IOVS Papers in Press. Published on September 13, 2012 as Manuscript iovs.12-10489

Genetic variants near *PDGFRA* are associated with corneal curvature in Australians

Aniket Mishra[1], Seyhan Yazar[2*], Alex W Hewitt[2, 3], Jenny A Mountain[4], Wei Ang[5], Craig E Pennell[5], Nicholas G Martin[1], Grant W Montgomery[1], Christopher J Hammond[6], Terri L Young[7], Stuart Macgregor[1], David A Mackey[2, 3]*

[1]Queensland Institute of Medical Research, Brisbane, Australia;
[2]Lions Eye Institute, University of Western Australia, Centre for Ophthalmology and Visual Science, Perth, Australia;
[3]Centre for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, Australia;
[4]Telethon Institute for Child Health Research, Centre for Child Health Research, University of Western Australia.
[5]School of Women's and Infants' Health, University of Western Australia, Perth 6009, Australia;
[6]Department of Twin Research and Genetic Epidemiology, King's College London, St. Thomas' Hospital, London,
[7]Center for Human Genetics, Duke University Medical Center, Durham, North Carolina, USA.

*Authors contributed equally.

Abstract

PURPOSE. Irregularity in corneal curvature (CC) is highly associated with various eye disorders such as keratoconus and myopia. Our sample had limited power to find genome-wide significant (5×10^{-8}) hits but good power for replication. Thus, we attempted to test whether alleles in the *FRAP1* and *PDGFRA* genes, recently found to be associated with CC in Asian populations, also influence CC in Australians of North European ancestry. We also report initial GWAS results for CC in Australians.

METHODS. Two population-based cohorts of 1788 Australian twins and their families, as well as 1013 individuals from a birth cohort from Western Australia were genotyped using genome-wide arrays. Following separate individual analysis and quality control, the results from each cohort underwent meta-analysis.

RESULTS. Meta-analysis revealed significant replication of association between rs2114039 and corneal curvature (P = 0.0045). The SNP rs2114039 near *PDGFRA* has been previously implicated in Asians. No SNP at the *FRAP1* locus was found to be associated in our Australian samples. No SNP surpassed the genome wide significance threshold of 5×10^{-8}. The SNP with strongest association was rs2444240 has ($p=3.658 \times 10^{-07}$), which is 31kb upstream to the *TRIM29* gene.

CONCLUSIONS. This study confirms a significant role of the *PDGFRA* gene in determining corneal curvature in the Australian population. It also highlights the putative association of the *TRIM29* locus with CC.

Introduction

Refraction of light through the cornea is the preliminary step in vision. This bending of light is highly dependent on the curvature of the cornea. Corneal astigmatism or an irregularity in corneal curvature (CC) leads to blurred uncorrected vision. Keratoconus is a disease characterised by a conical shaped cornea and irregular astigmatism.[1] Patients with this corneal condition often experience vision distortion, multiple images, and sensitivity to light. In addition to keratoconus, corneal irregularities are also associated with refractive error[2] and Marfan syndrome.[3]

Variation in corneal curvature is dependent on ethnic background,[4; 5] geographical as well as environmental conditions,[6] age[7] and stature.[7] CC is highly heritable,[8] with previous studies revealing heritability estimates ranging between 60% and 95%.[6; 8-11] Improved understanding of the genetic architecture of this biometric trait will aid in determining the molecular mechanisms of blinding eye disorders, and contribute to our ocular development and evolutionary biology knowledge.

Genome wide association studies (GWAS) have been successful in revealing the genetic variants behind various complex traits including age-related macular degeneration, type 2 diabetes as examples.[12-15] The only published GWAS for CC is from a Singaporean Asian population, in which the significant associations of single nucleotide polymorphisms (SNPs) in *FRAP1* and *PDGFRA* genes with corneal curvature were reported. [6] As is the case with other quantitative traits, CC is likely to be determined by many genes, with ever larger GWASs likely to lead to the identification of additional associated SNPs.[16] Furthermore, it is unknown whether genes found to be significantly associated with CC in Asian population would be relevant to other racial groups. In previous studies, ethnic and environmental background is an important determinant of CC. [17-19] Hence we aimed to test whether *PDGFRA* and *FRAP1* genes found to be associated with CC in Singaporean Asian population also determine CC in Australian of northern European ancestry. We also aimed to report initial GWAS on CC in Australians of northern European ancestry. We conducted two population based GWAS on

1788 Australian twins and their families,[20] as well as 1013 unrelated individuals from a population cohort from Western Australian.

Materials and Methods

Ethics statement

This study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the human research ethics committees of the University of Tasmania, Royal Victorian Eye and Ear Hospital, Queensland Institute of Medical Research, and University of Western Australia. Informed consent was obtained from parents with the child's assent or from adult participants before testing.

Twin cohorts

1788 individuals of 857 twin families were recruited from Australia. Recruitment of Twins was performed through the Twins Eye Study in Tasmania (TEST) and the Brisbane Adolescent Twin Study (BATS). [20] BATS participants ranged in age from 10 to 40 years and TEST participants ranged in age from 5 to 90 years. Corneal curvature was measured using a Humphrey-598 Automatic Refractor / Keratometer (Carl Zeiss Meditec, Inc., Miami, Florida, USA). The difference between curvature values of left and right eyes was not significant (t-test, p-value = 0.24).

Saliva or peripheral blood samples from subjects were used to extract DNA which was genotyped on the Illumina HumanHap 610W Quad arrays (Illumina Inc., San Diego, CA, USA). The majority of people from the BATS were genotyped by deCODE Genetics. TEST participants and a small number of BATS individuals were genotyped by the Centre for Inherited Disease Research (CIDR).

Filtering criteria for genotypic data were: minor allele frequency ≥ 1%, Hardy-Weinberg Equilibrium Test p-value ≥ 10^{-6}, SNP call rate > 95% or Illumina Beadstudio GenCall score ≥ 0.7. Quality control of SNPs gave 559,990 SNPs, which then underwent association testing in the twin cohorts.

Ancestral outliers were corrected by Principal Component Analysis (PCA) [21] using 'smartpca' program from v3.0 of EIGENSOFT. Australian twin data was compared with all populations in HapMap phase 3 and collection of five other GenomEUTWIN populations. [22; 23] Only PC1 and PC2 with highest Eigen values were considered to identify and filter outliers. PC1 reflects the difference between Africans and others whereas PC2 reflects difference between East Asian populations with others. As expected the Australian population clustered with Europeans with some individuals also showing Asian and African ancestry. To remove Australian population outliers like Australians with African or Asian ancestry, mean and standard deviation of collective European population viz., Hapmap CEU (Utah residents with Northern and Western European ancestry from the CEPH collection), TSI (Toscans in Italy) and GenomEUTWIN (European Twin Population), was calculated for reference PC1 and PC2 scores. Any individual which fell away from mean by >6 times their standard deviation on PC1 and PC2 were removed, with the remainder considered for further analysis.

Using the 'MACH v1.0.16b' and 'mimimac' packages, imputation of Australian Twin genotyped data was carried out using the 1000 Genomes haplotypes available for 283 European ancestry individuals in the August 2010 release of 1000 Genomes project. [24; 25] Around 482 thousand common genotyped markers were used in imputation which gave 11,914,767 imputed SNPs. Following quality control (R^2 >0.3 for each SNP), 8,016,011 SNPs underwent association analysis. The MERLIN program was used to perform association testing, taking into account relatedness of the twins and their families. [26] We used age and sex as co-variates.

Raine Eye Health Study

The 21-year review of the Western Australian Pregnancy Cohort (Raine) Study investigated ophthalmic health and established the Raine Eye Health Study (REHS). The Raine Study is one of the largest ongoing prospective cohort studies of pregnancy, childhood, adolescence and young adulthood. In 1989, 2,900 pregnant women were recruited at 16-18 weeks' gestation into a randomized clinical trial at King Edward Memorial Hospital, Perth, Western Australia for investigating effects of intensive ultrasound and Doppler studies in pregnancy outcomes. The cohort has been evaluated in detail during childhood (1, 2,3,5,8 and 10 years) and adolescence (14 and 17 years). Raine participants underwent a comprehensive ocular examination for the first time at the 21-year follow-up. The ocular examination included measurements of visual acuity, cycloplegic auto refraction, as well as several ocular biometric variables and multiple ophthalmic photographs (anterior and posterior segment). A total of 1344 subjects were examined in the 24-month period from March 2010 to February 2012.

Ocular biometric parameters including CC were measured with IOLMaster V.5 (Carl Zeiss Meditec AG, Jena, Germany). Three measurements of CC within 0.3D within each meridian with careful alignment and focus were recorded. Complete data was available from 1013 participants for analysis. The average age of the participants was 20.0 (range: 18-22) years. DNA samples from previous assessments and consents for GWAS studies were available for the participants.

Individual genotype data for 1494 participants were extracted from the genome-wide Illumina 660 Quad Array. Briefly, the genotyping was performed on the Illumina BeadArray Reader at the Centre for Applied Genomics (Toronto, Ontario, Canada) using 250 nanograms of DNA. Any pair of individuals who were related with a $\pi > 0.1875$ (in between second and third degree relatives – e.g. between half-sibs and cousins) was investigated, and the individual with the higher proportion of missing data was excluded from the 'clean' dataset (68 individuals excluded). Individuals who had low genotyping success (i.e. missing data) were excluded from the 'clean' dataset – a threshold of absent data > 3% was used for exclusion (16 individuals excluded). Additionally, if they had high

levels of heterozygosity then they were also excluded (heterozygosity < 0.30 excluded 3 individuals) as this may indicate sample contamination. In terms of genotyping success rates, we also excluded SNPs which did not satisfy a Hardy-Weinburg equilibrium p-value > 5.7x10-7 (919 markers), a call rate >95% (97,718 markers), and a minor allele frequency >0.01 (1%) (119,246 markers – includes CNV's). To account for population stratification, the first five principal components were calculated using a subset of 42,888 SNPS that were not in LD with each other. Principal component analysis was conducted using the EIGENSTRAT program.[21]

The MACH v1.0.16 (http://www.sph.umich.edu/csg/yli/mach/index.html) software was used for GWAS imputation on the 22 autosomes. Once the data were cleaned, a two step process was carried out using the CEU samples from HapMap phase2 build 36 release 22 (http://hapmap.ncbi.nlm.nih.gov/index.html.en) as a reference panel.

With PLINK[27] as an interface with R, a linear regression model was used to examine the association between SNPs and keratometry adjusted for age, sex and the first two principal components, which account for population stratification in this cohort.

Meta-analysis method

Meta-analysis increases the power to identify associations by increasing the sample size. The β-coefficients strategy of the program METAL was used to conduct meta-analysis.[28]

Genetic power calculation

GWAS power (type 1 error cutoff = 5×10^{-8}) and Replication Power for two SNP test (cutoff = 0.05/2) was calculated using Genetic Power Calculator program (http://pngu.mgh.harvard.edu/~purcell/gpc/).[29]

Results

After quality control, the population sizes of the BATS/TEST and Raine studies were 1788 and 1013 respectively. Summary statistics for both the populations under study are shown in Table 1.

With consideration of combined sample size, the GWAS power to detect variant that could explain at least 1% variation of CC is very low (power = 30%). Whereas replication power for significant replication cut off 0.05/2 is more than 99%. Hence, our primary emphasis was on replication of *FRAP1* and *PDGFRA* genes, reported Genome wide association with CC in Asians, in Australian population of northern European ancestry. We tested whether the most significant SNP in each gene was associated in our European ancestry samples. We found that SNP rs2114039 in *PDGFRA* was associated with corneal curvature (P= 0.0045). Figure 1 shows the recombination profile between the SNPs and PDGFRA locus. The effect size of the trait increasing allele was 0.02275 mm per copy of the T allele. The effect sizes across different studies shown in Figure 2a.However, SNP rs6540964 in gene *FRAP1* was not associated with curvature (P=0.2984), effect sizes are shown in Figure 2b.

Although our study does not have enough power to detect a genome-wide associated variant, we report the initial findings. The Independent association test on BATS/TEST and Raine data yielded a best SNP rs4552334 (P-value=2.5×10^{-6}) and rs11930632 (P-value 2.47×10^{-6}) respectively. We followed this analysis by meta-analysis of outcomes from the 1,704,858 SNPs common in both studies.

Results from the meta-analysis across the genome are displayed in Figure 3. The 25 most significant SNPs from the meta-analysis are shown in Supplementary Table 1.

As expected meta-analysis did not reveal any genome-wide associated SNPs - the most associated genotyped SNP rs2444240 had p-value of 3.658×10^{-07} at 120.040 Mb (build 37) on chromosome 11. This SNP had a slightly stronger signal in the Raine study as shown in Table 2. The nearest gene to

these SNPs is *TRIM29* on chromosome 11q23.3 region (NCBI build 37), which spans only 26kb. Recombination profile between the SNPs and *TRIM29* locus is shown in supplementary figure 1.

Discussion

We have conducted a genome-wide association study on corneal curvature in Australian population of northern European ancestry. Our sample showed that SNPs near *PDGFRA*, which were very recently shown to play a role in CC in Asians, also play a role in samples of Northern European Ancestry. The allele frequency at rs2114039 is similar (~0.3) across the range of European and Asian ancestry samples in the HapMap3 data. The variance explained by rs2114039 in our European ancestry sample for BATS/TEST and RAINE study was 0.6% and 0.1% respectively, which is somewhat lower than that seen in the Asian studies (1.8%, 11.1%, 4.9%, 7.5% for SP2, SIMES, SINDI and SCORM study respectively). However, the estimates of variance explained in the Asian studies are probably an overestimate of the true effect size due to this SNP being selected as one of the most significant results from a genome-wide scan. [30] An estimate of the effect size in an independent Asian population would allow us to determine if the effect size at this SNP truly differs in the two populations.

Although, our study did not identify any genome-wide significant loci, meta-analyzing across two Australian studies led to a more significant top SNP, with rs2444240 in *TRIM29* achieving a P value of 3.658×10^{-07}. Larger sample sizes are required to unambiguously identify novel loci. With reference to past literature, we found involvement of some of the genes near or within the top 25 SNPs with some related traits.[31-34] A Gene expression profile of human keratoconus suggests significant expression of *TRIM29* gene.[31] This differential expression of *TRIM29* gene could be responsible for conical shape of cornea. Apart from this, a linkage study on Ashkenazi Jewish families also supports a possible role of *TRIM29* in variation of CC by their report of linkage between 11q23 loci and myopia. [32] In addition, a gene expression study on focal loss of retinal ganglion cells suggest significant down

regulation of the *BCL11B* gene. [34] Studies on myopia in chicks already established that the flattened cornea is an outcome of ganglion cells destruction. [33] Further study needs to be done to investigate the possible role of *TRIM29* and *BCL11B* genes in determining CC.

In conclusion, our study of CC showed that a SNP in *PDGFRA*, recently implicated in this trait in Asians, also underlies trait variation in Australians of Northern European ancestry. The other gene reported to be associated with CC in Asian populations, *FRAP1*, did not show a significant effect in our samples. Although our study was underpowered to detect novel loci, we found some evidence that SNPs near *TRIM29* may play a role in determining CC. Our findings of SNPs at *TRIM29* and other regions should be replicated in further studies, with meta-analyses likely to prove important in further dissecting this important endophenotype.

Summary

Corneal curvature (CC) is a risk factor for various vision disorders including keratoconus and myopia. This study reports association of PDGFRA gene with CC in Australian population, previously, which found associated in Asian population. It also report initial GWAS findings on CC in Australian population with northern European ancestry.

Acknowledgements

The Australian Twin Registry is supported by an Australian National Health and Medical Research Council (NHMRC) Enabling Grant (2004–2009). Genotyping for part of the Australian sample was funded by an NHMRC Medical Genomics Grant. Genotyping for the remainder was performed by the National Institutes of Health (NIH) and CIDR as part of an NIH/NEI grant 1RO1EY018246, and we are grateful to Dr Camilla Day and staff. AWH and SM are supported by Australian NHMRC Career Development Awards. The authors acknowledge the funding and support of the NIH/NEI grant 1RO1EY018246, and NIH/Center for Inherited Diseases Research genotyping project grant (PI: Terri Young).

Ophthalmic examination of the Twins was funded by the Clifford Craig Medical Research Trust, Ophthalmic Research Institute of Australia (ORIA), American Health Assistance Foundation (AHAF), Peggy and Leslie Cranbourne Foundation, Foundation for Children, National Health and Medical Research Foundation Project Grant 350415 (2005–2007), Jack Brockhoff Foundation and the Pfizer Australia Senior Research Fellowship (DAM).

The core management of the Raine Study is funded by The University of Western Australia (UWA), The Telethon Institute for Child Health Research, Raine Medical Research Foundation, UWA Faculty of Medicine, Dentistry and Health Sciences, Women's and Infant's Research Foundation and Curtin University. Genotyping was funded by NHMRC project grant 572613. Support for the Raine Eye Health Study was provided by NHMRC Grant 1021105, Lions Eye Institute, the Australian Foundation for the Prevention of Blindness and Alcon Research Institute.

The QIMR authors thank the twins eye study participants and their families; Scott D. Gordon, Anjali K. Henders, Sarah E. Medland, Brian McEvoy, Dale R. Nyholt, Margaret J. Wright, Megan J. Campbell, and Anthony Caracella for their assistance in processing the Australian genotyping data. We are also grateful for Jane MacKinnon, Shayne Brown, Lisa Kearns, Sandra Staffieri, Olivia Bigault, Colleen Wilkinson, Julie Barbour, Byoung Sung Chu, Jonathan Ruddle Paul Sanfilippo, Cong Sun, Justin Sherwin, Robert Macmillan, Rachael Adams, Robyn Troutbeck, Ya Ling Ma, Christine Chen and Amy Cohn In addition, we appreciate the assistance in recruiting twins from Thanuja Gunasekera, Allison McKenzie, Anne-Louise Ponsonby, Terry Dwyer, James Dilger, Palma Ragno, Jenny Boadle, Kim Dorrell, Shyamali Dharmage, John Hopper and Jamie Craig.

The Raine Eye Health Study authors thank the Raine eye health study participants and their families. They also thank the Raine Study team, and ophthalmologists, orthoptists and medical students at Lions Eye Institute for cohort co-ordination and data collection, particularly: Hannah Forward, Charlotte McKnight, Alex Tan, Alla Soloshenko, Sandra Oates, and Diane Wood.

References

1. Rabinowitz, Y.S. (1998). Keratoconus. Surv Ophthalmol 42, 297-319.
2. Dirani, M., Islam, A., Shekar, S.N., and Baird, P.N. (2008). Dominant genetic effects on corneal astigmatism: the genes in myopia (GEM) twin study. Invest Ophthalmol Vis Sci 49, 1339-1344.
3. Maumenee, I.H. (1981). The eye in the Marfan syndrome. Trans Am Ophthalmol Soc 79, 684-733.
4. Ip, J.M., Huynh, S.C., Robaei, D., Kifley, A., Rose, K.A., Morgan, I.G., Wang, J.J., and Mitchell, P. (2008). Ethnic differences in refraction and ocular biometry in a population-based sample of 11-15-year-old Australian children. Eye (Lond) 22, 649-656.
5. Kleinstein, R.N., Jones, L.A., Hullett, S., Kwon, S., Lee, R.J., Friedman, N.E., Manny, R.E., Mutti, D.O., Yu, J.A., and Zadnik, K. (2003). Refractive error and ethnicity in children. Arch Ophthalmol 121, 1141-1147.
6. Han, S., Chen, P., Fan, Q., Khor, C.C., Sim, X., Tay, W.T., Ong, R.T., Suo, C., Goh, L.K., Lavanya, R., et al. (2011). Association of variants in FRAP1 and PDGFRA with corneal curvature in Asian populations from Singapore. Hum Mol Genet 20, 3693-3698.
7. Lim, L.S., Saw, S.M., Jeganathan, V.S., Tay, W.T., Aung, T., Tong, L., Mitchell, P., and Wong, T.Y. (2010). Distribution and determinants of ocular biometric parameters in an Asian population: the Singapore Malay eye study. Invest Ophthalmol Vis Sci 51, 103-109.
8. Klein, A.P., Suktitipat, B., Duggal, P., Lee, K.E., Klein, R., Bailey-Wilson, J.E., and Klein, B.E. (2009). Heritability analysis of spherical equivalent, axial length, corneal curvature, and anterior chamber depth in the Beaver Dam Eye Study. Arch Ophthalmol 127, 649-655.
9. Biino, G., Palmas, M.A., Corona, C., Prodi, D., Fanciulli, M., Sulis, R., Serra, A., Fossarello, M., and Pirastu, M. (2005). Ocular refraction: heritability and genome-wide search for eye morphometry traits in an isolated Sardinian population. Hum Genet 116, 152-159.
10. Hammond, C.J., Duncan, D.D., Snieder, H., de Lange, M., West, S.K., Spector, T.D., and Gilbert, C.E. (2001). The heritability of age-related cortical cataract: the twin eye study. Invest Ophthalmol Vis Sci 42, 601-605.
11. Lyhne, N., Sjolie, A.K., Kyvik, K.O., and Green, A. (2001). The importance of genes and environment for ocular refraction and its determiners: a population based study among 20-45 year old twins. Br J Ophthalmol 85, 1470-1476.
12. Klein, R.J., Zeiss, C., Chew, E.Y., Tsai, J.Y., Sackler, R.S., Haynes, C., Henning, A.K., SanGiovanni, J.P., Mane, S.M., Mayne, S.T., et al. (2005). Complement factor H polymorphism in age-related macular degeneration. Science 308, 385-389.
13. Sladek, R., Rocheleau, G., Rung, J., Dina, C., Shen, L., Serre, D., Boutin, P., Vincent, D., Belisle, A., Hadjadj, S., et al. (2007). A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 445, 881-885.
14. Yamazaki, K., McGovern, D., Ragoussis, J., Paolucci, M., Butler, H., Jewell, D., Cardon, L., Takazoe, M., Tanaka, T., Ichimori, T., et al. (2005). Single nucleotide polymorphisms in TNFSF15 confer susceptibility to Crohn's disease. Hum Mol Genet 14, 3499-3506.
15. Stranger, B.E., Stahl, E.A., and Raj, T. (2011). Progress and promise of genome-wide association studies for human complex trait genetics. Genetics 187, 367-383.
16. Visscher, P.M., Brown, M.A., McCarthy, M.I., and Yang, J. (2012). Five years of GWAS discovery. Am J Hum Genet 90, 7-24.
17. Wong, T.Y., Foster, P.J., Johnson, G.J., Klein, B.E., and Seah, S.K. (2001). The relationship between ocular dimensions and refraction with adult stature: the Tanjong Pagar Survey. Invest Ophthalmol Vis Sci 42, 1237-1242.

18. Fotedar, R., Wang, J.J., Burlutsky, G., Morgan, I.G., Rose, K., Wong, T.Y., and Mitchell, P. (2010). Distribution of axial length and ocular biometry measured using partial coherence laser interferometry (IOL Master) in an older white population. Ophthalmology 117, 417-423.
19. Pan, C.W., Wong, T.Y., Chang, L., Lin, X.Y., Lavanya, R., Zheng, Y.F., Kok, Y.O., Wu, R.Y., Aung, T., and Saw, S.M. (2011). Ocular biometry in an urban Indian population: the Singapore Indian Eye Study (SINDI). Invest Ophthalmol Vis Sci 52, 6636-6642.
20. Mackey, D.A., Mackinnon, J.R., Brown, S.A., Kearns, L.S., Ruddle, J.B., Sanfilippo, P.G., Sun, C., Hammond, C.J., Young, T.L., Martin, N.G., et al. (2009). Twins eye study in Tasmania (TEST): rationale and methodology to recruit and examine twins. Twin Res Hum Genet 12, 441-454.
21. Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E., Shadick, N.A., and Reich, D. (2006). Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 38, 904-909.
22. Peltonen, L. (2003). GenomEUtwin: a strategy to identify genetic influences on health and disease. Twin Res 6, 354-360.
23. McEvoy, B.P., Montgomery, G.W., McRae, A.F., Ripatti, S., Perola, M., Spector, T.D., Cherkas, L., Ahmadi, K.R., Boomsma, D., Willemsen, G., et al. (2009). Geographical structure and differential natural selection among North European populations. Genome Res 19, 804-814.
24. (2010). A map of human genome variation from population-scale sequencing. Nature 467, 1061-1073.
25. Li, Y., Willer, C.J., Ding, J., Scheet, P., and Abecasis, G.R. (2010). MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol 34, 816-834.
26. Abecasis, G.R., Cherny, S.S., Cookson, W.O., and Cardon, L.R. (2002). Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 30, 97-101.
27. Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., et al. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81, 559-575.
28. Willer, C.J., Li, Y., and Abecasis, G.R. (2010). METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics 26, 2190-2191.
29. Purcell, S., Cherny, S.S., and Sham, P.C. (2003). Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19, 149-150.
30. Goring, H.H., Terwilliger, J.D., and Blangero, J. (2001). Large upward bias in estimation of locus-specific effects from genomewide scans. Am J Hum Genet 69, 1357-1369.
31. Rabinowitz, Y.S., Dong, L., and Wistow, G. (2005). Gene expression profile studies of human keratoconus cornea for NEIBank: a novel cornea-expressed gene and the absence of transcripts for aquaporin 5. Invest Ophthalmol Vis Sci 46, 1239-1246.
32. Stambolian, D., Ibay, G., Reider, L., Dana, D., Moy, C., Schlifka, M., Holmes, T., Ciner, E., and Bailey-Wilson, J.E. (2004). Genomewide linkage scan for myopia susceptibility loci among Ashkenazi Jewish families shows evidence of linkage on chromosome 22q12. Am J Hum Genet 75, 448-459.
33. Fischer, A.J., Morgan, I.G., and Stell, W.K. (1999). Colchicine causes excessive ocular growth and myopia in chicks. Vision Res 39, 685-697.
34. Panagis, L., Zhao, X., Ge, Y., Ren, L., Mittag, T.W., and Danias, J. (2010). Gene expression changes in areas of focal loss of retinal ganglion cells in the retina of DBA/2J mice. Invest Ophthalmol Vis Sci 51, 2024-2034.

Tables

Table 1. Demographic details of study participants

	TEST and BATS	Raine Study
Number of subjects	1788	1013
Number of families	857	1013
Mean age in years	22.2	20.0
Range of age	5 to 90	18 to 22
Sex (% female)	1014 (56.7)	497 (49.1)
Mean corneal curvature, mm (SD)	7.63 (0.24)	7.72 (0.24)
Range of corneal curvature, mm	6.77 to 8.41	7.04 to 8.61

Table 1: Demographic details of study participants Corneal curvature was measured in millimeters (mm), corneal curvature mentioned here are average of the mean of corneal curvature measured vertically and horizontally for left and right eyes of participants respectively.

Table 2: Top 4 SNPs and their association results

Marker	Chr	Coordinate (build 36)	Nearest Gene	Alleles*	BATS/ TEST Effect	SE	BATS/ TEST P	RAINE Effect	SE	RAINE P	Weighted Effect	SE	Meta analysis P
rs2444240	11	119545652	*TRIM29*	T/G	0.030631	0.009544	1.28×10^{-03}	0.044733	0.011125	5.71×10^{-05}	-0.0364	0.0071	3.66×10^{-07}
rs494965	11	119558860	*TRIM29*	T/C	-0.0309	0.009392	1.12×10^{-03}	-0.04317	0.010944	9.35×10^{-05}	0.0365	0.0073	4.50×10^{-07}
rs470606	11	119533412	*TRIM29*	T/G	0.029068	0.009541	2.2×10^{-03}	0.045626	0.011163	4.41×10^{-05}	-0.0359	0.0071	5.66×10^{-07}
rs470373	11	119531481	*TRIM29*	T/C	0.029068	0.009541	2.2×10^{-03}	-0.04492	0.010922	4.61×10^{-05}	0.0362	0.0073	5.90×10^{-07}

*The first letter in the Alleles column is the effect allele for specified SNP, e.g., For SNP rs2444240; T is effect allele not G.

Figures

Figure 1: Association of variants at PDGFRA locus

Figure 1: Association of variants at the *PDGFRA* locus. The top SNP rs2114039 has a P value 4.549×10^{-03}. The red shading shows the degree of linkage disequilibrium between rs2444240 and neighboring SNPs. The light blue line displays the rate of recombination with scale on right hand axis.

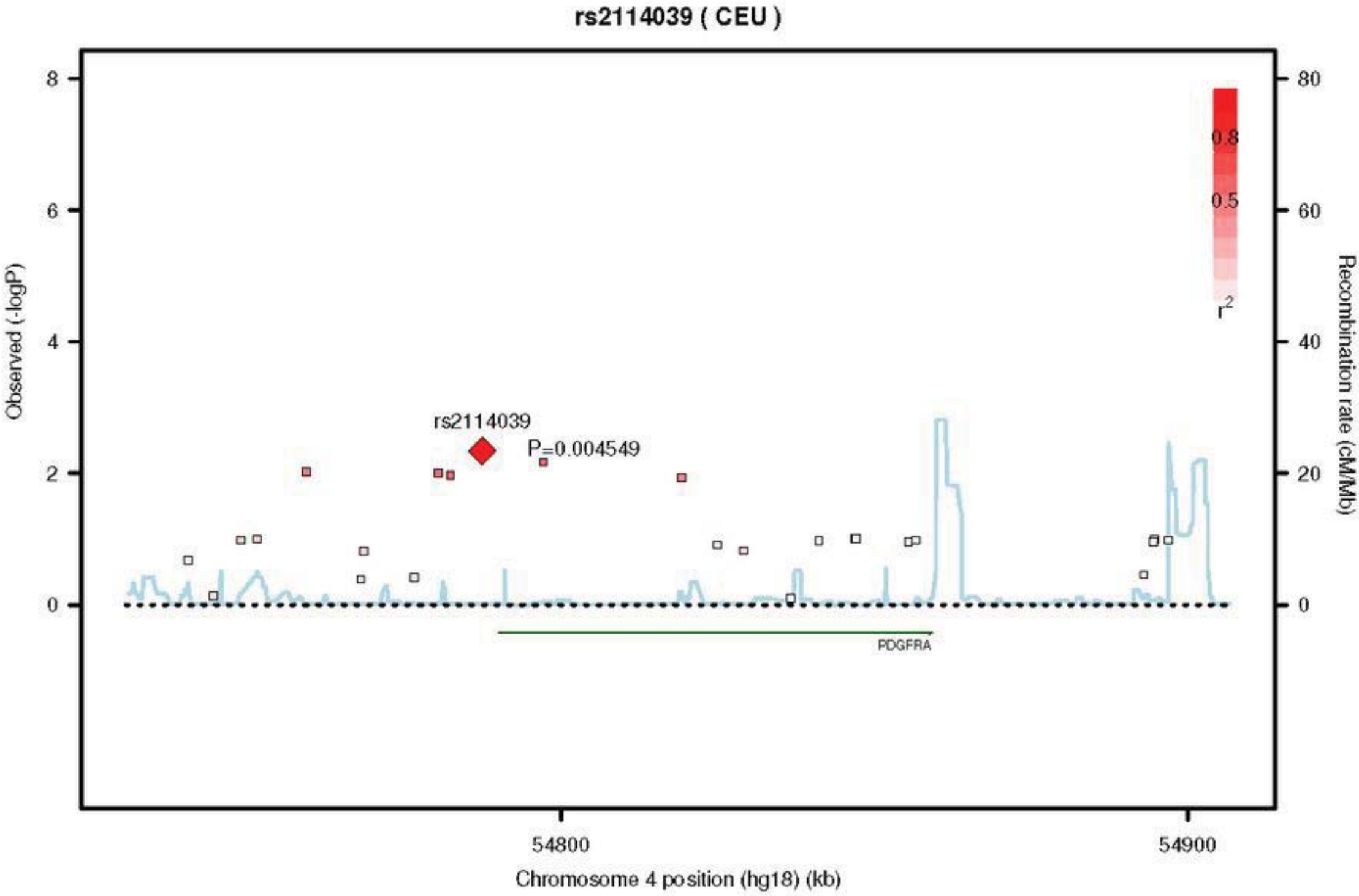
Figure 2a: Forest plot for PDGFRA SNP rs2114039

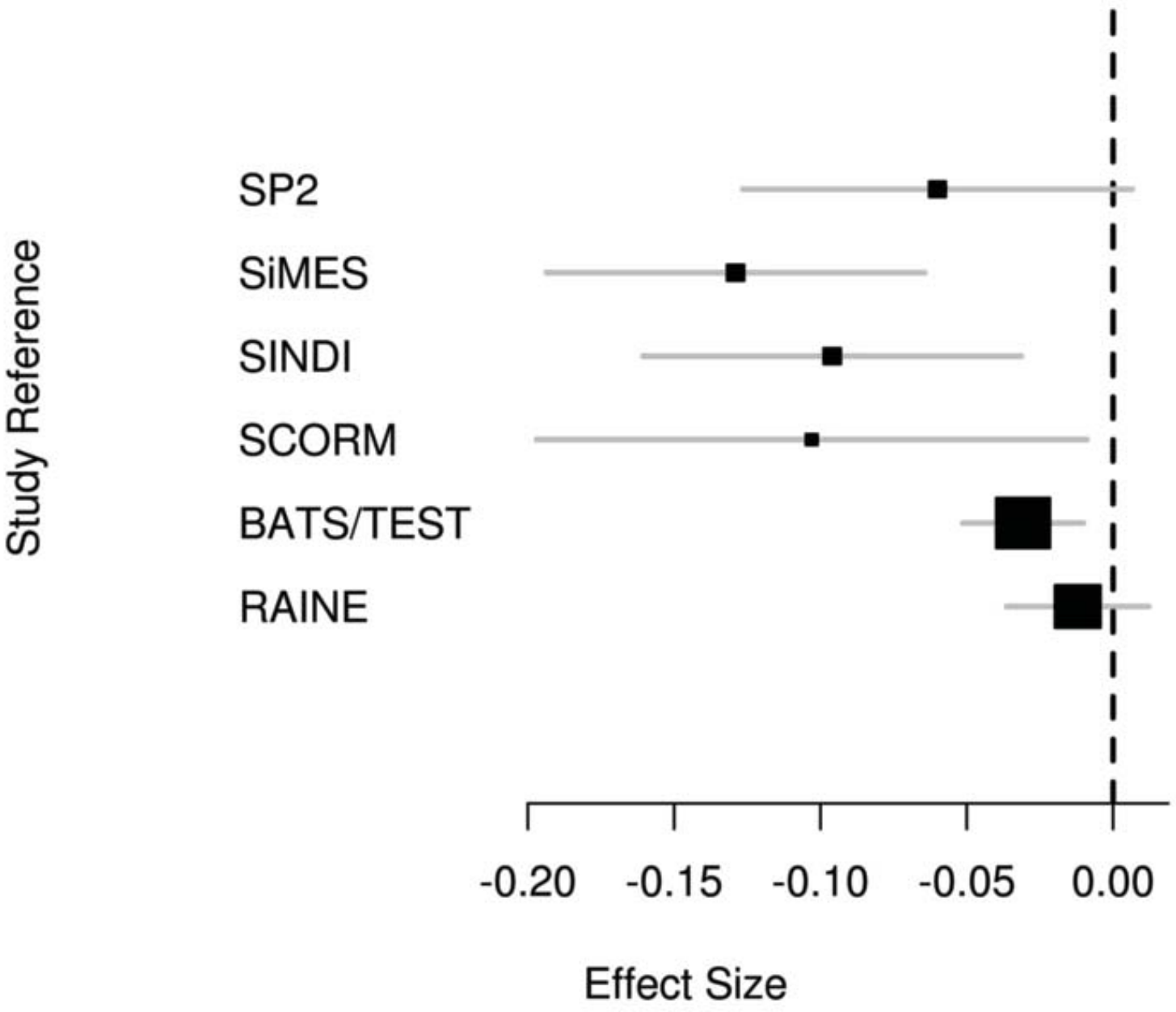
Figure 2b: Forest plot for FRAP1 SNP rs6540964

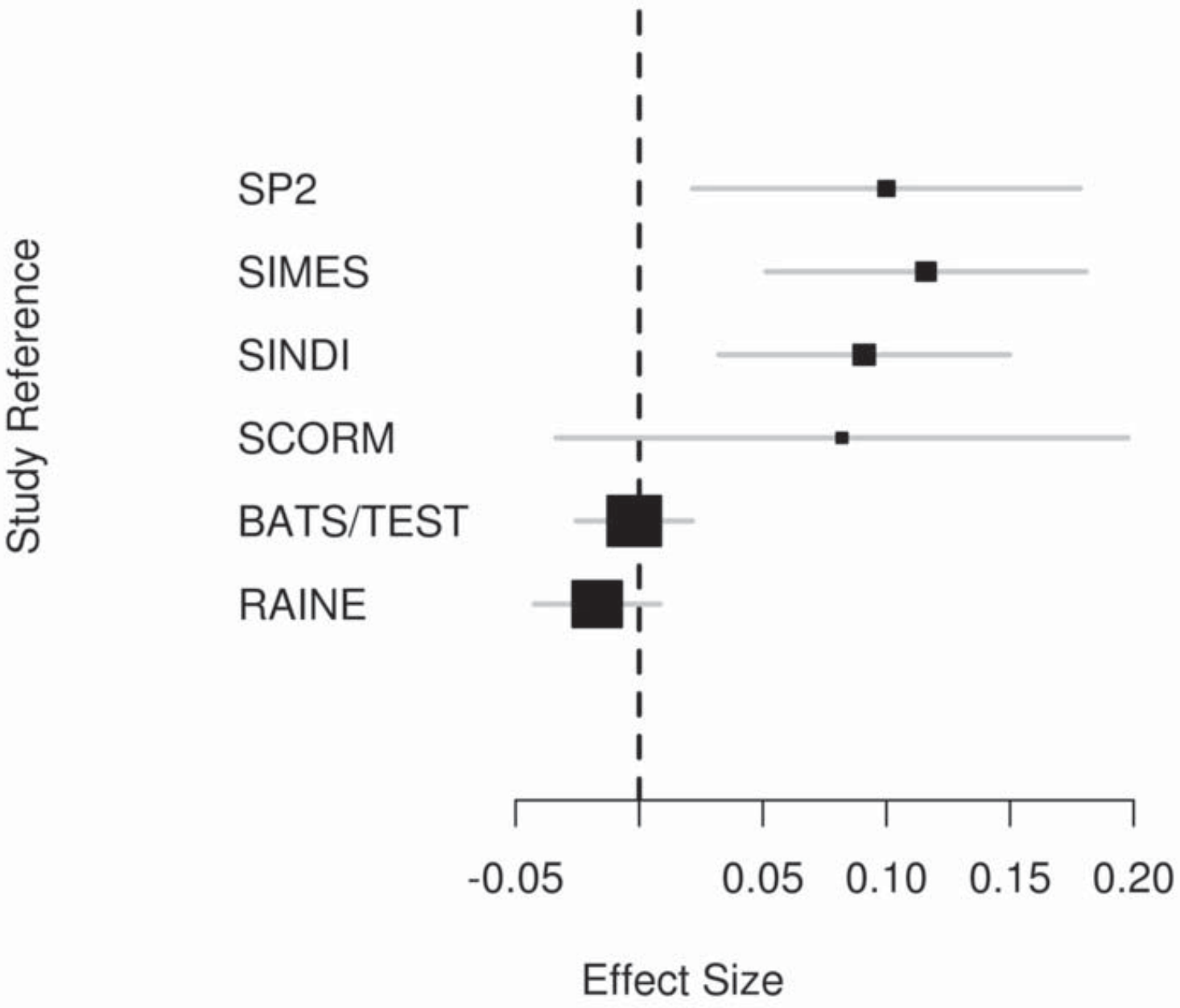
Figure2: Forrest plot. It shows effect size distribution in different studies for (2a) *PDGFRA* SNP rs2114039 and (2b) *FRAP1* SNP rs6540964. The top four studies are reported by corneal curvature studies in an Asian population. The last two studies demonstrate the effect size distribution for SNPs found in our analysis of corneal curvature GWAS on Australians with Northern European Ancestry.

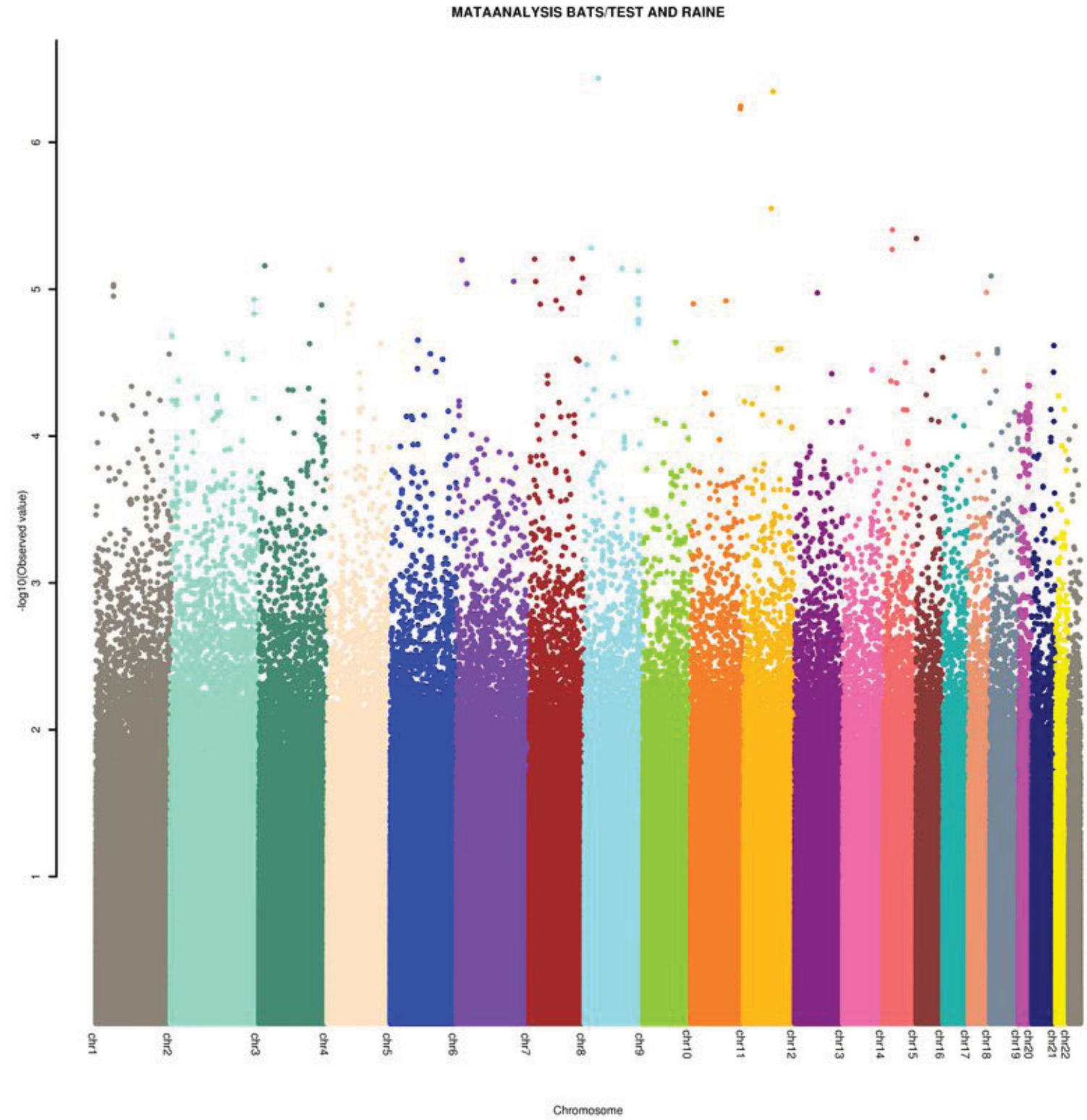
Figure 3: Manhattan Plot

Figure 3: Manhattan plot. This shows SNP p-value distribution of meta-analysis results with respect to the chromosome.


rs2114039 (CEU)
Observed (-logP)
Recombination rate (cM/Mb)
8
6
4
2
0
80
60
40
20
0
0.8
0.5
r2
rs2114039
P=0.004549
PDGFRA
54800
54900
Chromosome 4 position (hg18) (kb)


Study Reference
SP2
SiMES
SINDI
SCORM
BATS/TEST
RAINE
-0.20
-0.15
-0.10
-0.05
0.00
Effect Size


Study Reference
SP2
SIMES
SINDI
SCORM
BATS/TEST
RAINE
-0.05
0.05
0.10
0.15
0.20
Effect Size


MATAANALYSIS BATS/TEST AND RAINE
-log10(Observed value)
1
2
3
4
5
6
chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chr20
chr21
chr22
Chromosome