Malay and Japanese populations. Further evaluation of causal variants and underlying pathway mechanisms may contribute to early identification of children at highest risk of developing myopia, and eventually lead to appropriate interventions to retard the progression of myopia.

Materials and Methods

Discovery cohorts

Singapore Chinese Eye Study (SCES). SCES is an ongoing population-based cross-sectional survey of eye diseases in Chinese adults aged 40 to 80 years residing in the Southwestern part of Singapore. The study began in 2007 and a detailed description was published elsewhere [59]. In brief, a total of 2,226 residents in the Southwestern area of Singapore completed comprehensive ophthalmologic examinations, including visual acuity assessments, refraction, lens and retinal imaging, and slit lamp examinations. Genome-wide genotyping was performed in 1,952 individuals. Completed post quality control (QC) data for GWAS were available for 1,860 adults with AL measurements.

Singapore Cohort study of the Risk factors for Myopia (SCORM). A total of 1,979 children in grades 1, 2, and 3 from three schools in Singapore were recruited from 1999 to 2001 [17]. The children were examined on their respective school premises annually by a team of eye care professionals. The GWAS was conducted in a subset of 1,116 Chinese children [14,60]. The phenotype used in this study was based on the AL measured on the 4th annual examination of the study (children at age 10 to 12 years). Complete post-filtering data on AL measurements and SNP data were available in 929 children.

Singapore Malay Eye Study (SiMES). SiMES is a population-based cross-sectional survey of eye diseases in Malay adults aged 40 to 80 years living in Singapore. It was conducted between August of 2004 and June of 2006 [61]. A total of 4,168 Malay residents in the Southwestern area of Singapore were identified and invited for a detailed ocular examination where 3,280 (78.7%) participated. Genome-wide genotyping was performed in 3,072 individuals [62,63]. Complete post-filtering data for GWAS with AL measurements were available for 2,155 subjects.

Validation cohorts for high myopia

Japan dataset 1. The Japan dataset 1 consisted of 483 high myopia cases and 1,194 general healthy population controls. High myopia status was determined primarily on the basis of AL≥28 mm for both eyes, which corresponded to the spherical equivalent (SE) cut-off of at least −9.00 D [64]. Cases were recruited at the Center for Macular Disease of Kyoto University Hospital, the High Myopia Clinic of Tokyo Medical and Dental University, and the Fukushima Medical University Hospital. Details of the data have been reported elsewhere [13]. The population controls were recruited at the Aichi Cancer Center Research Institute.

Japan dataset 2. The Japan dataset 2 was comprised of 504 high myopia cases (SE≤−9.00 D in either eye) and 550 non-highly myopic controls (SE≥−3.00 D in both eyes). Less stringent thresholds were adopted for controls for the purpose of ease of recruitment from the clinics. Given the large phenotypic separation between the cases and controls, and assumption of homoscedasticity across genotype categories, such a study design using the extreme on one end (i.e. SE≤−9.00 D) but sampling less extreme controls (i.e. SE≥−3.00 D) still provides sufficient statistical power to detect the true positive signals in the association study [65]. Cases were recruited at the Yokohama City University and Okada Eye Clinic. Controls were obtained from the Yokohama City University and Tokai University Hospital.

Measurements of AL, refractive error, and covariates

All the studies used a similar protocol for ocular phenotype measurements. For subjects in SCES and SiMES, AL for both eyes were measured using optical laser interferometry (IOLMaster V3.01, Carl Zeiss; Meditec AG Jena, Germany) [59,61]. Children in the SCORM study underwent AL measurements using the A-scan ultrasound biometry machine (Echoscan US-800; Nidek Co, Tokyo, Japan) [17]. For subjects in the Japan dataset 1, applanation A-scan ultrasongraphy (UD-6000, Tomey, Nagoya, Japan) or partial coherence interferometry (IOLMaster, Carl Zeiss Meditec, Dublin, CA) were used to measure AL. AL was assessed using a portable A-scan Biometer/pachymeter (AL-2000, Tomey, Negoya, Japan) for the participants in the Japan dataset 2.

Non-cycloplegic refraction in SCES and SiMES as well as cycloplegic refraction in SCORM (three drops of 1% cyclopentolate at 5 minutes apart) were measured by autorefractor (Canon RK-5, Tokyo, Japan) [66]. For subjects in the Japan dataset 2, refraction was measured using auto-refraction ARK-730A (NIDEK), ARK-700A (NIDEK) and KR-8100P (TOPCON). SE was calculated as the sphere power plus half of the cylinder power for each eye.

To perform the genetic association of high myopia in SCES and SiMES, we used the definition adopted by the Japan case-control studies and defined high myopia cases as subjects having SE≤−9.0 D in at least one eye, and non high-myopia controls as samples with SE≥−3.0 D in both eyes. For children from SCORM aged 10 to 12 years, cases were defined as SE≤−6.0 D for at least one eye, while controls were defined as SE≥−1.0 D for both eyes; this is approximately equivalent to the projected SE of −9.0 and −3.0 respectively at university age based on the estimated annual progression rate in SE of −0.6 D for Chinese myopic children and −0.3 D in the controls [67]. Given the small sample sizes of high myopia cases identified in our population-based cohorts, in the supplementary analysis, we further applied the commonly adopted criteria of SE≤−6.0 D in either eye as cases. Controls were defined as SE≥−1.0 D in both eyes. For SCORM children, we retained the same criteria in both analyses. The detailed definitions of cases and controls are described in Table S4.

Age, gender, height and level of education were obtained from all Singapore participants who underwent ophthalmologic examination. Education was measured on an ordinal scale from no formal education to the highest educational level. For participants in SCORM, the education of the child was defined by the level of educational attainment of the father, as a marker of socioeconomic status.

Ethics

All studies followed the principle of the Declaration of Helsinki. Study procedures and protocols were approved by the Institutional Review Board of each local institution involved in the study. In all cohorts, participants provided written, informed consent at the recruitment into the studies. Informed written consent was obtained from adult participants, and from the parents of the SCORM children.

Animal study approval was obtained from the SingHealth IACUC (AAALAC accredited). All procedures performed in this study complied with the Association of Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmology and Vision Research.

Genotyping and data quality control in discovery cohorts

For SCES, a total of 1,952 venous blood-derived samples were genotyped using Illumina Human 610 Quad Beadchips (Illumina Inc., San Diego, US) according to the manufacturer's protocols. Samples which failed genotyping or with low call rate (<95%, n = 11), with excessive heterozygosity (defined as sample heterozygosity exceeding 3 standard deviations from the mean sample heterogzygosity; n = 3), with gender discrepancies (n = 2) were excluded, as were cryptically related samples identified by the identity-by-state (IBS) (n = 41) and population structure in the principal components analyses (PCA) (n = 6). The criteria to define cryptically related samples and outliers with population structure in the discovery cohorts are described in the following paragraph. After the removal of the samples, SNP QC was then applied on a total of 579,999 autosomal SNPs for the 1,889 post-QC samples. SNPs were excluded based on (i) high rates of missingness (>5%) (n = 26,437); (ii) monomorphism or minor allele frequency (MAF)<1% (n = 59,633); or (iii) genotype frequencies deviating from Hardy-Weinberg Equilibrium (HWE) defined as HWE P-value$<10^{-6}$ (n = 1,821). This yielded 492,108 autosomal SNPs. Those individuals with missing data on phenotypes were further removed (n = 29). Finally, 492,108 SNPs in 1,860 samples were available for analyses.

For SCORM, 1,116 DNA samples (1,037 from buccal swab and 79 from saliva) were genotyped on the Illumina HumanHap 550 Beadchips and 550 Duo Beadarrays. A total of 108 samples were excluded, comprising (i) 70 samples with call rates below 98%; (ii) 6 with poor genotyping quality; (iii) 11 samples identified from sibships; (iv) 18 with inconsistent gender information; and (v) 3 due to population structure. This left a total of 1,008 samples for further SNP QC. Based on 514,849 autosomal SNPs, we excluded 32,669 markers if they had missing genotype calls >5%, MAF<1%, or significantly deviated from HWE ($P<10^{-6}$) [14]. A final set of 929 samples with 482,180 post-QC SNPs and completed AL measurement were included in analyses.

For SiMES, 3,072 DNA samples were genotyped using the Illumina Human 610 Quad Beadchips. The detailed QC procedures were provided elsewhere [68]. In brief, we omitted a total of 530 individuals due to: (i) subpopulation structure (n = 170); (ii) cryptic relatedness (n = 279); (iii) excessive heterozygosity or high missingness rate >5% (n = 37); and (iv) gender discrepancy (n = 44). After the removal of the samples, SNP QC was then applied on a total of 579,999 autosomal SNPs for the 2,542 post-QC samples. SNPs were excluded based on: (i) high rates of missingness (>5%) (n = 26,343); (ii) monomorphism or MAF<1% (n = 34,891); or (iii) genotype frequencies deviating from HWE ($P<10^{-6}$) (n = 3,645). This yielded 515,120 SNPs after the same SNP QC criteria. Individuals without valid measurements for AL were further removed (n = 387). After the above filtering criteria, 515,120 SNPs in 2,155 samples were available for association analyses.

In our discovery cohorts, IBS was estimated with the genome-wide SNP data using PLINK software to assess the degree of recent shared ancestry for a pair of individuals [69]. For a pair of putatively-related samples defined as an identity by descent (IBD) value greater than 0.185 [70], we removed one individual from each pair of monzygotic twins/duplicates, parent-offspring or full-siblings etc. Population structure was ascertained using PCA with the EIGENSTRAT program and genetic outliers were defined as individuals whose ancestry was at least 6 standard deviations from the mean on one of the top ten inferred axes of variation [71].

For SiMES Malays, we also excluded the samples falling in the main clusters of PCA plots of the Chinese and Indians ethnic groups, as described in the previous study [68]. In SiMES, we noticed some degree of admixture in genetic ancestry of Malays and thus adjusted for ancestry along the top five axes of variation, as the spread of principal component scores was greater for the top five eigenvectors in the bivariate plots of PCA (Figure S3), The top ten principal components explained a small percentage of the global genetic variability of 1.3% while top five explained 1.0%, suggesting, all together, they had minimal effects on our association analyses.

Validation cohorts for high myopia

High myopia cases in the Japan dataset 1 were genotyped using Illumina Human-Hap550 and 660 chips [13], while controls in the Japan dataset 1 were genotyped on Illumina Human-Hap610 chips. Subjects in the Japan dataset 2 were genotyped on the Affymetrix GeneChip Human Mapping 500 K Array Set (Affymetrix Inc., Santa Clara, US). For SNPs not available on the Affymetric chips (rs43737678, rs10779363 and rs7544369), genotyping was performed with TaqMan 5′ exonuclease assays using primers supplied by Applied Biosystems (Foster City, US). The probe fluorescence signal was detected using the TaqMan Assay for Real-Time PCR (7500 Fast Real-Time PCR System, Applied Biosystems).

Gene expression in a mouse model of myopia

Experimental myopia was induced in B6 wild-type (WT) mice (n = 36) by applying a −15.00 D spectacle lens on the right eye (experimental eye) for 6 weeks since post-natal day 10. The left eyes were uncovered and served as contra-lateral fellow eyes. Age matched naive mice eyes were used as independent control eyes (n = 36). Each eye was refracted weekly using the automated infrared photorefractor as described previously [72]. AL was measured by AC- Master, Optic low coherence interferometry (Carl-Zeiss), in-vivo at 2, 4 and 6 weeks after the induction of myopia [73]. The minus-lens-induced eyes after six weeks were significantly associated with increased AL and myopic shift in refraction of <−5.00 D as compared to independent control eyes (n = 36, $P=3.00\times10^{-6}$ for AL, and 2.05×10^{-4} for refraction). Eye tissues were collected at 6 weeks post myopia induction for further analyses.

Total RNA was isolated from pooled cryogenically ground mouse neural retina (retina), retinal pigment epithelium (RPE) and sclera for three batches using TRIzol Reagent (Invitrogen, Carlsbad, CA) with each batch (n = 6) comprising the myopic eye, fellow eye and control eye. RNA concentration and quality were assessed by the absorbance at 260 nm and the ratio of absorbance ratio at 260 and 280 nm respectively, using Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies, Wilmington, DE). RNA was purified using the RNeasy Mini kit (Qiagen, GmbH).

500 ng of purifed RNA was reverse-transcribed into cDNA using random primers and reagents from iScriptTM select cDNA synthesis kit (Bio-rad Laboratories, Hercules, CA). The pseudogene *ZC3H11B* (zinc finger CCCH type containing 11B) is not characterized in the mouse genome, therefore we examined a similar gene *ZC3H11A* (zinc finger CCCH type containing 11A) in mice. *ZC3H11A* in mice and *ZC3H11B* in humans are highly conserved with 79% nucleotide similarity by BLAST alignment analysis (http://blast.ncbi.nlm.nih.gov). We used quantitative Real-Time PCR (qRT-PCR) to validate the gene expression. qRT-PCR primers (Table S5) were designed using ProbeFinder 2.45 (Roche Applied Science, Indianapolis, IN) and this was performed using a Lightcycler 480 Probe Master (Roche Applied Science, Indianapolis, IN). The reaction was run in a Lightcycler 480 for 45 cycles under the following conditions: 95°C for 10 s, 56°C for 10 s and 72°C for 30 s. Gene expressions in the retina, RPE and sclera after six weeks of myopic eyes and the fellow eyes were compared to the control eyes. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an endogenous internal control.

Immunohistochemistry

Whole mouse eyes (6 weeks minus lens treated myopic, contralateral fellow and independent control eyes, n = 6 per type) were embedded in frozen tissue matrix compound at −20°C for 1 hour. Prepared tissue blocks were sectioned with a cryostat at 6 microns thicknesses and collected on clean polysine™ glass slides. Slides with the sections were air dried at room temperature (RT) for 1 hour and fixed with 4% para-formaldehyde for 10 min. After washing 3X with 1x PBS for 5 minutes, 4% bovine serum albumin (BSA) diluted with 1x PBS was added as a blocking buffer. The slides were then covered and incubated for 1 hour at RT in a humid chamber. After rinsing with 1x PBS, a specific primary antibody raised in rabbit against *ZC3H11A, SLC30A10* and raised in goat against *LYPLAL1* (Abcam, Cambridge, UK) diluted (1:200) with 4% BSA was added and incubated further at 4°C in a humid chamber overnight. After washing 3X with 1x PBS for 10 min, fluorescein-labeled goat anti-rabbit secondary antibody (1:800, Invitrogen-Molecular Probes, Eugene, OR) and fluorescein-labeled rabbit anti-goat secondary antibody (1:800, Santa Cruz Biotechnology, Inc. CA, USA) was applied respectively and incubated for 90 min at RT. After washing and air-drying, slides were mounted with antifade medium containing DAPI (4,6-diamidino-2-phenylindole; Vectashield, Vector Laboratories, Burlingame, CA) to visualize the cell nuclei. Sections incubated with 4% BSA and omitted primary antibody were used as a negative control. A fluorescence microscope (Axioplan 2; Carl Zeiss Meditec GmbH, Oberkochen, Germany) was used to examine the slides and capture images. Experiments were repeated in duplicates from three different samples.

Gene expression in human tissues

GAPDH, ZC3H11B, SLC30A10, and LYLPLAL1 were run using 10 ul reactions with Qiagen's PCR products consisting of 1.26 ul H_2O, 1.0 ul 10X buffer, 1.0 ul dNTPs, 0.3 ul MgCl, 2.0 ul Q-Solution, 0.06 ul taq polymerase, 1.0 ul forward primer, 1.0 ul reverse primer and 1.5.0 ul cDNA. The reactions were run on a Eppendorf Mastercycler Pro S thermocycler with touchdown PCR ramping down 1°C per cycle from 72°C to 55°C followed by 50 cycles of 94°C for 0:30, 55°C for 0:30 and 72°C for 0:30 with a final elongation of 7:00 at 72°C. All primer sets were designed using Primer3 [74]. The gel electrophoresis was run on a 2% agarose gel at 70 volts for 35 minutes. The primers were run on a custom tissue panel including Clontech's Human MTC Panel I, Fetal MTC Panel I and an ocular tissue panel. The adult ocular samples were obtained from normal eyes of an 82-year-old Caucasian female from the North Carolina Eye Bank, Winston-Salem, North Carolina, USA. The fetal ocular samples were from 24-week fetal eyes obtained by Advanced Bioscience Resources Inc., Alameda, California, USA. All adult ocular samples were stored in Qiagen's RNA*later* within 6.5 hours of collection and shipped on ice overnight to the lab. Fetal eyes were preserved in RNA*later* within minutes of harvesting and shipped over night on ice. Whole globes were dissected on the arrival day. Isolated tissues were snap-frozen and stored at −80°C until RNA extraction. RNA was extracted from each tissue sample independently using the Ambion *mir*Vana total RNA extraction kit. The tissue samples were homogenized in Ambion lysis buffer using an Omni Bead Ruptor Tissue Homogenizer per protocol. Reverse transcription reactions were performed with Invitrogen SuperScript III First-Strand Synthesis kit.

Statistical analysis

The primary analysis was performed on the AL quantitative trait. As a strong correlation exists in AL measurements from both eyes ($r>0.9$), we used the mean AL across both eyes in the GWAS analysis, as was recommended in a review [75]. Linear regression was used to interrogate the association of each SNP with AL after adjusting for age, gender, height and level of education, under the assumption of an additive genetic effect where the genotypes of each SNP are coded numerically as 0, 1 and 2 for the number of minor alleles carried. In addition, for SiMES, the top five principal components of genetic ancestry from the EIGENSTRAT PCA were also included as covariates to account for the effects of population substructure as described in genotype QC section [60]. Association tests between each genetic marker and phenotype were carried out using PLINK software [69] (version 1.07). Analyses were also repeated without adjustment for education level or height for the purpose of comparison.

In the discovery phase, we conducted a meta-analysis of GWAS results from 3 cohorts for AL using a weighted-inverse variance approach by fixed-effect modeling in METAL (http://www.sph.umich.edu/csg/abecasis/metal). In the secondary analyses, SNPs that have been identified from the primary analyses were tested for association with high myopia onset (as a binary trait) and SE (as a quantitative trait). For Singapore cohorts, the association analyses adjusted for the same covariates as the primary analyses within a linear regression and logistic regression framework respectively. For Japan case-control datasets, only age and gender were included as covariates in the model for high myopia, as the other covariates were not available.

The regional association plots were constructed by SNAP (http://www.broadinstitute.org/mpg/snap). Haploview 4.1 (http://www.broad.mit.edu/mpg/haploview) was used to visualize the LD of the genomic regions. Genotyping quality of all reported SNPs has been visually evaluated by the intensity clusterplots. The coordinates reported in this paper are on NCB136 (hg18).

For functional studies in the myopic mouse model, gene expression of all three identified genes in control and experimental groups was quantified using the $2^{-\Delta\Delta Ct}$ method [76]. The standard student's t-test was performed to determine the significance of the relative fold change of mRNA between the myopic eyes of the experimental mice with the independent age-matched controls.

Supporting Information

Figure S1 Principal Component Analysis (PCA) of discovery cohorts SCES, SCORM and SiMES with respect to the four population panels in phase 2 of the HapMap samples (CEU - European, YRI – African, CHB – Chinese, JPT – Japanese) (A), and with respect to two reference population panels CHB and JPT (B–D). (A) Principal components 1 versus 2; the principal components (PCs) were calculated with SCES, SCORM, SiMES and four HapMap panels on the thinned set of 102,122 SNPs ($r^2<0.2$). (B) Principal components 1 versus 2; (C) Principal components 1 versus 3; (D) Principal components 1 versus 4. For (B–D), the PCs were calculated with SCES, SCORM, SiMES and HapMap Asian population panels on the thinned set of 86,516 SNPs ($r^2<0.2$).
(PDF)

Figure S2 Quantile-Quantile (Q-Q) plots of *P*-values for association between all SNPs and AL in the individual cohort (A) SCES, (B) SCORM, (C) SiMES, and combined meta-analysis of the discovery cohorts (D) SCES+SCORM+SiMES.
(PDF)

Figure S3 Principal Component Analysis (PCA) was performed in SiMES to assess the extent of population structure. Each figure represents a bivariate plot of two principal components from the

PCA of genetic diversity within SiMES on the thinned set of 83,585 SNPs ($r^2<0.2$). The first 5 principal components were used as covariates to account for population structure.
(PDF)

Table S1 Characteristics of high myopia cases and controls in three Singapore cohorts.
(DOCX)

Table S2 Association between genetic variants at chromosome 1q41 and high myopia in the meta-analysis of five cohorts.
(DOCX)

Table S3 Association between genetic variants at chromosome 1q41 and spherical equivalent (SE) in the meta-analysis of three Asian cohorts.
(DOCX)

Table S4 Definitions and numbers of high-myopia cases and controls used in the main and supplementary association analyses for high myopia.
(DOCX)

Table S5 Gene accession number in the nucleotide sequence database (NCBI), and qRT-PCR primer sequences in mice genome.
(DOCX)

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Author Contributions

Conceived and designed the experiments: TLY T-YW Y-YT S-MS. Performed the experiments: VAB AM IN WL CEHH FH YZ DC HI KY. Analyzed the data: QF VAB XZ C-YC. Contributed reagents/materials/analysis tools: C-CK L-KG Y-JL KO-M KM FM EV MS NM RWB E-ST NY TA TLY T-YW Y-YT S-MS. Wrote the paper: QF VAB Y-YT S-MS. Critically reviewed the manuscript: C-YC C-CK L-KG E-ST TLY T-YW.

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ORIGINAL INVESTIGATION

Large scale international replication and meta-analysis study confirms association of the 15q14 locus with myopia. The CREAM consortium

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Abstract Myopia is a complex genetic disorder and a common cause of visual impairment among working age adults. Genome-wide association studies have identified susceptibility loci on chromosomes 15q14 and 15q25 in Caucasian populations of European ancestry. Here, we present a confirmation and meta-analysis study in which we assessed whether these two loci are also associated with myopia in other populations. The study population comprised 31 cohorts from the Consortium of Refractive Error and Myopia (CREAM) representing 4 different continents with 55,177 individuals; 42,845 Caucasians and 12,332 Asians. We performed a meta-analysis of 14 single nucleotide polymorphisms (SNPs) on 15q14 and 5 SNPs on 15q25 using linear regression analysis with spherical equivalent as a quantitative outcome, adjusted for age and

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sex. We calculated the odds ratio (OR) of myopia versus hyperopia for carriers of the top-SNP alleles using a fixed effects meta-analysis. At locus 15q14, all SNPs were significantly replicated, with the lowest P value 3.87×10^{-12} for SNP rs634990 in Caucasians, and 9.65×10^{-4} for rs8032019 in Asians. The overall meta-analysis provided P value 9.20×10^{-23} for the top SNP rs634990. The risk of myopia versus hyperopia was OR 1.88 (95 % CI 1.64, 2.16, $P < 0.001$) for homozygous carriers of the risk allele at the top SNP rs634990, and OR 1.33 (95 % CI 1.19, 1.49, $P < 0.001$) for heterozygous carriers. SNPs at locus 15q25 did not replicate significantly (P value 5.81×10^{-2} for top SNP rs939661). We conclude that common variants at chromosome 15q14 influence susceptibility for myopia in Caucasian and Asian populations world-wide.

Introduction

Refractive errors are common optical defects of the visual system. An important refractive error is myopia (near-sightedness), which occurs when the eye elongates beyond the focal plane. The prevalence of myopia is high, affecting about one-third of the world's population, and reaching over 70 % in certain Asian ethnic groups (He et al. 2004; Kempen et al. 2004; Lin et al. 2004; Vitale et al. 2008; Wu et al. 2001). High degrees of myopia are associated with pathologic ocular changes, such as myopic macular degeneration, retinal detachment, and glaucoma (Curtin and Karlin 1971; McBrien and Gentle 2003; Saw 2006; Saw et al. 2005; Tano 2002). Due to the limited treatment options, myopia is a common cause of visual impairment (Tano 2002; Young 2009).

Refractive errors, and myopia in particular, are complex genetic traits with a largely unknown etiology. Established environmental factors are education, early reading, and reduced outdoor exposure (Dirani et al. 2009; Ip et al. 2008; McBrien et al. 2008; Morgan and Rose 2005; Rose et al. 2008; Saw et al. 2001; Young 2009). Although heritability estimates are high [50–90 % (Young et al. 2007)], the search for myopia genes is still ongoing. Previous linkage and association studies have led to the identification of at least 18 myopia (MYP) loci, 10 additional chromosomal regions, and several candidate genes (Baird et al. 2010; Young 2009). Replication of these associations has been inconsistent, and their application to the general population is limited (Baird et al. 2010).

Recent genome-wide association studies (GWAS) reported several susceptibility loci for refractive error and

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myopia (Hysi et al. 2010; Li et al. 2011a, b; Nakanishi et al. 2009; Shi et al. 2011; Solouki et al. 2010). Solouki et al. (2010) and Hysi et al. (2010) were the first to perform a GWAS in a general Caucasian population, and identified susceptibility loci on chromosomes 15q14 and 15q25, respectively. In both studies, carriers of single nucleotide polymorphism (SNP) rs634990 at 15q14 (OR 1.83, 95 % CI 1.42–2.36) and of SNP rs8027411 at 15q25 (OR 1.16, 95 % CI 1.02–1.28) had a higher risk of myopia. Confirmation of these findings was obtained in various replication studies (Hayashi et al. 2011; Hysi et al. 2010; Solouki et al. 2010). However, these replication cohorts were relatively limited in size, increasing the chance of a type 1 error.

To address potential inaccuracies and to investigate generalizability, we investigated the associations between refractive error, and the 15q14 and 15q25 susceptibility loci in a large international replication and meta-analysis study (Consortium of Refractive Error and Myopia, CREAM) including 31 cohorts with various ethnicities from 4 different continents.

Results

Meta-analysis of allelic effects on spherical equivalent (SE)

Complete data on refractive error and genome-wide SNPs were available in all 29 population-based studies comprising 49,364 subjects: 42,224 Caucasians and 7,140 Asians (Table 1; Fig. 1, Supplementary Table 1). This includes the previously reported discovery set consisting of 15,608 (Solouki et al. 2010) and 17,608 subjects (Hysi et al. 2010), respectively.

Table 2 shows the results of the meta-analysis of the 14 SNPs (Hysi et al. 2010; Solouki et al. 2010) at locus 15q14 and 5 SNPs at locus 15q25. The frequency of the effect allele C for top SNP rs634990 at locus 15q14 ranged from 0.38 to 0.64, while frequency of the effect allele A for top SNP rs939661 at 15q25 showed a larger variation, ranging from 0.28 to 0.63 (Supplementary Figure 1). The sample size of each SNP per study is provided in Supplementary Table 1. For locus 15q14, the magnitude and direction of the effects were consistent in all cohorts except Croatia Vis and SIMES. For locus 15q25, there was less consistency; for top SNP rs939661 8 cohorts—both Caucasian and Asian (Australian Twins, Croatia Split, Croatia Vis, EGCUT, FITSA, GHS II, ORCADES, and SIMES)—had a regression beta coefficient in the opposite direction to that of the other studies.

For locus 15q14, the replication set, consisting of all studies except the ones previously used in the discovery analysis, showed a statistically significant association between SE and all SNPs with a best P value 4.53×10^{-14} for top SNP rs634990. Confirmation was achieved in 23 out of 25 Caucasian studies (overall P 3.87×10^{-12} for SNP rs634990), and in 3 out of 4 Asian studies (overall

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P 2.21 $\times$ 10^{-3} for SNP rs634990). Meta-analysis of the discovery and replication cohorts together provided P value 9.20 $\times$ 10^{-23} for SNP rs634990.

For locus 15q25, neither Caucasian nor Asian validation studies replicated the original association. Meta-analysis of the combined set of the 5 SNPs yielded a lowest P 1.22 $\times$ 10^{-4} for SNP rs939661. As a subsequent analysis, we investigated locus 15q25 in more detail, and tested another 26 SNPs in 26 out of 29 cohorts (no data available in ALSPAC, AREDS 1, and EGCUT). This set of SNPs was not replicated either, however, meta-analysis including the discovery cohort was still significant (best P 2.07 $\times$ 10^{-4} for SNP rs1915726; Supplementary Table 3).

Meta-analysis of risk of myopia for top SNP

Genotype distributions for rs634990 at locus 15q14 were available for 28 out of 31 studies (all but FITSA, Australian Twins, and SORBS). There was no evidence of heterogeneity in the analyses of homozygote carriers [χ^2 21.35 (d.f. 26), P 0.724, I^2 0.0 %] or heterozygote carriers [χ^2 24.22 (d.f. 26), P 0.564, I^2 0.0 %]. Therefore, only results from fixed effects meta-analysis were used. Figure 2 shows the forest plots for the risk of myopia for homozygous and heterozygous carriers of the top SNP rs634990. The OR of moderate to high myopia (SE ≤ -3 D) versus moderate to high hyperopia (SE $\geq +3$ D) was 1.88 (95 % CI 1.64, 2.16, $P < 0.001$) for homozygous carriers of the risk allele at the top SNP rs634990, and 1.33 (95 % CI 1.19, 1.49, $P < 0.001$) for heterozygous carriers.

Discussion

Chromosome 15q was first implicated in refractive error and myopia by genome-wide analysis of two large studies located in Northern Europe (Hysi et al. 2010; Solouki et al. 2010). Here, in an international meta-analysis consisting of 31 independent studies from the CREAM consortium, we provide further support that the association with locus 15q14 is robust and present in both Caucasians and Asians. We combined the results with those of the initial study into a powerful meta-analysis of highly associated SNPs with a total study population of 55,177 participants. The combined results showed that all tested SNPs for locus 15q14 were associated with refractive errors, and that homozygous carriers of the top SNP rs634990 had approximately twice the risk of myopia. SNPs at the other locus, 15q25, could not be convincingly replicated.

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This study has strengths and limitations. Major strengths of the study include the sample size and the inclusion of different ethnicities. The CREAM consortium represents the largest study on refractive error known to date. Previous replication studies have not been large scaled and focused on populations of the same ancestry (Gao et al. 2012; Lu et al. 2011; Wang et al. 2011). Another advantage of our study is the incorporation of clinical relevant endpoints such as high myopia and high hyperopia. Among the limitations are differences in designs and methods of the studies. (1) Population-based as well as case control studies were incorporated. However, the latter were only two (Kyoto Study and SORBS) and both had results within the same range as the population-based studies. (2) Different types of equipment and measurement methods were used to detect refractive error. These differences are generally subtle, and are not likely to cause false findings. (3) Various methods of genotyping and imputation were used, and genotyping was not complete in all studies. All SNPs at 15q14 had similar effect; thus, we do not think this has influenced these associations. SNPs at 15q25 showed larger variation, and the incomplete genotyping may have underpowered this analysis.

Earlier replication of the 15q14 locus was reported by Hayashi et al. (2011) in a Japanese sample of high myopic probands and controls. In a comparison of 1,125 high myopes (axial length >26.1 mm) versus 1,295 controls, the risk of high myopia was increased for the carriers of the initial top SNP rs634990 [OR 1.84 in homozygotes (95 % CI 1.44–2.36)]. Taken together with the current findings, this suggests that 15q14 plays a role in both common and high myopia.

The 15q14 associated region contains two interesting genes that are both well expressed in the retina, *GJD2* and *ACTC1*. *GJD2* encodes the Connexin36 protein, which plays a crucial role in the transmission and processing of visual signals in the retina by enabling intercellular transport of small molecules and ions in photoreceptors, amacrine and bipolar cells (Deans et al. 2002; Guldenagel et al. 2001; Kihara et al. 2009; Striedinger et al. 2005). We speculated that the protein encoded by the other candidate gene, *ACTC1*, could play a role in scleral remodeling, given the fact that similar actin proteins have been shown to be increased in developing myopic tree shrew eyes (Jobling et al. 2009). Previous *GJD2* (Solouki et al. 2010) and *ACTC1* (unpublished data) direct sequencing experiments did not reveal a functional variant, but the 15q14 locus appeared to harbor regulatory elements which may influence transcription of these genes (Solouki et al. 2010).

The 15q25 region contains the interesting candidate gene *RASGRF1*, which is highly expressed in the retina and has previously been implicated in photoreception and visual sensory processes (Fernandez-Medarde et al. 2009; Jones and Moses 2004). The association with this locus and gene is not robust, since none of the initial SNPs replicated significantly, and determination of more SNPs did not increase significance. A type 1 error may explain the initial finding. Another potential cause for the non-replication is a large variation in allele frequencies. The range of allele frequencies at 15q25 (0.28–0.63) was only slightly larger

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Table 1 Descriptives of all study cohorts

Study	*n*	Mean age (SD)	Age range	Men (%)	Mean SE (SD)
1958 British Birth Cohort	1,658	42 (0.0)	40–50	54.2	−0.96 (2.00)
AGES Reykjavik	2,986	76.3 (5.4)	60–80+	35.3	1.22 (2.05)
ALSPAC	3,804	15.4 (0.3)	14.25–17.08	47.2	−0.38 (1.28)
AREDS 1	816	79.5 (5.1)	60–80+	43.5	0.68 (1.94)
AREDS 2	1,506	68.0 (4.7)	55–81	41.1	0.54 (2.25)
Australian Twins	1,819	22.2 (12.7)	5–90	44.0	−0.22 (1.28)
Blue Mountains Eye Study	1,574	64 (7.9)	50–80+	43.4	0.59 (1.96)
Croatia Split	366	49.8 (14.4)	18–85	46.0	−1.83 (1.83)
Croatia Vis Island	544	55.8 (14.0)	18–83	40.0	−0.16 (1.93)
Croatia Korcula Island	836	56.0 (13.8)	18–98	35.0	−0.25 (1.92)
ERF	2,032	48.5 (14.3)	18+	43.1	0.07 (2.13)
EGCUT	338	34.8 (15.2)	18–85	36.9	−2.60 (2.00)
Finnish Twin Study on Aging	127	68.2 (3.8)	63–76	0.0	1.68 (1.54)
Framingham Eye Study	1,500	55.5 (9.0)	20–80	42.5	−0.17 (2.40)
Gutenberg Health Study I	2,745	55.7 (11)	35–74	51.5	−0.38 (2.44)
Gutenberg Health Study II	1,142	55.0 (10.9)	35–74	49.8	−0.41 (2.58)
KORA	1,867	55.6 (11.7)	35–84	49.6	−0.29 (2.27)
MESA	1,462	62 (9.4)	46–86	49.5	−0.28 (2.62)
ORCADES	505	54.8 (13.7)	22–88.5	43.0	0.01 (2.14)
Rotterdam Study 1	5,328	68.5 (8.6)	55+	41.3	0.86 (2.45)
Rotterdam Study 2	2,009	64.2 (7.4)	55+	45.9	0.48 (2.51)
Rotterdam Study 3	1,970	56.0 (5.5)	45+	43.9	−0.35 (2.62)
OGP Talana	623	44.5 (21.1)	5–89	51.8	−0.15 (1.78)
SCORM	929	10.8 (0.8)	10–15	48.0	−2.02 (2.26)
SiMES	2,226	57.7 (10.8)	40–80	49.3	−0.08 (1.98)
SINDI	2,055	55.7 (8.7)	40–80+	51.2	0.01 (2.13)
SP2	1,930	47.5 (10.9)	20–80	45.4	−1.67 (2.89)
TwinsUK	4,270	55.0 (12.0)	20–82	7.4	−0.39 (2.73)
Young Finns	397	37.6 (5.2)	25–50	45.0	−1.20 (2.29)
Kyoto Study	5,192	na	na	na	na
Cases	1,143	58.4 (14.3)	20–91	33.3	−10.50 (6.44)
Controls 1	3,120	58.5 (13.6)	20–90	61.7	na
Controls 2	929	38.8 (11.8)	0–74	41.3	na
SORBS	621	na	na	na	na
Cases	100	45.4 (6.6)	18–40	36.4	na
Controls	521	28.3 (15.16)	18–80	45.0	na

than at 15q14 (0.38–0.64) in our consortium, making this an unlikely explanation (Supplementary Figure 1). Finally, population stratification within cohorts did not appear to play a major role, since only two cohorts had significant principal components, which were addressed in the analyses.

Other GWAS loci were only found for high myopia in Asian case control studies, and they were located on chromosomes 11q24.1 (Nakanishi et al. 2009), 5p15 (Li et al. 2011a), 4q25 (Li et al. 2011b), and 13q12.12 (Shi et al. 2011). The locus on chromosome 5p15 harbors the excellent candidate gene *CTNND2* which is involved in retinal morphogenesis, adhesion, retinal cell architecture integrity (Duparc et al. 2006; Paffenholz et al. 1999), and was replicated in subjects of the same ethnicity (Lu et al. 2011). Replication studies for the 4q25 (Gao et al. 2012) and 11q24.1 (Wang et al. 2011) loci were only successful in case of the 4q25 locus; these loci did not have prominent candidate genes.

What should be the next steps? For 15q14, comprehensive resequencing of the entire associated region and the flanking genes can reveal the responsible gene

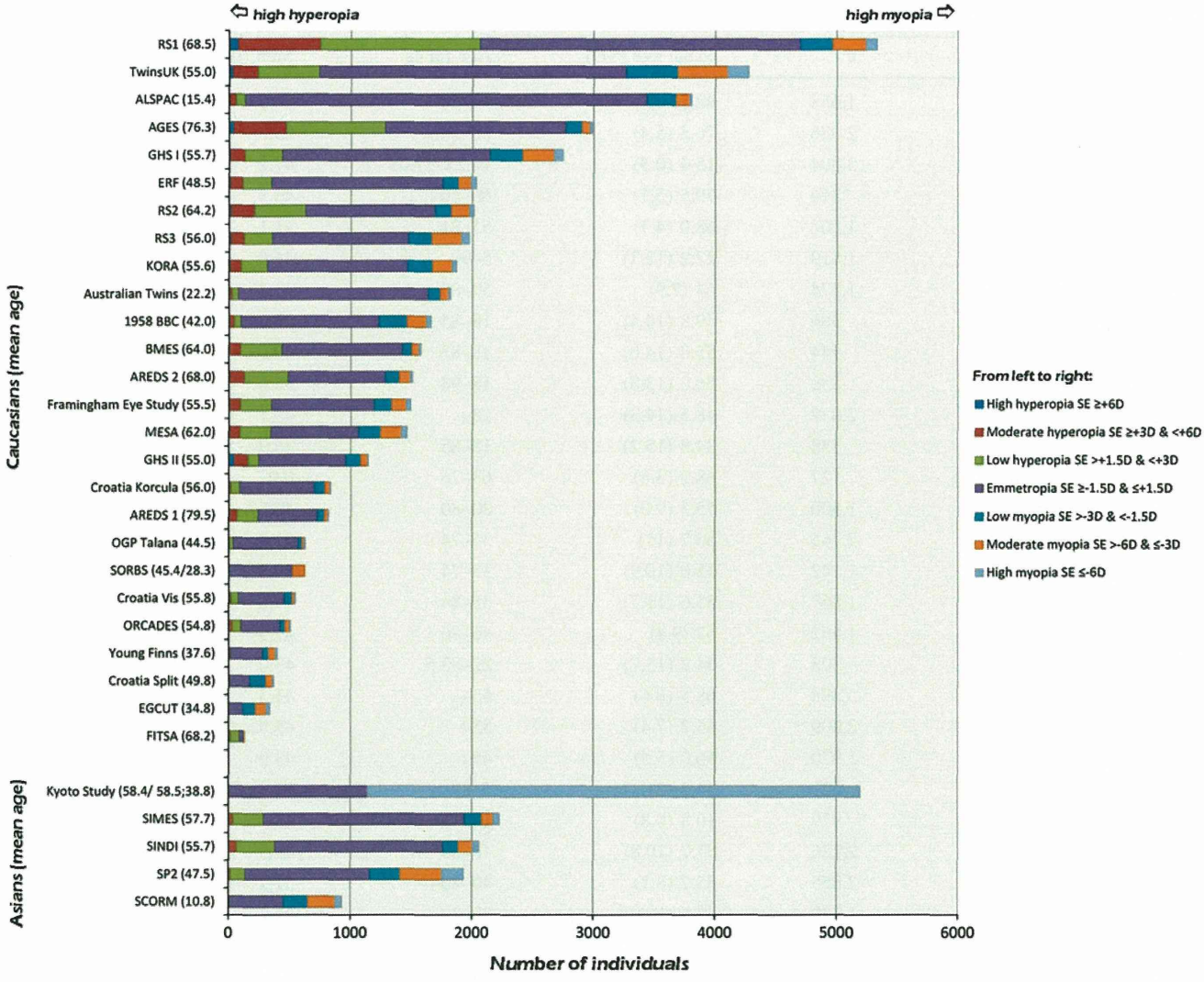

Fig. 1 Mean age and distribution of spherical equivalent in all study cohorts

defects which determine the association. Novel techniques such as next-generation sequencing are promising in this regard. Functional studies in knockout animals will shed light on potential protein effects. Finally, evaluation of gene-environment interactions may explain phenotypic variation and help identify high risk groups. For myopia genetics in general, performance of a genome-wide meta-analysis is a logical next step. The current CREAM collaboration is an excellent platform for this project.

In summary, we have convincingly demonstrated that common variants at chromosome 15q14 influence susceptibility for myopia in both Caucasian and Asian populations around the world. Identification of functional variants and responsible genes that explain this association will provide more insight in the complex etiology of myopia.

Materials and methods

Subjects and phenotyping

A total of 31 study cohorts from the Consortium of Refractive Error and Myopia (CREAM) participated in this meta-analysis. 29 population-based as well as 2 case–control studies were included. General methods, descriptives and phenotyping and genotyping methods of the study cohorts can be found in Table 1, the Supplementary Material and Supplementary Table 1, respectively. In short, 22 cohorts consisted of Caucasian, and 5 of Asian study subjects. All studies were performed with the approval of their local Medical Ethics Committee, and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Table 2 Meta-analysis of allelic effects on spherical equivalent at locus 15q14 and 15q25

SNP	Position	Effect allele	Non effect allele	Freq.	Discovery (n = 15,608)[a]			Replication (n = 33,755)[b]			Caucasian (n = 26,615)[c]		
					beta	se	P	beta	se	P	beta	se	P
Locus 15q14													
rs634990	32793365	C	T	0.49	−0.23	0.03	1.35×10^{-14}	−0.09	0.01	4.53×10^{-14}	−0.08	0.01	3.87×10^{-12}
rs560766	32788234	A	G	0.48	−0.20	0.03	4.82×10^{-12}	−0.09	0.01	3.53×10^{-14}	−0.08	0.01	3.91×10^{-12}
rs524952	32793178	A	T	0.48	−0.23	0.03	1.19×10^{-14}	−0.08	0.01	9.05×10^{-13}	−0.08	0.01	1.07×10^{-11}
rs688220	32786167	A	G	0.48	−0.20	0.03	4.43×10^{-12}	−0.08	0.01	1.01×10^{-13}	−0.08	0.01	1.38×10^{-11}
rs580839	32786121	A	G	0.48	−0.20	0.03	4.39×10^{-12}	−0.08	0.01	1.05×10^{-13}	−0.08	0.01	1.34×10^{-11}
rs11073060	32777143	A	C	0.48	−0.21	0.03	1.12×10^{-12}	−0.08	0.01	2.46×10^{-13}	−0.08	0.01	2.47×10^{-11}
rs4924134	32781857	G	A	0.45	−0.21	0.03	1.20×10^{-12}	−0.08	0.01	3.01×10^{-13}	−0.08	0.01	2.96×10^{-11}
rs7176510	32786771	T	C	0.45	−0.20	0.03	1.70×10^{-11}	−0.09	0.01	8.31×10^{-14}	−0.08	0.01	7.81×10^{-12}
rs619788	32782398	A	C	0.44	−0.20	0.03	3.94×10^{-12}	−0.08	0.01	2.21×10^{-13}	−0.08	0.01	2.29×10^{-11}
rs7163001	32777866	A	G	0.44	−0.21	0.03	1.26×10^{-12}	−0.08	0.01	6.28×10^{-13}	−0.08	0.01	4.16×10^{-11}
rs11073059	32776966	A	T	0.44	−0.21	0.03	1.98×10^{-12}	−0.08	0.01	8.78×10^{-13}	−0.08	0.01	4.85×10^{-11}
rs11073058	32776918	T	G	0.44	−0.20	0.03	2.23×10^{-12}	−0.08	0.01	8.52×10^{-13}	−0.08	0.01	4.84×10^{-11}
rs685352	32795627	G	A	0.46	−0.21	0.03	4.55×10^{-13}	−0.08	0.01	4.32×10^{-12}	−0.08	0.01	2.09×10^{-10}
rs8032019	32778782	G	A	0.40	−0.19	0.03	1.00×10^{-10}	−0.08	0.01	5.81×10^{-12}	−0.08	0.01	7.00×10^{-10}
SNP	Position	Effect allele	Non effect allele	Freq.	Discovery (n = 17,806)[a]			Replication (n = 31,557)[b]			Caucasian (n = 24,417)[c]		
					beta	se	P	beta	se	P	beta	se	P
Locus 15q25													
rs939661	77218118	A	G	0.51	−0.15	0.03	3.85×10^{-9}	−0.02	0.01	5.81×10^{-2}	−0.02	0.01	7.73×10^{-2}
rs939658	77238924	G	A	0.51	−0.15	0.03	1.85×10^{-9}	−0.02	0.01	1.60×10^{-1}	−0.02	0.01	2.16×10^{-1}
rs17175798	77251015	C	T	0.51	−0.15	0.03	1.99×10^{-9}	−0.02	0.01	1.81×10^{-1}	−0.01	0.01	2.38×10^{-1}
rs8033963	77242405	C	C	0.51	−0.15	0.03	1.86×10^{-9}	−0.01	0.01	2.18×10^{-1}	−0.02	0.01	2.20×10^{-1}
rs8027411	77248084	T	G	0.51	−0.15	0.03	2.07×10^{-9}	−0.01	0.01	2.49×10^{-1}	−0.02	0.01	2.16×10^{-1}

SNP	Position	Effect allele	Non effect allele	Freq.	Asian (n = 7,140)[d]			Meta-analysis (n = 49,363)[e]		
					beta	se	P	beta	se	P
Locus 15q14										
rs634990	32793365	C	T	0.49	−0.12	0.04	2.21×10^{-3}	−0.11	0.01	9.20×10^{-3}
rs560766	32788234	A	G	0.48	−0.12	0.04	1.47×10^{-3}	−0.10	0.01	1.03×10^{-21}
rs524952	32793178	A	T	0.48	−0.18	0.07	9.52×10^{-3}	−0.10	0.01	2.00×10^{-21}
rs688220	32786167	A	G	0.48	−0.12	0.04	9.80×10^{-4}	−0.10	0.01	3.44×10^{-21}
rs580839	32786121	A	G	0.48	−0.12	0.04	1.10×10^{-3}	−0.10	0.01	3.51×10^{-21}
rs11073060	32777143	A	C	0.48	−0.12	0.04	1.45×10^{-3}	−0.10	0.01	5.13×10^{-21}
rs4924134	32781857	G	A	0.45	−0.12	0.04	1.60×10^{-3}	−0.10	0.01	5.57×10^{-21}
rs7176510	32786771	T	C	0.45	−0.12	0.04	1.74×10^{-3}	−0.10	0.01	6.09×10^{-21}
rs619788	32782398	A	C	0.44	−0.12	0.04	1.54×10^{-3}	−0.10	0.01	6.97×10^{-21}
rs7163001	32777866	A	G	0.44	−0.11	0.04	2.81×10^{-3}	−0.10	0.01	1.41×10^{-20}
rs11073059	32776966	A	T	0.44	−0.11	0.04	3.64×10^{-3}	−0.10	0.01	2.63×10^{-20}
rs11073058	32776918	T	G	0.44	−0.11	0.04	3.50×10^{-3}	−0.10	0.01	2.68×10^{-20}
rs685352	32795627	G	A	0.46	−0.11	0.04	4.14×10^{-3}	−0.10	0.01	8.10×10^{-20}
rs8032019	32778782	G	A	0.40	−0.13	0.04	9.65×10^{-4}	−0.10	0.01	1.78×10^{-18}
Locus 15q25										
rs939661	77218118	A	G	0.51	−0.03	0.04	4.86×10^{-1}	−0.04	0.01	1.22×10^{-4}
rs939658	77238924	G	A	0.51	−0.04	0.05	3.94×10^{-1}	−0.04	0.01	4.32×10^{-4}
rs17175798	77251015	C	T	0.51	−0.05	0.06	3.70×10^{-1}	−0.04	0.01	6.12×10^{-4}

Table 2 continued

SNP	Position	Effect allele	Non effect allele	Freq.	Asian (n = 7,140)[d]			Meta-analysis (n = 49,363)[e]		
					beta	se	P	beta	se	P
rs8033963	77242405	C	C	0.51	−0.01	0.04	8.42×10^{-1}	−0.04	0.01	9.37×10^{-4}
rs8027411	77248084	T	G	0.51	0.00	0.04	9.12×10^{-1}	−0.03	0.01	1.14×10^{-3}

Freq average frequency

[a] For the 15q14 locus: RS1, RS2, RS3, ERF, TwinsUK; for the 15q25 locus: TwinsUK, RS1, RS2, RS3, ERF, 1958 British Birth Cohort, Australian Twins (adult samples only)

[b] For the 15q14 locus: 1958 British Birth Cohort, AGES, ALSPAC, AREDS 1, AREDS 2, Australian Twins, BMES, Croatia Split, Croatia Vis, Croatia Korcula, EGCUT, FITSA, Framingham, GHS I, GHS II, KORA, MESA, ORCADES, OGP Talana, SCORM, SiMES, SINDI, SP2, Young Finns; for the 15q25 locus: AGES, ALSPAC, AREDS 1, AREDS 2, BMES, Croatia Split, Croatia Vis, Croatia Korcula, EGCUT, FITSA, Framingham, GHS I, GHS II, KORA, MESA, ORCADES, OGP Talana, Young Finns, SCORM, SiMES, SINDI, SP2

[c] For the 15q14 locus: 1958 British Birth Cohort, AGES, ALSPAC, AREDS 1, AREDS 2, Australian Twins, BMES, Croatia Split, Croatia Vis, Croatia Korcula, EGCUT, FITSA, Framingham, GHS I, GHS II, KORA, MESA, ORCADES, OGP Talana, Young Finns; for 15q25 locus: AGES, ALSPAC, AREDS 1, AREDS 2, BMES, Croatia Split, Croatia Vis, Croatia Korcula, EGCUT, FITSA, Framingham, GHS I, GHS II, KORA, MESA, ORCADES, OGP Talana, Young Finns

[d] Asian replication: SP2, SIMES, SINDI, SCORM

[e] All studies

All studies used a similar protocol for phenotyping. Exclusion criteria were age ≤10 years, and bilateral cataract surgery, laser refractive procedures or other intra-ocular procedures which might alter refraction. Eligible participants underwent a complete ophthalmologic examination including a non-dilated measurement of refractive error (Table 1) of both eyes. Spherical equivalent was calculated according to the standard formula (SE = sphere + ½ cylinder), and the mean of two eyes was used for analysis. When data from only one eye were available, the SE of this eye was used. SE was categorized into low (SE from −1.5 to −3 D), moderate (SE from −3 to −6 D) and high (SE of −6 D or lower) myopia; and also into low (SE from +1.5 to +3 D), moderate (SE from +3 to +6 D) and high (SE of +6 D or higher) hyperopia. Emmetropia was defined as SE equal to or between −1.5 and +1.5 D.

Genotyping and imputation

DNA was extracted according to standard procedures, and genotyping and imputation of SNPs across the entire genome was performed using various methods (Table 1). Samples with a low call rate, with excess autosomal heterozygosity, with sex-mismatch, or outliers identified by the identity-by-state clustering analysis were excluded.

Statistical analysis

Meta-analysis of allelic effects on spherical equivalent

We selected 19 SNPs within loci 15q14 (14 SNPs) and 15q25 (5 SNPs) with a P value of $<10^{-6}$ from two previous GWAS (Hysi et al. 2010; Solouki et al. 2010). Linear regression models with a 1 degree of freedom trend test were used to examine associations with SE as a quantitative trait outcome, adjusting for age and gender and significant principal components if applicable. From all population-based cohorts, we obtained effect allele, non effect allele, regression coefficient beta, standard error, P value, minor allele and minor allele frequency for each of these SNPs. METAL for Linux was used to perform a meta-analysis on betas and standard errors for all SNPs. First, discovery cohorts (Hysi et al. 2010; Solouki et al. 2010) and replication studies were analyzed separately, followed by a combined meta-analysis. As a second analysis, 26 additional SNPs within the same linkage disequilibrium (LD) block were selected and tested for association using the procedures mentioned above. For these analyses, Bonferroni corrected P values (0.05/number of tested SNPs) of 3.57×10^{-3} for 15q14, and 1.0×10^{-2} (5 SNPs, Table 2) or 1.92×10^{-3} (26 SNPs, Table 3 Supplementary Material) for 15q25 were considered statistically significant.

Meta-analysis of risk of myopia for top SNP

From all population-based and case control studies, we obtained genotype distributions of the replicated top SNPs. We calculated heterogeneity (χ^2, I^2 calculated and corresponding P values) between studies, crude OR with corresponding 95 % CI and P value of moderate and high myopia versus moderate and high hyperopia with a random as well as fixed effects meta-analysis using Stata 11. When these analyses provided similar outcomes, data from fixed effect analysis were used. For studies without subjects with high or moderate hyperopia, emmetropia was used as a

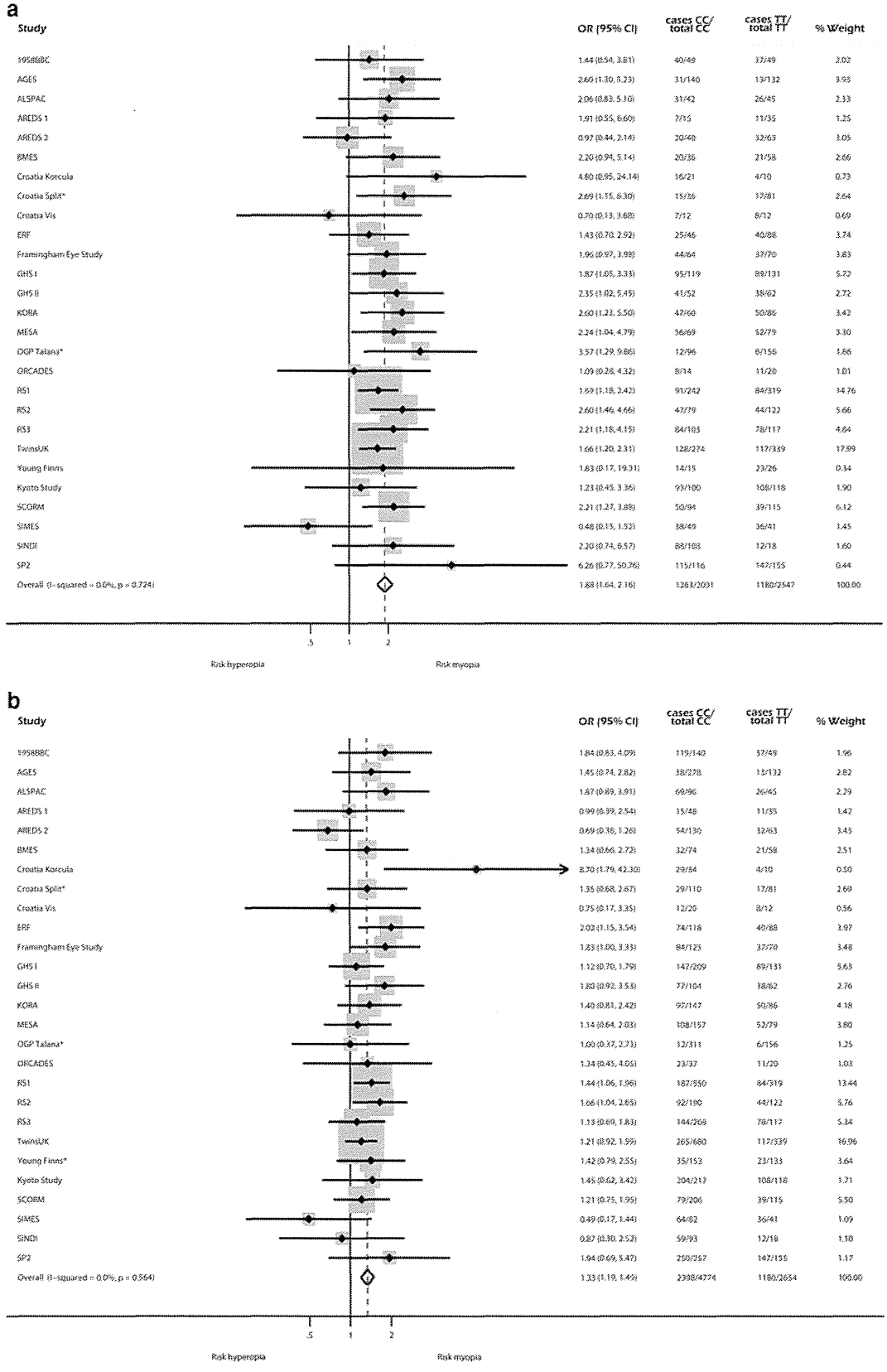

Fig. 2 Forest plots of odds ratios of myopia (spherical equivalent ≤ -3 diopters) versus hyperopia (spherical equivalent $\geq +3$ diopters) for top SNP rs634990. *For studies without subjects with high or moderate hyperopia, emmetropia was used as a reference group. **a** Homozygotes carriers of alleles TT versus CC for SNP rs634990. **b** Heterozygotes carriers of alleles TT versus TC for SNP rs634990

reference group. A standard P value of <0.05 was considered statistically significant.

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Ethical standards The experiments comply with the current laws in which they were performed.

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B-Type Natriuretic Peptide as an Independent Correlate of Nocturnal Voiding in Japanese Women

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Aims: To investigate whether objective cardiovascular parameters have an independent association with nocturnal voiding in women. **Methods:** Thirty-two parameters derived from questionnaires, and anthropometric, physiological and biochemical measures of 5,980 women were applied for analysis. Nocturnal voiding was assessed by the International Prostate Symptom Score and the Overactive Bladder Symptom Score. We measured variables including previously reported correlates of nocturnal voiding, such as age, a history of hypertension, and a history of diabetes, as well as those focusing on cardiovascular function, such as the cardio-ankle vascular index, the augmentation index, the ankle-brachial index, plasma B-type natriuretic peptide (BNP), and C-reactive protein (CRP). **Results:** Age [odds ratio (OR): 1.058, $P < 0.001$], length of sleep (OR: 1.194, $P < 0.001$), sleeplessness (OR: 2.841, $P < 0.001$), urgency (OR: 1.528, $P < 0.001$), log(BNP) (OR: 2.031, $P < 0.001$), waist circumference (OR: 1.037, $P = 0.002$), body mass index (OR: 0.935, $P = 0.007$), menopause (OR: 1.503, $P = 0.043$), and history of hypertension (OR: 1.225, $P = 0.029$) were independently associated with nocturnal voiding ≥ 2 times. Age ($\beta = 0.256$, $P < 0.001$), urgency ($\beta = 0.195$, $P < 0.001$), sleeplessness ($\beta = 0.181$, $P < 0.001$), length of sleep ($\beta = 0.088$, $P < 0.001$), log(BNP) ($\beta = 0.072$, $P < 0.001$), waist circumference ($\beta = 0.086$, $P < 0.001$), and low-density lipoprotein-cholesterol ($\beta = -0.038$, $P = 0.003$) were significantly correlated with the severity of nocturnal voiding. **Conclusions:** Plasma BNP, which represents cardiac load, is strongly associated with the prevalence and severity of nocturnal voiding in Japanese women, as well as previously known correlates including age, urgency, quality and quantity of sleep, and obesity. *Neurourol. Urodynam. 31:1266–1271, 2012.* © 2012 Wiley Periodicals, Inc.

Key words: B-type natriuretic peptide; epidemiology; nocturia; nocturnal voiding

INTRODUCTION

Nocturia is one of the most bothersome lower urinary tract symptoms (LUTS) in the elderly.[1] The causes of this symptom are multifold and are usually attributable to decreased nocturnal bladder capacity, increased nocturnal urine volume, and/or sleep problems.[2] Previous epidemiological studies have demonstrated that there are many correlates to nocturnal voiding, which include a past history of hypertension, diabetes, stroke, arrhythmia, sleeplessness, urinary urgency, and a large body mass index (BMI).[2,3] Several of these factors are components of metabolic syndrome (METS), and are related to cardiovascular disease (CVD).

Although each component of METS, hypertension, diabetes, dyslipidemia, and obesity, is an independent risk factor of CVD, synergistic effects as a risk of CVD are observed if combined.[4] Several studies have described the components of METS plus smoking as "vascular risk factors," and have reported the association between LUTS and these factors.[5,6] Therefore, it has been suggested that nocturnal voiding has a relationship with METS and/or cardiovascular conditions. However, no previous studies have shown a direct association between LUTS including nocturnal voiding and objective cardiovascular conditions, which could be assessed using several parameters including blood pressure (diastolic, systolic, and central), heart rate, the cardio-ankle vascular index (CAVI), augmentation index (AI), and ankle-brachial index (ABI).

The main purpose of this study was to investigate whether various objective parameters representing cardiovascular conditions are independent correlates for the prevalence and severity of nocturnal voiding when analyzed with previously known correlates, using data from a community-indwelling population in Japan.

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