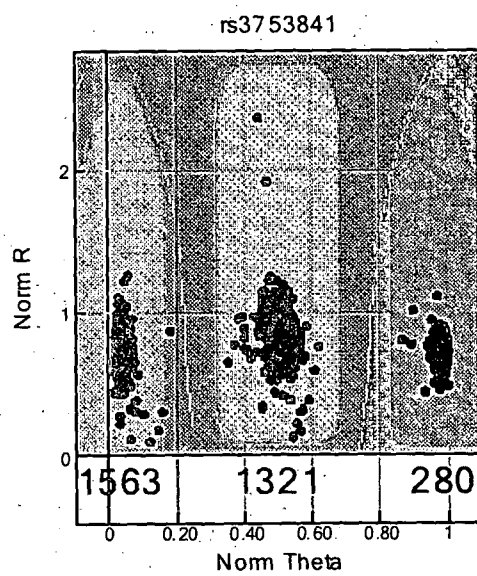
12/12



(A)



(B)



(C)

Figure 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2013/000355

A. CLASSIFICATION OF SUBJECT MATTER

C12Q 1/68 (2006.01) C07H 21/00 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, MEDLINE, HCAPLUS, BIOSIS, BIOTECHABS, EMBASE for Glaucoma, PACG, mutation, SNP, marker, PLEKHA7, COL11A1, PCMTD1, ST18, RS11024102, RS3753941, RS3753841, RS1015213, chromosome and like terms. GenomeQuest for SEQ ID NOs. 1-10 at 100% identity filtered with keywords glaucoma and PACG. SEQ ID NOs. 11-13 at 90% identity.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
27 September 2013

Date of mailing of the international search report
27 September 2013

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2013/000355

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/SG2013/000355
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/078599 A2 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 12 July 2007 Abstract; 0014; 00139; SEQ ID No: 3217	8-9, 11 and 13-15
X	US 2002/0198371 A1 (WANG) 26 December 2002 Title; Abstract; Claim 1; SEQ ID No. 122394	8-9 and 12-15
A	WO 1999/054493 A2 (UNIVERSITY OF ALBERTA) 28 October 1999 Whole Document	1-15
A	US 2007/0172919 A1 (SARFARAZI et al.) 26 July 2007 Whole Document	1-15
P,X	AWADALLA, M. S. et al., 'Association of genetic variants with primary angle closure glaucoma in two different populations', Public Library of Science One, June 2013, Issue 6, e67903. Abstract	1-15
P,X	DAY, A. C. et al., 'Genotype-phenotype analysis of SNPs associated with primary angle closure glaucoma (rs1015213, rs3753841 and rs11024102) and ocular biometry in the EPIC-Norfolk Eye Study', British Journal of Ophthalmology, March 2013, Vol. 97, Pages 704-707. Title; Abstract	1-15

Supplemental Box

Continuation of: Box III

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

Note that PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

The ISA has found that there are different inventions based on the following features that separate the claims into distinct groups:

Group I: Claims 1-15 (all in part) are directed to methods, probes and a kit for predicting and/or determining susceptibility to primary angle closure glaucoma in a subject, comprising determining the genotype of the subject for at least one single nucleotide polymorphism associated with primary angle closure glaucoma; wherein the single nucleotide polymorphism is *PLEKHA 7* rs11024102.

Group II: Claims 1-15 (all in part) are directed to methods, probes and a kit for predicting and/or determining susceptibility to primary angle closure glaucoma in a subject, comprising determining the genotype of the subject for at least one single nucleotide polymorphism associated with primary angle closure glaucoma; wherein the single nucleotide polymorphism is *COL11A1* rs3753941.

Group III: Claims 1-15 (all in part) are directed to methods, probes and a kit for predicting and/or determining susceptibility to primary angle closure glaucoma in a subject, comprising determining the genotype of the subject for at least one single nucleotide polymorphism associated with primary angle closure glaucoma; wherein the single nucleotide polymorphism is rs1015213.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a priori*.

However, the ISA believes that a search and examination for the second and third inventions will not involve more than negligible additional search and examination effort over that for the first invention and so no additional search fee is required in order to search and examine that invention. Consequently, all three inventions have been searched and commented on.

INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/SG2013/000355	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2007/078599 A2	12 Jul 2007	JP 2009519710 A	21 May 2009
		US 2007161031 A1	12 Jul 2007
		WO 2007078599 A2	12 Jul 2007
US 2002/0198371 A1	26 Dec 2002	None	
WO 1999/054493 A2	28 Oct 1999	AU 3843299 A	08 Nov 1999
		CA 2325663 A1	28 Oct 1999
		EP 1071823 A2	31 Jan 2001
		JP 2002512041 A	23 Apr 2002
		NZ 507787 A	29 Aug 2003
		US 2003013087 A1	16 Jan 2003
		WO 9954493 A2	28 Oct 1999
		ZA 200005707 A	12 Jun 2001
US 2007/0172919 A1	26 Jul 2007	None	
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2009)			