Identification of a Candidate Gene for Astigmatism

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PURPOSE. Astigmatism is a common refractive error that reduces vision, where the curvature and refractive power of the cornea in one meridian are less than those of the perpendicular axis. It is a complex trait likely to be influenced by both genetic and environmental factors. Twin studies of astigmatism have found approximately 60% of phenotypic variance is explained by genetic factors. This study aimed to identify susceptibility loci for astigmatism.

METHODS. We performed a meta-analysis of seven genome-wide association studies that included 22,100 individuals of European descent, where astigmatism was defined as the number of diopters of cylinder prescription, using fixed effect inverse variance-weighted methods.

RESULTS. A susceptibility locus was identified with lead single nucleotide polymorphism rs3771395 on chromosome 2p13.3 (meta-analysis, $P = 1.97 \times 10^{-7}$) in the *VAX2* gene. *VAX2* plays an important role in the development of the dorsoventral axis of the eye. Animal studies have shown a gradient in astigmatism along the vertical plane, with corresponding changes in refraction, particularly in the ventral field.

CONCLUSIONS. This finding advances the understanding of refractive error, and provides new potential pathways to be evaluated with regard to the development of astigmatism. (*Invest Ophthalmol Vis Sci.* 2013;54:1260–1267) DOI: 10.1167/iovs.12-10463

Astigmatism is a common refractive error that reduces vision, where the curvature and refractive power of the cornea in one meridian are less than those of the perpendicular axis. There are two components of astigmatism that can be independently measured, refractive astigmatism (also called noncorneal astigmatism) and corneal astigmatism; this study deals with refractive astigmatism. Astigmatism is an important clinical problem because it is common; prevalence ranges between 20% and 29.3% among adults in Europe and 36.2% among subjects 20 years old and older in the United States,[1–3]
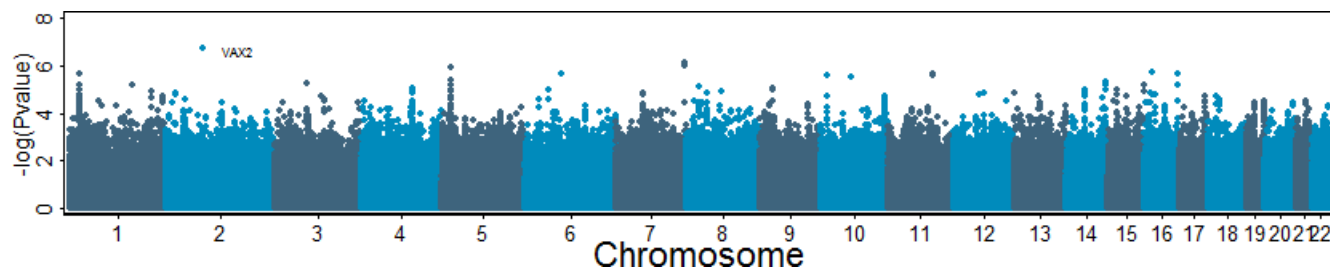
FIGURE 1. Manhattan plot of 2.8 million single nucleotide polymorphisms (SNPs) for a meta-analysis of seven cohorts for astigmatism. The –log10 *P* values are plotted against position in each chromosome. Chromosomes are shown in alternating contrasts for clarity. The strongest association is on chromosome 2.

with a significant social and economic impact, and is a risk factor for amblyopia and anisometropia.[4,5] Astigmatism is commonly associated with other refractive errors.[6] Understanding causes of astigmatism might also provide some insights into keratoconus, the most common reason for corneal transplantation in Europe.

The cause of astigmatism is complex and not fully understood. Few environmental risk factors have been reported, although a Singapore study found the number of hours playing video games was associated with more severe astigmatism in school children between 7 and 9 years old.[7] Previous studies have reported that the risk of developing astigmatism doubles in first-degree relatives of individuals with astigmatism.[8-10] Family and twin studies have determined a broad sense heritability of approximately 60%,[2,11,12] which suggests a significant genetic contribution to astigmatism.

There are, however, no genes known that influence astigmatism risk, and given the poor understanding of the pathways involved in its development, we felt that the hypothesis-free approach of genome-wide association studies (GWAS), which has been shown to be very successful in identifying common variants associated with common diseases and traits,[13] seemed appropriate. To date, GWAS have identified susceptibility variants in corneal astigmatism in an Asian meta-analysis[14] and myopia/hyperopia.[15-17]

In order to explore the putative susceptibility loci that underlie astigmatism, we conducted a meta-analysis of seven GWAS (TwinsUK, Rotterdam Eye Study, 1958 British birth cohort, and Australian cohorts) with a total of 22,100 individuals.

MATERIALS AND METHODS

Study Populations

UK Twin Cohort. The UK twin (TwinsUK) cohort consists of 5654 genotyped subjects (5158 females and 496 males) belonging to 3601 families, ranging between 16 and 84 years old (SD ±12.2 years). Twins were recruited through the TwinsUK Adult Twin Registry held at St. Thomas' Hospital, London, where they were invited to undergo an eye examination. Details of the registry have been described elsewhere.[18] Informed consent was obtained, and the research adhered to tenets of the Declaration of Helsinki. Twins were examined between January 1998 and September 2009.

All twin pairs underwent nondilated refraction using a Humphrey-670 (Humphrey Instruments, San Leandro, CA) automatic refractor (1998–2002) and subsequently an ARM-10 autorefractor (Takagi Seiko, Japan).[12] An automatic refractor measures refractive error by detection of infrared light aligned through the pupil and reflected back by the

retina. The astigmatism was calculated as the absolute value of cylinder (Ast = |cylinder|), and a posteriori, it was normalized by applying the inverse normal transformation. Here we decided to use absolute cylinder value rather than polar value as the former is an untransformed value and, thus, a direct measure of cylinder power and includes all types of astigmatism, not only with-the-rule (WTR) and against-the-rule (ATR) astigmatism but also oblique astigmatism, in contrast to the polar value, which is a transformed value[19] and excludes oblique astigmatism (transforms oblique astigmatism to values of zero). Mean astigmatism was 0.76 diopters (D) (±0.69) with a range of 0 to 6.625. Astigmatism presented a leptokurtic distribution with a right skew of 2.48 (Supplementary Table S1 [see Supplementary Material and Supplementary Table S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10463/-/DCSupplemental]).

Dutch Cohorts: Rotterdam Studies I-III and Erasmus Rucphen Family Study. Dutch cohorts comprised four different populations: three were population-based Rotterdam Study (RS-I, RS-II, and RS-III),[20] and one was the family based Erasmus Rucphen Family (ERF) study. RS-I included 5513 subjects ranging from 55 to 99 years old. Baseline ophthalmologic examinations took place between 1991 and 1993 and included 6775 subjects. Individuals were excluded if they had undergone bilateral cataract surgery, laser refractive procedures, or other intraocular procedures which might alter refraction. The RS-II cohort consisted of 2000 new subjects between the ages of 55 and 95 years old. Baseline examinations were carried out from 2000 to 2002 and follow-up examinations were from 2004 to 2005. RS-III cohort consisted of 3434 new individuals, 45 to 97 years old. Baseline examinations took place between 2006 and 2009. The last Dutch cohort, ERF, consisted of 2032 living descendents, ranging between 18 and 86 years old from 22 families.

All measurements of astigmatism were taken through nondilated pupils, using an automated measurement of refractive error (model RM-A2000 autorefractor; Topcon, Tokyo, Japan), with the approval of the Medical Ethics Committee of Erasmus University, and all participants gave written informed consent in accordance with the Declaration of Helsinki. Astigmatism was calculated by the same formula: inverse normal transformation of the absolute of the mean cylinder. RS-I, -II, -III and ERF cohorts present a leptokurtic distribution ranging from 0 to 7.5 D (mean, 0.97 D); 0 to 5.625 D (mean, 0.91 D); 0 to 5.875 D (mean, 0.83 D); and 0 to 5.37 D (mean, 0.609 D), respectively, with a right skew of 1.97, 1.79, 2.01, and 2.25, respectively (Supplementary Table S1 [see Supplementary Material and Supplementary Table S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10463/-/DCSupplemental]).

Australian Cohort. The Australian Twin Eye study included subjects examined as part of the Twin Eye Study in Tasmania and the Brisbane Adolescent Twin Study, between 2004 and 2009. In total this study included 1809 subjects from 786 families, 18 years old or older at the time of the examination. Ethical approval was obtained from the Royal Victorian Eye and Ear Hospital, University of Tasmania, Australian

Twin Registry, and Queensland Institute of Medical Research and adhered to tenets of the Declaration of Helsinki. Subjects underwent cycloplegia (following instillation of tropicamide 1%), and refraction for both eyes was measured using a Humphrey-598 automatic refractor (Carl Zeiss Meditec, Inc., Miami, FL). The normalized value, after applying the inverse normal transformation, of the absolute of the mean cylinder of both eyes was used for analysis. The distribution of absolute cylinder was similar to the one used in the other cohorts, leptokurtic distribution with a right skew of 2.8. Absolute cylinder ranged between 0 and 4 D, and the mean was 0.38 D ($\pm$0.01) (Supplementary Table S1 [see Supplementary Material and Supplementary Table S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10463/-/DCSupplemental]).

1958 British Birth Cohort. The 1958 British birth cohort was a prospective population-based study that initially included 17,000 newborn children whose births occurred within the first week of March 1958. All participants gave informed written consent to participate in genetic association studies, and the study was approved by the South East Multicentre Research Ethics Committee (MREC), Oversight Committee for the biomedical examination of the 1958 British birth cohort and adhered to tenets of the Declaration of Helsinki. Biomedical examination protocols were approved by the South East MREC.

The phenotype for this cohort was absolute cylinder (mean of both eyes) measured with noncycloplegic autorefraction (Retinomax 2; Nikon). Absolute cylinder was normalized a posteriori, using inverse normal transformation. A total of 1658 randomly chosen subjects from this cohort, all 44 to 45 years old, were included in the GWAS. This group included 760 females and 898 males, and the average astigmatism value was 0.49 ($\pm$0.49 D), ranging from 0 to 5.625 D. The distribution of astigmatism was leptokurtic with a right skew of 3.2 (Supplementary Table S1 [see Supplementary Material and Supplementary Table S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10463/-/DCSupplemental]).

Genotyping and Quality Control

TwinsUK Cohort. Genotyping was carried out using a combination of Illumina arrays (Illumina, Inc., San Diego, CA): HumanHap 300k Duo HumanHap 610-Quad, and 1M-Duo and 1.2M-Duo. The imputation was performed with reference to HapMap (http://www.hapmap.org, available in the public domain by the International HapMap project) release 22 CEU (Utah residents with Northern and Western European ancestry from the CEPH collection) using IMPUTE version 2.[21] In total, this panel has complete information for 2.6 million single nucleotide polymorphisms (SNPs), of which up to 874,733 SNPs are directly genotyped (HumanHap300: 303,940 SNPs; HumanHap610Q: 553,487 SNPs; and HumanHap1M and 1.2M: 874,733 SNPs) for all individuals. As part of quality control, SNPs were excluded if they had a call rate $\leq$97%, a minor allele frequency (MAF) $\leq$0.05, or significant deviations from Hardy-Weinberg equilibrium (HWE) ($P \leq 10^{-6}$). A principal component analysis was performed in order to confirm the genetic ancestry of this cohort by comparison to standard HapMap Phase 2 population controls.

Dutch Cohorts: RS I-III and ERF. In the RS-I cohort, genotyping was performed using Infinium II HumanHap 550 chip version 3.0 array (Illumina); the RS-II cohort was genotyped using HumanHap 550 Duo Arrays and Human 610 Quad Arrays (Illumina), and the RS-III cohort was genotyped using Human 610 Quad Arrays (Illumina) only. For ERF, DNA was genotyped with one of four different platforms (Illumina 6K, Illumina 318K, Illumina 370K, and Affymetrix 250K). The genotyped set was imputed using MACH,[22] resulting in an analysis of a total of 2.5 million SNPs from 530,683 genotyped SNPs (RS cohorts) and 495,478 genotyped SNPs (ERF). Exclusion criteria for SNPs were a MAF $\leq$0.01, an HWE of $P < 10^{-6}$, or an SNP call rate $\leq$90%.

TABLE. Summary Statistics of the 10 Top Associated SNPs of Seven Independent Studies of Astigmatism

SNP	Chr.	Position B36 (bp)	Risk Allele	Nonrisk Allele	MAF	Closest Gene	TwinsUK			RS-I			RS-II		
							Beta	SEM	P	Beta	SEM	P	Beta	SEM	P
rs3771395	2	70,986,522	G	A	0.16	VAX2	−0.043	0.035	2.21E-01	−0.125	0.029	1.28E-05	−0.108	0.046	2.05E-02
rs10226930	7	155,530,056	C	T	0.07	SHH	NA	NA	NA	−1.511	0.417	2.89E-04	−1.578	0.478	9.72E-04
rs7802427	7	155,522,186	C	T	0.06	SHH	NA	NA	NA	−1.452	0.414	4.59E-04	−1.609	0.484	8.81E-04
rs795544	5	13,851,559	G	T	0.17	DNAH5	0.104	0.029	2.79E-04	0.077	0.024	1.17E-03	0.076	0.039	4.83E-02
rs12445126	16	17,647,193	G	A	0.09	XYLT1	−0.098	0.043	2.47E-02	−0.055	0.033	9.36E-02	−0.052	0.055	3.45E-01
rs9445732	6	67,305,131	G	T	0.05	NA	1.245	0.491	1.13E-02	0.512	1.050	6.26E-01	2.200	1.154	5.66E-02
rs11644988	16	85,042,632	G	A	0.12	FOXF1	0.258	0.086	2.87E-03	0.074	0.040	6.44E-02	0.093	0.071	1.89E-01
rs11645033	16	85,042,762	G	A	0.09	FOXF1	0.250	0.086	3.61E-03	0.074	0.040	6.46E-02	0.094	0.071	1.83E-01
rs7743168	6	67,314,603	A	G	0.13	NA	1.592	0.653	1.18E-02	0.515	1.083	6.34E-01	2.256	1.173	5.45E-01
rs485842	11	95,662,862	A	G	0.261	MAML2	0.020	0.025	4.22E-01	0.078	0.020	8.61E-05	0.069	0.033	3.76E-02

Beta, effect size; Chr., chromosome; MAF, global minor allele frequency in dbSNP; NA, missing value; SEM, beta standard error of the mean.

Australian Cohort. The Australian cohort was genotyped using the Human Hap610 Quad array. The genotyped panel was imputed using MACH[22] with HapMap data obtained from people of northern and western European ancestry, resulting in a total of 2.3 million SNPs, including 513,908 SNPs with complete genotype information. For quality control, SNPs were excluded in accordance with a series of quality control filters, including an SNP call rate <95%, a MAF <0.01, and a P value for HWE test of $<10^{-6}$. Genotypic data for non-European ancestral outliers were excluded using a principal component analysis by comparing Australian twin data with 16 global populations taken from HapMap Phase 3.

1958 British Birth Cohort. Genotyping was carried out primarily using a Human Omni 1M-duo chip (Illumina), which contains 910,582 SNPs ($n = 1000$). Exclusion criteria for SNPs were MAF <0.02, SNP call rate <95%, and HWE $P < 1 \times 10^{-4}$. Additional genotypes ($n = 658$) were obtained using SNP Array version 6.0 (Affymetrix, Inc., Santa Clara, CA), HumanHap 550 Duo Arrays, and Cardio-Metabochip (Illumina), where most of the samples were genotyped two or more times using different chips.

Statistical Analysis

Association was analyzed using GenABEL[23] in the TwinsUK cohort, GRIMP[24] in the RS I–III cohorts, ProbABEL software[25] in the ERF study, Merlin[26] in the Australian study, and PLINK[27] in the 1958 British birth cohort. The presence of heterogeneity was calculated using Cochran's Q and I[2] test statistic.[28,29]

Summary statistics (effect sizes) for the risk (minor) allele were combined from seven family and population-based association studies involving Caucasian individuals of European ancestry in the TwinsUK, Dutch (RS-I, RS-II, RS-III, and ERF), Australian, and 1958 British birth cohorts. Meta-analysis was performed using the fixed effect inverse variance-weighted method in genome-wide association meta-analysis (GWAMA) software tool for meta-analysis of whole-genome association data.[30]

Quantile-quantile (Q-Q) plots were used to evaluate the overall significance of the genome-wide association results and the potential effect of population stratification. The genomic control inflation factor was calculated for the overall samples as described previously.[31] The genomic control values for the directly genotyped SNPs were 1.003 (TwinsUK), 1.054 (RS-I), 1.012 (RS-II, -III), 1.037 (ERF), 1.01 (Australian cohort), and 1.002 (British birth cohort). The linkage disequilibrium (LD) patterns (including McVean's fine scale recombination rate and squared correlation coefficient) were investigated for the associated loci including the susceptibility loci by using data from HapMap project.

RESULTS

No genome-wide significant evidence for association was observed for any single cohort, assuming a threshold P value of 5×10^{-8} as previously suggested[32-34]; therefore, we proceeded with the meta-analysis. A meta-analysis of seven cohorts (TwinsUK, RS-I, RS-II, RS-III, ERF, Australian, and 1958 British birth cohorts) consisting of 22,100 individuals of European descent was conducted. After genomic control correction for each component study, the association data for 2.8 million autosomal SNPs were combined into a fixed effect additive model meta-analysis using inverse variance-weighted model. The genomic control inflation for the overall samples was small ($\lambda_{gc} = 1.018$), suggesting that the observed results were not confounded by population stratification which is reflected in the alignment of the Q-Q plot of the meta P values (Supplementary Fig. S1 [see Supplementary Material and

Table. Extended

| SNP	RS-III Beta	SEM	P	ERF Beta	SEM	P	1958 British Cohort Beta	SEM	P	Australian Twins Beta	SEM	P	Meta-Analysis Beta	P
rs3771395	−0.062	0.048	1.94E-01	−0.067	0.049	1.20E-01	−0.086	0.056	1.23E-01	−0.36	0.051	4.83E-01	−0.082	1.97E-07
rs10226930	−0.879	0.640	1.70E-01	−0.107	1.665	1.44E-03	NA	NA	NA	NA	NA	NA	−1.375	7.76E-07
rs7802427	−0.886	0.644	1.69E-01	−0.105	1.715	1.47E-03	NA	NA	NA	NA	NA	NA	−1.362	1.06E-06
rs795544	0.040	0.039	3.16E-01	0.012	0.040	2.10E-01	0.079	0.046	8.30E-02	−0.018	0.044	6.73E-01	0.063	1.19E-06
rs12445126	−0.126	0.055	2.09E-02	−0.108	0.060	1.11E-01	−0.143	0.063	2.36E-02	−0.110	0.059	6.38E-02	−0.088	1.75E-06
rs9445732	2.165	1.093	4.76E-02	1.205	0.376	9.98E-01	NA	NA	NA	NA	NA	NA	1.284	2.03E-06
rs11644988	0.194	0.066	3.10E-03	0.309	0.182	2.63E-02	NA	NA	NA	0.141	0.077	6.71E-02	0.128	2.07E-06
rs11645033	0.195	0.066	3.07E-03	0.308	0.183	2.63E-02	NA	NA	NA	0.141	0.077	6.71E-02	0.127	2.16E-06
rs7743168	2.174	1.105	4.92E-02	1.222	0.381	9.98E-01	NA	NA	NA	NA	NA	NA	1.379	2.24E-06
rs485842	0.054	0.033	1.02E-01	0.036	0.035	6.84E-01	NA	NA	NA	0.055	0.038	1.45E-01	0.055	2.25E-06
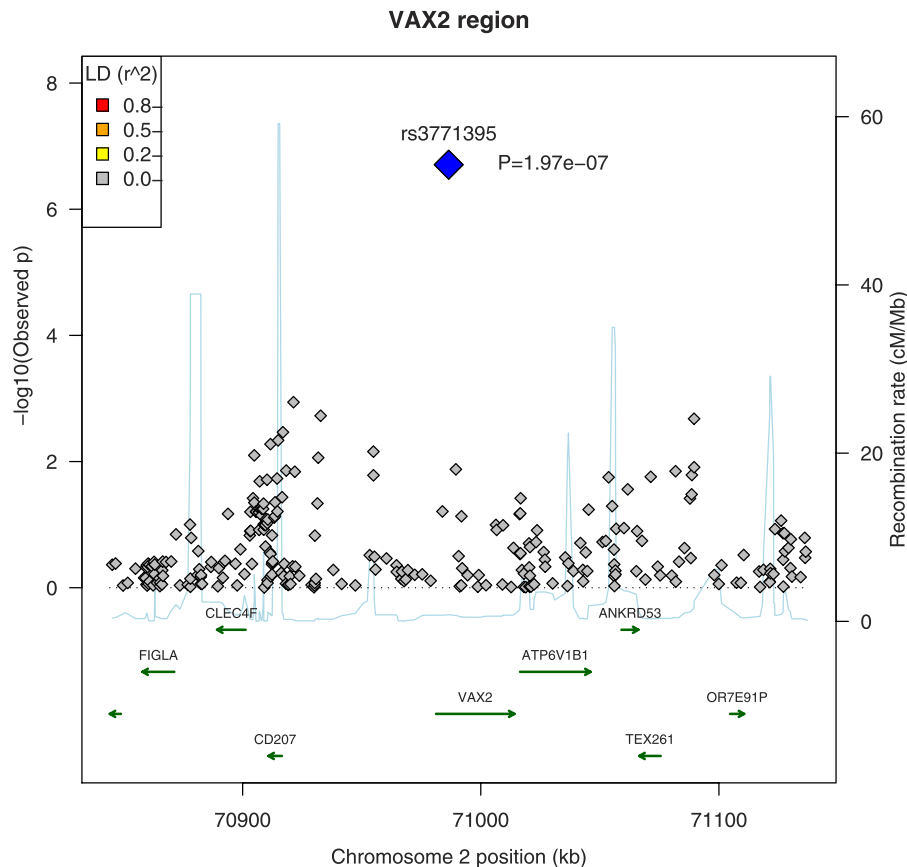
FIGURE 2. Regional association plot for the 2p13.3 (*VAX2*) risk loci region. Meta-analysis –log *P* values were plotted against the chromosomal map position. The color of each SNP reflects its r^2 value, with the top SNP (*blue* and genotyped across all cohorts), changing from *gray* to *yellow*, *orange*, and *red* with increasing r^2 value. The lead SNP is not in LD with any of the surrounding SNPs in the region. Estimated McVean's fine-scale recombination rates (from HapMap Phase II) are plotted in *light blue*. Gene annotations were adapted from the University of California at Santa Cruz genome browser.

Supplementary Fig. S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10463/-/DCSupplemental]). There was also no evidence of significant heterogeneity across studies, I^2 being between 1% and 7% for all SNPs meta-analyzed (heterogeneity for the most significantly associated markers is shown in Supplementary Table S2 [see Supplementary Material and Supplementary Table S2, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10463/-/DCSupplemental]).

There were a number of loci associated with astigmatism at various levels of statistical significance, although none met the strictest criteria of conventional genome-wide significance (Fig. 1, Table, and Supplementary Table S3 [see Supplementary Material and Supplementary Table S3, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-10463/-/DCSupplemental]). The SNP with the strongest association was rs3771395 (meta-analysis, $P = 1.97 \times 10^{-7}$), which was followed by two SNPs in perfect LD with each other: rs10226930 and rs7802427 (meta-analysis, $P = 7.76 \times 10^{-7}$ and $P = 1.06 \times 10^{-6}$, respectively), and in turn followed by rs795544 ($P = 1.19 \times 10^{-6}$), rs12445126 ($P = 1.75 \times 10^{-6}$), and rs9445732 ($P = 2.03 \times 10^{-6}$). The lead SNP lies in an approximately 121.4-kb LD block region, demarcated by recombination hot spots, with no other SNPs correlated and associated with astigmatism (Fig. 2). This LD block on chromosome 2p13.3 region overlaps with the first intron of the ventral anterior homeobox 2 (*VAX2*) gene (Fig. 2). This is a

small gene with a span of 32.9 kb, which encodes a homeobox protein. The rs795544 and rs12445126 SNPs are intergenic, situated 232 kb downstream of the *SHH* gene; rs795544 is located within intron 28 of the *DNAH5* gene; rs12445126 is located 175 kb downstream of the *XYLT1* gene, and rs9445732 falls in a desert region.

The direction of the effect was negative for the G allele of rs3771395 SNP, representing a protective factor for astigmatism (Fig. 3). The magnitude of the effect for the top loci tended to be slightly higher in the RS-I and -II cohorts (betas = −0.125 and −0.108, respectively) and the 1958 British birth cohort (beta = −0.086) than in the other studies (betas = −0.062, −0.067, −0.043, and −0.036 for RS-III, ERF, TwinsUK, and Australian cohorts, respectively). This SNP is common in the CEU HapMap populations, with a MAF of 0.12 across all cohorts.

DISCUSSION

Genetic susceptibility to astigmatism is poorly understood. Here we report a multistage study of over 22,000 individuals for the purpose of identifying genetic variants underpinning astigmatism. Although none of the 2 million markers analyzed met the stringent multiple-testing criteria for genome-wide
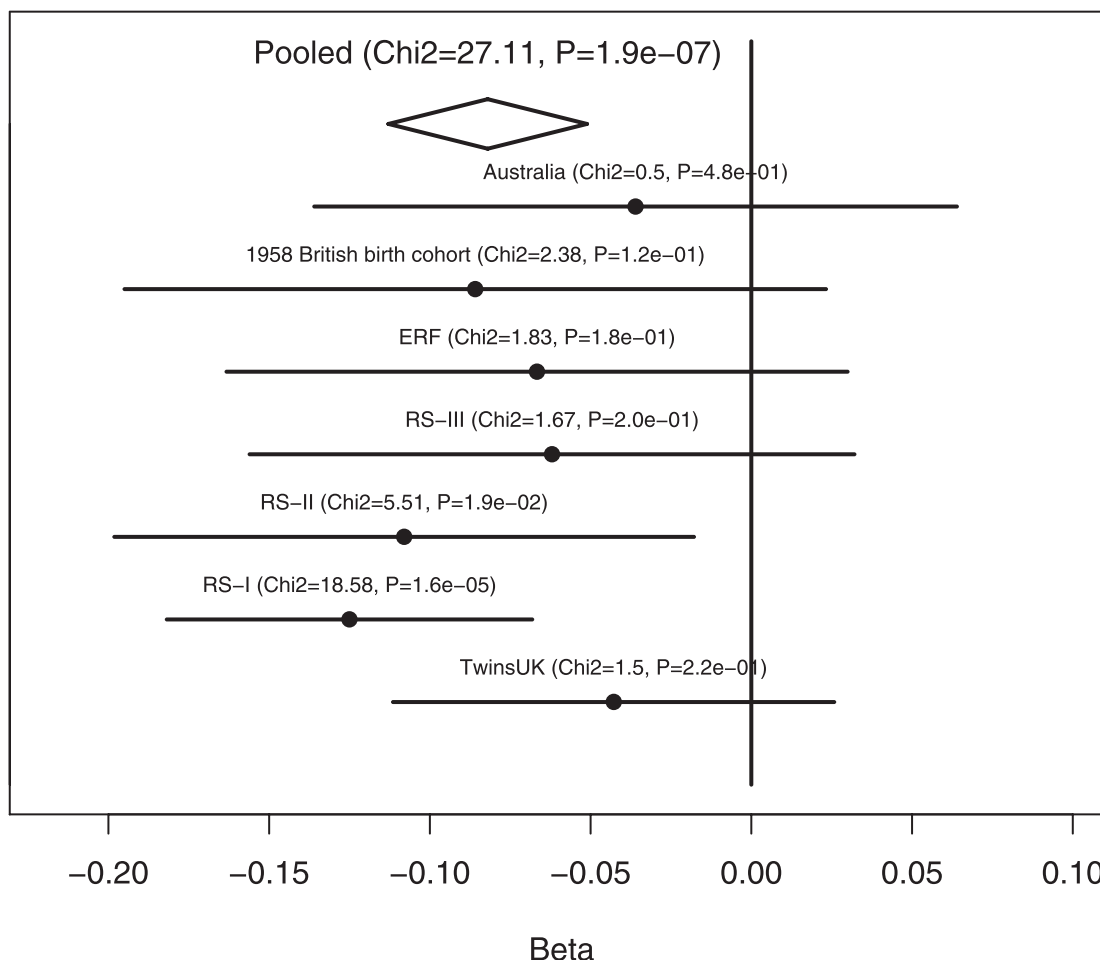
rs3771395

FIGURE 3. Forest plot showing the beta coefficients (effect size) for the top associated SNP rs3771395 in the various populations studied. Pooled data are represented as a *diamond. Vertical line* (beta = 0) indicates no association with astigmatism.

statistical significant association, our analysis has identified the *VAX2* gene, a candidate gene involved in susceptibility to astigmatism because of the highly suggestive statistical evidence (meta-analysis, $P = 1.97 \times 10^{-7}$) and biological plausibility deriving from its known functionality.

VAX2 is an eye-specific homeobox gene, explicitly involved in the development of the ventral eye.[35] This and the *VAX1* gene are members of a subgroup of homeobox-containing genes, the *VAX* subfamily. *VAX2* expression is restricted to the ventral region of the prospective neural retina in vertebrates and is required for ventral eye specification.[35-37] It controls the patterning of the dorsoventral (DV) axis of the eye, and misexpression of *VAX2* in chicks and mice determines a ventralizing effect on the developing eye and retinotectal projections along the DV axis, with a resulting abnormal eye phenotype.[37,38] The ventralization process is achieved through repression of the *PAX6* gene by *VAX2* expression.[39] Studies in amphibians have shown that fluctuations in astigmatism, correlated with changes of the refractive power along the vertical meridian, are particularly prevalent in the ventral visual field.[40,41] The mechanism underpinning astigmatism in the ventral field is not understood, although astigmatism is induced by the corneal surface, in which the curvature tends to decrease from dorsal to ventral aspects.

VAX2 also plays an important role in controlling retinoic acid (RA) metabolism in the developing eye in vertebrates, particularly in maturation of the retina,[42] but RA is also involved in development of the cornea.[43] RA has been shown to influence eye growth in animal models.[44] RA is mainly produced by the choroid, and its synthesis is altered by form deprivation with diffusers or by introduction of defocusing lenses in front of the eye. It is part of a signal cascade from retina to sclera which results in changing ocular elongation and therefore influences refractive error.[44]

Another gene of potential interest is the sonic hedgehog (*SHH*) gene, essential for the proper development and patterning of several vertebrate tissues including the eye. *SHH* is associated with several ocular disorders in humans, such as cyclopia, anophthalmia, microphthalmia, and coloboma.[45-47] Nanophthalmos (a small-eye phenotype) was reported to be associated with irregular astigmatism and corneal steepening.[48]

Recently, a genome-wide meta-analysis for corneal astigmatism in five Asian case-control studies identified a susceptibility locus in the platelet-derived growth factor receptor (*PDGFRA*) gene on chromosome 4q12.[14] Our study failed to replicate any of the top associated signals with corneal astigmatism (SNPs rs17084051, $P = 0.40$; rs17084051, $P = 0.52$; rs2307049, $P = 0.51$; rs7660560, $P = 0.56$; rs2228230, $P = 0.08$; rs4864872, $P = 0.08$; rs3690, $P = 0.10$). There are various reasons for the failure to replicate: different study designs; different LD patterns across ethnicities (resulting in differences in allele frequencies for specific SNPs); distinct underlying genetic mechanisms between corneal and refractive astigmatism.

Effects conferred by individual loci in highly complex diseases are often too small to be detected by population panels such as those used by the current generation of GWAS. Even pooling of results from multiple populations, totaling over 22,000 subjects in this study, has not afforded formal genome-wide statistical significance. Clearly, even larger studies will be needed to identify risk variants. Large collaborative efforts, such as the Consortium of Refractive Error and Myopia (CREAM) study involving 31 cohorts from four continents, with over 40,000 individuals, are in progress and may replicate and validate our findings and identify further susceptibility loci for astigmatism. Potentially, pursuing alternative phenotypes pertaining to astigmatism such as polar values, which take into account the axis of astigmatism, could be of benefit. WTR astigmatism is common in children, and with age there is a shift toward increasing ATR (and therefore decreasing WTR) astigmatism; subjects with corneas that retain WTR astigmatism may "resist" gravity, and subjects with ATR gravity may have more lax corneas, making the axis physiologically relevant. Additionally, there is evidence suggesting astigmatism axis is related to the level of ametropia, astigmats with higher spherical ametropia are more likely to have WTR axes.[6] It is biologically plausible that genes might influence the amount of WTR astigmatism more than overall astigmatism, and genes underlying WTR astigmatism could also be indirectly associated with high levels of ametropia including myopia. Alternatively an association test fitting a dominant model could also enrich GWAS signals, as dominant genetic effects account for most of the astigmatism genetic variance in heritability studies.[12]

In summary, we have presented a meta-analysis of astigmatism in seven Caucasian populations and identified a locus associated with astigmatism, but not at genome-wide level of significance, in the *VAX2* gene region, which is a biologically plausible candidate gene. This finding may allow new insights into astigmatism and advance the understanding of refractive error. However, functional studies will be required to confirm a role for *VAX2* in development of astigmatism.

Acknowledgments

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