Genome-wide association study identifies genetic risk underlying primary rhegmatogenous retinal detachment

Mirna Kirin1,†, Aman Chandra2,3, David G. Charteris2, Caroline Hayward4, Susan Campbell4, Ivana Celap5, Goran Bencic6, Zoran Vatavuk6, Iva Kirac7, Allan J. Richards8,9, Albert Tenesa4,10, Martin P. Snead11, Brian W. Fleck12, Jaswinder Singh12, Steven Harsum2, Robert E. MacLaren2, Anneke I. den Hollander13,14, Malcolm G. Dunlop4, Carel B. Hoyng13, Alan F. Wright4, Harry Campbell1, Veronique Vitart4,†,∗ and Danny Mitry1,†

1Centre for Population Health Sciences, University of Edinburgh, Edinburgh EH8 9AG, UK, 2Moorfields Eye Hospital, City Road, London EC1 V2PD, UK, 3UCL Institute of Ophthalmology, Bath Street, London EC1 V9EL, UK, 4MRC Human Genetics Unit, IGMM, University of Edinburgh, Edinburgh EH4 2XU, UK, 5Clinical Institute of Chemistry, 6Department of Ophthalmology and, 7Surgical Oncology Department, University Hospital for Tumours, University Clinical Hospital ‘Sestre Milosrdnice’, 10000 Zagreb, Croatia, 8Molecular Genetics and Medical Genetics, Cambridge University NHS Foundation Trust, Hills Road, Cambridge CB2 0QQ, UK, 9Department of Pathology, University of Cambridge, Cambridge CB2 1QP, UK, 10The Roslin Institute, The University of Edinburgh, Edinburgh EH25 9RG, UK, 11Vitreoretinal Service, BOX 41, Cambridge University NHS Foundation Trust, Hills Road, Cambridge, UK, 12Princess Alexandra Eye Pavilion, Edinburgh, UK, 13Department of Ophthalmology and 14Department of Human Genetics, Radboud University Nijmegen Medical Centre, 6500 HB Nijmegen, the Netherlands

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Rhegmatogenous retinal detachment (RRD) is an important cause of vision loss and can potentially lead to blindness. The underlying pathogenesis is complex and incompletely understood. We applied a two-stage genetic association discovery phase followed by a replication phase in a combined total of 2833 RRD cases and 7871 controls. The discovery phase involved a genome-wide association scan of 867 affected individuals and 1953 controls from Scotland, followed by genotyping and testing 4347 highest ranking or candidate single nucleotide polymorphisms (SNPs) in independent sets of cases (1000) and controls (2912) of Dutch and British origin. None of the SNPs selected reached a Bonferroni-corrected threshold for significance ($P < 1.27 \times 10^{-7}$). The strongest association, for rs12960119 ($P = 1.58 \times 10^{-7}$) located within an intron of the SS18 gene. Further testing was carried out in independent case–control series from London (846 cases) and Croatia (120 cases). The combined meta-analysis identified one association reaching genome-wide significance for rs267738 (OR = 1.29, $P = 2.11 \times 10^{-8}$), a missense coding SNP and eQTL for CERS2 encoding the protein ceramide synthase 2. Several of the top signals showing suggestive significance in the combined meta-analysis encompassed genes with a documented role in cell adhesion or migration, including SS18, TIAM1, TSTA3 and LDB2, which warrant further investigation. This first genetic association study of RRD supports a polygenic component underlying RRD risk since 27.4% of the underlying RRD liability could be explained by the collective additive effects of the genotyped SNP from the discovery genome-wide scan.

†These authors contributed equally to this work.

*To whom correspondence should be addressed. Tel: +44 1316511071; Fax: +44 1314678456; Email: veronique.vitart@igmm.ed.ac.uk

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INTRODUCTION

Despite advances in surgical treatment, rhegmatogenous retinal detachment (RRD) remains an important cause of visual loss. It accounts for up to 4% of the blind and partial sight registrations in Ireland (1). Following the formation of a break in the retina, the neural retina separates from the underlying retinal pigment epithelium (RPE) due to the egress of fluid from the vitreous cavity into the subretinal space. This leaves the neurosensory retina (NSR) deprived of nutrients and oxygen, severely reducing visual function, especially when the macula is affected. Pathological changes at the vitreoretinal interface are critical both to the development of a retinal break and to the progression of retinal detachment, since liquefied vitreous passes through the open break and the NSR is separated from the underlying RPE. The adhesion between the NSR and RPE is relatively weak since no junctional attachments exist between these two layers (2). The adhesion is maintained by the physical interconnection of the RPE cells’ apical microvilli with the outer segments of NSR photoreceptors, mechanical support from the interphotoreceptor matrix and the net fluid transport from the retina to the choroid. Once these forces are overwhelmed, the subretinal space expands, allowing the fluid to accumulate and retinal detachment to occur. The exact mechanisms involved in this complex and synergistic adhesion system are incompletely understood.

The annual incidence of RRD is \( \approx \) 10–15 cases per 100 000 individuals (3), and prevalence \( \approx \) 1%, (4) with notable differences between ethnic groups (5–7). The rate of RRD has been reported to be higher in elderly patients (5,8,9) and in men compared with women (6,10–13). Other risk factors for RRD include high myopia, lattice retinal degeneration, trauma and previous cataract surgery.

A genetic predisposition to RRD is suggested by a number of inherited disorders (with known causal genetic variants) presenting RRD as an associated feature and by familial aggregation studies (14,15). Cases of non-syndromic familial RRD, inherited in a dominant fashion, have also been ascribed to mutations in the \textit{COL2A1} gene (a known Stickler’s syndrome gene [MIM 609508]) (16,17). Very little is known about the genes underlying non-syndromic RRD in the general population and no previous study has investigated the role of common genetic variants. We conducted a genome-wide association study of RRD using a two-stage discovery phase followed by a replication phase (Fig. 1). A full genome-wide scan was first performed with close to 300 000 single nucleotide polymorphisms (SNPs) in a large collection of Scottish RRD cases (\( n = 867 \)) (18) and ethnically matched controls (\( n = 1953 \)) (19).

Figure 1. Our study included a two-stage discovery phase followed by a replication phase where the seven most promising findings were brought forward for testing. GWAS was conducted in a Scottish study in the first stage of the discovery phase. The best ranked SNPs were selected for follow-up and complemented with candidate gene markers. Four thousand and three hundred and forty-seven SNPs were successfully genotyped and analyzed in the UK and Dutch studies in the second stage of the discovery phase, and results from both discovery stages were combined in a meta-analysis (significance threshold for discovery phase meta-analysis was set to \( 1.27 \times 10^{-7} \)). An overall combined meta-analysis of discovery (two stages) and replication phases was completed for the seven SNPs carried all along (significance threshold set at \( 2.5 \times 10^{-8} \)). Studies are labeled according to the location of the center(s) where the RRD cases were collected.
results were then tested in independent sets of cases and controls of Dutch (252 cases; 320 controls) and British origin (748 cases; 2592 controls). Replication and follow-up was sought through the use of further samples from populations of English (846 cases; 2737 controls) and Croatian descent (120 cases; 269 controls). In total, 2833 cases and 7871 controls were analyzed in this study.

RESULTS

Association analysis

Discovery phase
A genome-wide association study (GWAS) was carried on 867 RRD cases and 1953 controls from Scotland, using 299 737 genotyped SNPs, after applying stringent quality control criteria. GWAS results showed a very slight excess of low P-values compared with those expected under the null hypothesis of no association (Fig. 2 and Supplementary Material, Fig. S2). One SNP, rs10510663, achieved genome-wide significance after correction for multiple testing ($P = 1.88 \times 10^{-7}$). This marker is located on chromosome 3p22.3, upstream of the ARPP21 gene. The full GWAS results corresponding to Figure 2 are available Supplementary Material, Table S7.

The most significant SNPs associated with RRD by GWAS plus some selected candidate gene markers (see section ‘Methods’ and Supplementary Material, Table S1) were then genotyped on a custom-made Illumina iSelect array in an additional 1000 RRD cases (Discovery stage 2; 252 Dutch and 748 UK individuals). The meta-analysis of the SNP association data from these two steps constituted the discovery phase and involved analyses of three distinct case–control cohorts (of Scottish, British and Dutch ancestry). A stringent genome-wide significance threshold based on the number of SNPs (of Scottish, British and Dutch ancestry). A stringent genome-wide significance threshold based on the number of SNPs (of Scottish, British and Dutch ancestry). A stringent genome-wide significance threshold based on the number of SNPs (of Scottish, British and Dutch ancestry). A stringent genome-wide significance threshold based on the number of SNPs (of Scottish, British and Dutch ancestry). A stringent genome-wide significance threshold based on the number of SNPs (of Scottish, British and Dutch ancestry). A stringent genome-wide significance threshold based on the number of SNPs (of Scottish, British and Dutch ancestry).

In the replication phase, we followed up the six most significant SNPs identified in this extended pathway analysis (MCL1-RAE1, BMP7-SETDB1, rs6070015-rs267738; SETDB1-PPA1, rs267738-rs7097067; TIAM1-TrkB, rs81232771-rs913444). The latter is strongly supported by experimental evidence of a protein–protein interaction (21).

Replication phase
In the replication phase, we followed up the six most significantly associated markers displaying low heterogeneity across either the UK ($P = 0.86$), Dutch ($P = 0.24$) and its association $P$-value after meta-analysis of discovery studies increased to be $>10^{-4}$. The most significantly associated marker after this stage was rs12960119, with an association $P$-value of $1.58 \times 10^{-7}$, just below the genome-wide significance threshold. This marker is located on chromosome 18q11.2 within an intron of the JS18 gene (OR = 1.46; 1.26–1.67 95% CI). The magnitude and direction of the signals were consistent across all three populations, translating into a low heterogeneity score across studies ($I^2 = 0$; $P = 0.67$). Cases showed an excess of the rs12960119 minor allele G compared with controls in all three cohorts (Table 2). Another marker (rs7234959), located upstream of the JS18 gene, displayed a suggestive $P$-value in the meta-analysis ($P = 2.68 \times 10^{-5}$), strengthening the validity of an association signal in this region. Most suggestive SNPs ($P < 10^{-4}$) also showed comparable directions of effect across all three populations (Table 1).

The genes putatively underlying the association signals were identified using a protocol implemented in GRAIL (20) (several genes were assigned to one association signal—Table 1) and inter loci functional networks sought out using the pathway analysis tool implemented in IPA (Ingenuity® Systems, www.ingenuity.com, last accessed 04/09/2012). Analysis using only the six most significant SNPs (additive model) as index SNPs revealed one highly significant network ($P = 10^{-62}$) involving 35 molecules in total, 21 of which were present in the top six signals (Supplementary Material, Fig. S3). This network showed enrichment for molecules involved in cell death, DNA replication, recombination and repair, and haematological system development and function. Additional pathway analysis was carried out using the 23 top results ($P < 3.10^{-4}$) from the discovery meta-analysis (Table 1). Four direct interactions were identified in this extended pathway analysis (MCL1-RAE1, rs267738-rs6070015; BMP7-SETDB1, rs6070015-rs267738; SETDB1-PPA1, rs267738-rs7097067; TIAM1-TrkB, rs81232771-rs913444). The latter is strongly supported by experimental evidence of a protein–protein interaction (21).

![Figure 2. Manhattan plot (A) and Quantile–quantile (B) plot for the discovery stage 1 genome-wide scan results. Statistics from a logistic regression adjusting for age sex and co-ancestry, performed on 867 Scottish RRD cases and 1953 controls (SOCCS participants) using 299 737 genotyped SNPs which passed quality control, are displayed. In the Manhattan plot (A) $-\log10(P$-value) is plotted on the x-axis, and chromosomal location on the y-axis. In the QQ plot (B), distributions of observed and expected Chi-square values are compared.](https://example.com/figure2.png)
<table>
<thead>
<tr>
<th>SNP</th>
<th>Minor allele</th>
<th>CHR</th>
<th>Position (build36)</th>
<th>CEU minor allele freq</th>
<th>OR (95% CI)</th>
<th>Direction</th>
<th>Heterogeneity</th>
<th>GRAIL input genes</th>
<th>Genes in LD block $r^2 &gt; 0.8$ with index SNP</th>
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<tbody>
<tr>
<td>rs12960119</td>
<td>G</td>
<td>18</td>
<td>21 868 701</td>
<td>0.075</td>
<td>1.46 (1.26–1.67)</td>
<td>++</td>
<td>0.67</td>
<td>PSM48, TAF4B, SS18</td>
<td>SS18, PSAM8, CERS2</td>
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<td>rs267738</td>
<td>G</td>
<td>1</td>
<td>149 207 249</td>
<td>0.275</td>
<td>0.79 (0.71–0.87)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs955943</td>
<td>A</td>
<td>4</td>
<td>16 123 506</td>
<td>0.067</td>
<td>1.54 (1.27–1.87)</td>
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<td>0.67</td>
<td>LDB2</td>
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<td>22 671 126</td>
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<td>++</td>
<td>0.73</td>
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<td>8</td>
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<td>0.425</td>
<td>1.12 (1.11–1.31)</td>
<td>++</td>
<td>0.25</td>
<td>TAC3</td>
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<tr>
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<td>21</td>
<td>31 921 294</td>
<td>0.075</td>
<td>1.43 (1.21–1.69)</td>
<td>++</td>
<td>0.21</td>
<td>TIA1, SOD1, SCAF4</td>
<td>SOD1, SCAF4</td>
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<tr>
<td>rs11259960</td>
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<td>15</td>
<td>81 379 707</td>
<td>0.200</td>
<td>1.35 (1.17–1.54)</td>
<td>++</td>
<td>0.41</td>
<td>HOMER2</td>
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<tr>
<td>rs2368106</td>
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<td>2</td>
<td>180 815 911</td>
<td>0.125</td>
<td>1.35 (1.17–1.54)</td>
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<td></td>
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<tr>
<td>rs7234959</td>
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<td>18</td>
<td>21 816 517</td>
<td>0.083</td>
<td>1.34 (1.17–1.53)</td>
<td>++</td>
<td>0.77</td>
<td>TAF4B, PSMA8, SS18</td>
<td>SS18, PSAM8, CERS2</td>
</tr>
<tr>
<td>rs6070015</td>
<td>A</td>
<td>20</td>
<td>55 192 062</td>
<td>0.067</td>
<td>1.67 (1.31–2.13)</td>
<td>++</td>
<td>0.9084</td>
<td>RAE1, SPO11, BMP7, RMB3</td>
<td>BMP7</td>
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<tr>
<td>rs9134444</td>
<td>A</td>
<td>9</td>
<td>87 051 470</td>
<td>0.250</td>
<td>1.22 (1.11–1.34)</td>
<td>++</td>
<td>0.94</td>
<td>TRK8</td>
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<td>rs4893905</td>
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<td>2</td>
<td>180 867 194</td>
<td>0.100</td>
<td>1.33 (1.16–1.52)</td>
<td>+</td>
<td>0.59</td>
<td>CW22, UB2E3</td>
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<tr>
<td>rs1477441</td>
<td>A</td>
<td>5</td>
<td>22 569 230</td>
<td>0.124</td>
<td>1.31 (1.15–1.49)</td>
<td>++</td>
<td>0.87</td>
<td>CDH12</td>
<td></td>
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<tr>
<td>rs12193473</td>
<td>G</td>
<td>6</td>
<td>121 301 788</td>
<td>0.124</td>
<td>1.31 (1.10–1.31)</td>
<td></td>
<td></td>
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<tr>
<td>rs218843</td>
<td>A</td>
<td>6</td>
<td>121 388 196</td>
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<td>1.20 (1.10–1.31)</td>
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<td></td>
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<tr>
<td>rs4715056</td>
<td>G</td>
<td>6</td>
<td>47 977 103</td>
<td>0.275</td>
<td>0.81 (0.73–0.90)</td>
<td>++</td>
<td>0.59</td>
<td>GPR115, OPN5, CD2AP, GPR11</td>
<td>GPR115, OPN5, CD2AP, GPR11</td>
</tr>
<tr>
<td>rs8217896</td>
<td>G</td>
<td>1</td>
<td>22 988 636</td>
<td>0.242</td>
<td>1.24 (1.12–1.37)</td>
<td>++</td>
<td>0.82</td>
<td>EPB2</td>
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<tr>
<td>rs10515162</td>
<td>C</td>
<td>5</td>
<td>73 090 012</td>
<td>0.050</td>
<td>0.72 (0.61–0.85)</td>
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<td></td>
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<tr>
<td>rs11181447</td>
<td>A</td>
<td>12</td>
<td>41 026 298</td>
<td>0.150</td>
<td>1.28 (1.13–1.45)</td>
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<td></td>
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<tr>
<td>rs12202993</td>
<td>A</td>
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<td>121 330 001</td>
<td>0.375</td>
<td>1.20 (1.10–1.31)</td>
<td>++</td>
<td>0.30</td>
<td>C6orf170</td>
<td>MANIA, C6orf170</td>
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<td>rs643531</td>
<td>A</td>
<td>6</td>
<td>47 927 523</td>
<td>0.208</td>
<td>0.79 (0.70–0.89)</td>
<td>++</td>
<td>0.91</td>
<td>GPR115, OPN5, CD2AP, GPR111</td>
<td>OPN5, PTCHD4</td>
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<tr>
<td>rs6035211</td>
<td>C</td>
<td>11</td>
<td>19 008 513</td>
<td>0.117</td>
<td>1.30 (1.14–1.47)</td>
<td></td>
<td></td>
<td></td>
<td>SLC24A3</td>
</tr>
</tbody>
</table>

Odds ratios with 95% confidence intervals are given with respect to the minor allele for an additive model of allelic effect, association P-values, direction of the minor effect in, respectively, the Netherlands, UK and Scotland study (+ increasing risk, – decreasing) and the heterogeneity P-values are also displayed. Genes potentially underlying each SNP signal as identified by a protocol within the GRAIL software and genes falling within the higher LD block tagged by the top associated SNP (defined by markers in high LD with it, $r^2 > 0.8$, based on based on CEU haplotypes HapMap3 build36 using the SNAP webtool http://www.broadinstitute.org/mpg/snap/ldplot.php, date last accessed 14/04/2013) are listed.

*Intergenic association signal, flanking genes are indicated.
studies (rs12960119, rs267738, rs955943, rs8132771, rs2045084, rs1074463—Table 1) and in addition, rs913444, the next highest ranking with low heterogeneity across studies marker with the highest minor allele frequency (to increase replication power). Furthermore, the product of the gene associated with this additional marker, TrkB, is known to bind and phosphorylate the product of TIA1 (21), a gene close to the rs8132771 association signal. A brief description of the genes included in or flanking the corresponding association signals is reported in Supplementary Material, Table S3. The seven selected markers were typed in 846 cases from London and 120 cases from Croatia (Fig. 1). All but one marker (rs1074463) displayed same direction of effect in the replication analysis compared with those in the discovery analysis, and one of them reached the Bonferroni-corrected significance threshold of 0.0035 (Supplementary Material, Tables S4 and S5). This marker, rs2045084, is located on chromosome 8q24.3, 949 bp 3’ of TSTA3 and 7.8 kb 5’ of PYCRL gene and displayed a statistically significant association under a dominant model (P = 9 × 10^{-4}, OR = 1.31) and nominally significant association under an additive model (P = 0.028, OR = 1.13). The next best associated marker, rs267738, is located on chromosome 1q21.3 within an exon of the ceramide synthase 2 (CERS2) gene and displayed a nominally significant association under both additive and dominant models (additive P = 7.1 × 10^{-3}, OR = 0.83). rs12960119, which almost reached genome-wide significance in the discovery phase, did not replicate in the London cohort (P = 0.37 additive effect). The minor allele of this marker (allele G, frequency = 0.092) was, however, associated with increased risk, as in the previous analysis, but the effect was smaller in size (OR additive model = 1.07, OR dominant model = 1.07). This reflected the frequency of the G allele of rs12960119 in the Wellcome Trust Case Control Consortium (WTCCC) NBS controls used in this replication analysis being higher (MAF = 0.087) than that of the CEU HapMap population (MAF = 0.075) or the WTCCC 1958BC control sample used in the discovery stage (MAF = 0.078), whereas the minor allele frequencies were comparable in cases. This marker was successfully genotyped in 109 cases and 265 controls from Croatia. It did not reach statistical significance (P = 0.36) in this sample due to the small sample size, but the direction and size of the effect (OR = 1.23; 0.79–1.91 95% CI) were comparable with those in the discovery cohorts, with the minor allele frequency of the risk allele higher in cases (MAF = 0.14) compared with the controls (MAF = 0.11).

**Combined analysis**

Results for the seven SNPs analyzed through both discovery and replication steps were combined into an overall meta-analysis (Table 3) and this yielded one variant (rs267738) reaching the conventional genome-wide significance threshold and the next most significant SNP (rs2045084) falling just below our initial GWAS threshold (dominant model P-value = 2.59 × 10^{-7}; threshold set at 1.27 × 10^{-8}). SNP rs267738, within the CERS2 gene, showed the strongest association signal under a dominant effect model (P = 2.11 × 10^{-8}). The SNP effect size was consistent in both direction and magnitude across all four populations (Fig. 3), with cases showing an excess of the major allele homozygote genotype AA (OR = 0.78; 0.71–0.85 95% CI). The signal in this region was further supported by an additional SNP (rs267733) showing suggestive significance in the discovery phase meta-analysis (P = 5.29 × 10^{-5}). This marker is located within the gene ANXA9, which is 5’ of CERS2. The next most significant SNP association in the combined meta-analysis (rs2045084) is located 3’ of the TSTA3 gene, and showed some heterogeneity between studies (I^2 = 32.8, P = 0.22, Fig. 3). The association was not significant in the UK discovery cohort [P = 0.55, OR = 1.09 (0.85–1.40 95% CI)].

The rs12960119 SNP, located in the SS18 gene, which almost reached genome-wide significance at the discovery phase, did not replicate in the London cohort and did not reach genome-wide significance in the overall meta-analysis (P = 2.04 × 10^{-6}; dominant model; P = 2.49 × 10^{-6} additive model). Heterogeneity in the overall meta-analysis was high under both models (I^2 = 50.2, P = 0.09, under a dominant model; I^2 = 49.6, P = 0.09, under an additive model) and contrasted with

### Table 2. rs12960119 minor allele (G) effect across the three discovery cohorts

<table>
<thead>
<tr>
<th>Population</th>
<th>Allele frequency (cases/controls)</th>
<th>OR (95% CI) additive model</th>
<th>OR (95% CI) dominant model</th>
<th>P-value (additive/dominant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scottish</td>
<td>0.115/0.086</td>
<td>1.42 (1.16–1.75)</td>
<td>1.40 (1.30–1.73)</td>
<td>0.0009/0.003</td>
</tr>
<tr>
<td>UK</td>
<td>0.104/0.077</td>
<td>1.41 (1.13–1.76)</td>
<td>1.46 (1.17–1.82)</td>
<td>0.002/0.0008</td>
</tr>
<tr>
<td>Dutch</td>
<td>0.117/0.075</td>
<td>1.76 (1.13–2.76)</td>
<td>1.90 (1.17–3.1)</td>
<td>0.013/0.009</td>
</tr>
</tbody>
</table>

rs12960119 allele frequencies, odds ratios and association P-values for the minor G allele by discovery population.

### Table 3. Combined discovery and replication phases meta-analysis results

<table>
<thead>
<tr>
<th>SNP</th>
<th>Additive model</th>
<th>OR</th>
<th>L95% CI</th>
<th>U95% CI</th>
<th>P-value</th>
<th>Direction</th>
<th>Dominant model</th>
<th>OR</th>
<th>L95% CI</th>
<th>U96% CI</th>
<th>P-value</th>
<th>Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs267738</td>
<td>0.81</td>
<td>0.75</td>
<td>0.88</td>
<td>1.43E-07</td>
<td>-</td>
<td>-</td>
<td>0.78</td>
<td>0.71</td>
<td>0.85</td>
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<td>2.11E-08</td>
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</tr>
<tr>
<td>rs2045084</td>
<td>1.16</td>
<td>1.09</td>
<td>1.24</td>
<td>2.85E-06</td>
<td>++++++</td>
<td>1.25</td>
<td>1.15</td>
<td>1.36</td>
<td>2.59E-07</td>
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Odds ratios with 95% confidence intervals and direction of effect with respect to the SNP minor allele. Direction of the effect is given for, respectively, the Netherlands, UK, Scotland, London and Croatia studies.
the homogeneity of association signal in the discovery phase, where three diverse study populations had been used.

**Genetic risk score**

Each of these markers contributed only a modest amount to individual risk of RRD. To assess the combined effect of our top-ranked markers, we prioritized five of the SNPs that were followed up at the replication phase (see section ‘Methods’) and combined them in a genetic risk score using a simple allele counting approach. The difference in a mean number of risk alleles between cases and controls was statistically significant in all studies using the Mann–Whitney test (discovery Dutch \( P = 2.05 \times 10^{-5} \); discovery Scottish \( P = 2.19 \times 10^{-11} \); discovery UK \( P = 4.1 \times 10^{-6} \); replication London \( P = 2.4 \times 10^{-3} \)). Figure 4 illustrates the distribution of the risk alleles in our four-independent studies, displaying, in each study, a higher proportion of cases compared with controls among individuals carrying four or more risk alleles. The odds ratios calculated relative to the median number of risk alleles in the controls for each population show a significantly increased RRD risk (OR > 1) for the individuals with five or more risk alleles: with odds ratios of at least two in the discovery cohorts and > 1.22 in the London replication cohort (Supplementary Material, Fig. S4).

**Variation in disease liability explained**

Following stringent QC procedures and using the method of Yang et al., implemented in the Genome Wide Complex Trait Analysis (GCTA) software, 27.4% of the variance in underlying disease liability could be assigned to the combined effect of the markers tagged by the primary genome-wide scan SNPs. The proportion of the variance explained remained comparable when different minor allele frequencies and missing genotype cut-offs were used (Table 4). The signal was not due to
population structure within Scottish cases and controls since it was not found when the Scottish Colorectal Cancer Study (SOCCS) control group was artificially split into cases and controls (liability transformed estimate = 0.000001, SE = 0.23). These GCTA estimates further supported the evidence of a modest polygenic contribution by common genetic variants in RRD.

**Discussion**

This is the first large-scale genome-wide association study ever performed for RRD, a condition which continues to result in significant vision loss. Our results support a polygenic etiology for this condition and identified several common contributory risk variants of small effect.

A genome-wide significant signal of association with RRD was found for the marker rs267738. This is a missense (Glu to Ala) coding SNP located within the CERS2 gene, in a gene-rich region. The index SNP has been associated with the expression level of CERS2 in lymphoblastoid cell lines (exon eQTL for CERS2) making the regulation of CERS2 expression a strong functional candidate for the RRD association. CERS2 encodes the protein CerS2, the most abundantly and ubiquitously expressed member of the ceramide synthase family (22). Ceramide-enriched membrane domains amplify diverse signals and are critically involved in inducing apoptosis (23).

*In vitro*, ceramides have been shown to mediate apoptosis of mammalian photoreceptors (24,25) and RPE cell lines (26–28). In a mouse model of retinitis pigmentosa, inhibition of ceramide synthesis slowed disease progression and mutation in the ceramide kinase-like gene CERKL has been implicated in a recessive form of retinitis pigmentosa in humans (29). Increased cellular ceramide levels were found in brains of patients with Batten’s disease (30) and in retinas of patients with Farber’s disease (31). A feature of both diseases is blindness, although this is due to retinal degeneration rather than detachment. An association signal with RRD in this region is supported by an additional association with SNP rs267733 (P = 5.3 × 10^{-5}; dominant model), located 11 kb from CERS2. A variant in high LD (rs267734; r^2 = 1) with this SNP has previously been reported to be associated with chronic kidney disease (32).

Within or near each of the five suggestive signals of the combined analysis (2.5 × 10^{-8} < P combined < 10^{-4}) was a gene expressed in the retina, several of which with a documented role in cell adhesion or migration (Supplementary Material, Table S3 for genes within suggestive signals). Both TSTA3 and SS18 can modify integrins. Integrins are transmembrane glycoproteins that bind extracellular matrix components to the actin cytoskeleton and activate a signaling cascade leading to cytoskeletal reorganization and cell adhesion. The products of LDB2 and TIAM1, the later close to- not within- a suggestive signal (rs8132771), have a role in cytoskeletal reorganization. TIAM1 encodes a guanine nucleotide exchange factor which activates a small GTPase of the rho family and modulates changes in cytoskeleton downstream of membrane receptor stimulation. These receptors include TrkB (21), encoded by a gene within another suggestive signal, but also adhesion molecules such as β1 integrins (33,34).

While single marker associations were weak, association with RRD jointly using the five markers showing the most significant and consistent association was significant in all four studies (Scottish discovery, Dutch discovery, UK discovery, London replication) so that the combined genetic score was a potentially useful predictor of disease. Using all the genotyped SNP additive effects from the discovery genome-wide scan jointly, explained 27.4% of the underlying liability to RRD, supporting a moderate polygenic contribution to disease status (solely due to the common variants tested in this scan). It has been suggested that estimating the proportion of variance explained that is due to all SNPs jointly may be inflated due to the confounding effect of population structure (35). However, we believe that this should not be a strong contributory factor in our discovery cohort as the variance explained in the controls alone, randomly labeled as cases and controls, was negligible. The ancestry clusters drawn in RRD cases and Scottish controls were overlapping (Supplementary Material, Fig. S1) and care was taken to remove systematic differences between RRD cases and controls due to the different genotyping platforms used (36).

There were a number of limitations to this study. As we followed up only a subset of the variants tested in the GWAS discovery step, variants which displayed a non-statistically significant association at this first stage may have been missed and yet could have been statistically significant in the larger sample size of the combined data sets (false negatives due to lack of power). Furthermore, there was a substantial failure rate (14%) of the SNPs tested on the customized iSelect Illumina platform in the follow-up discovery step. We tried to minimize the effect of this by (re)genotyping the most significant markers not represented by a proxy SNP; however, true associations could still have been missed. A further limitation was the

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use of controls from studies or repositories that were genotyped independently using different genotyping platforms. This can create confounding but true associations should remain consistent across the case–control sets assembled. The controls were unselected with regard to phenotype therefore a number of RRD cases might be expected within the control series given the relatively high prevalence of RRD (0.8%) (15). Since the number of affected RRDs may have fluctuated across control series by chance, this may partly explain the heterogeneity across studies. Furthermore, significant population structure has been reported in two of the WTCCC control cohorts, 1958 BC and NBS (35), that we used in the discovery and replication stages, respectively, making it a possible explanation for the non-replication of the SS18 locus. NBS controls had a 1.2% higher allele frequency for rs12960119 compared with that in the HapMap CEU population, and a 0.9% higher allele frequency than that in the other control group used in the discovery step (1958 Birth Cohort). The fact that only a subset of SNPs had been typed in the RRD cases used after the stage 1 discovery prevented us from correcting for population structure for the matched RRD cases and controls in the usual manner (by adjusting for ancestry principal components). Heterogeneity of the RRD phenotype is an additional concern as other underlying pathologies usually precede its development, e.g. posterior vitreous detachment, high myopia, lattice degeneration. Larger studies will be useful to tease apart the possible overlap between these pathologies. Nevertheless, results from the published GWAS on myopia (37,38) and lattice degeneration (39) did not show any overlap with our most significant associations and neither the SS18, CERS2 or TIAM1 signal strengths were affected by removing high myopes from the RRD cases in the primary analysis (Supplementary Material, Table S6) although the TSTA3, TrkB and LDB2 associations may be influenced by myopia. This study was not designed to explore the association of rare variants with RRD, and given the fraction of underlying disease liability not accounted for by the common variants tested in this study, the search for rare variants influencing RRD is warranted. Finally, given our sample size, we had limited power to detect and replicate variants of moderate effect sizes.

Despite these limitations, this first genetic association study of RRD supports a polygenic component underlying RRD risk, and identified several common variants of moderate effect contributing to risk. Additional studies will be required to confirm the individual variants highlighted in this study and additional genotyping and increased sample size will be needed to extend this first exploration of the genetic basis of RRD. Since the pathogenesis of RRD is not fully understood, the identification of genes influencing it should make an important contribution to furthering our understanding of the disorder and in time lead to better prevention and treatment.

**MATERIALS AND METHODS**

**Study subjects**

All participating studies received approval from their local ethics committees and followed the tenets of the Declaration of Helsinki.

**Retinal detachment cases**

All Scottish cases [aged 9–91 (mean = 58.91)] were recruited as a part of a population-based epidemiology study of RRD recently carried out in Scotland (18). All incident cases of RRD reported in any of the six vitreoretinal surgical sites (Ayr, Glasgow, Edinburgh, Dundee, Aberdeen and Inverness) were invited to participate in the study. The diagnosis of RRD was based on a case definition of ‘a full thickness break in the NSR with a surrounding area of sub-retinal fluid extending greater than 2 disc diameters’ (12,40–42). All other types of retinal detachment (exudative, tractional and combined) and re-detachments cases regardless of the duration of attachment post-operatively were excluded. Also excluded were participants with a cataract surgery within 2 years of the detachment diagnostic or with known syndromic features. 6.9% of RRD Scottish subjects evaluated reported an affected first degree relative (15). Care was taken that cases from London, UK n = 457 in the discovery phase (aged 9–92, mean = 57.87); n = 846 in the replication phase (aged 14–90, mean = 58.58), Cambridge, UK (n = 173, aged 15–91, mean = 61.38), Nijmegen, the Netherlands (n = 252; aged 12–95, mean = 59.92) and Zagreb, Croatia (n = 120, aged 20–83, mean = 59.96) were collected using the same phenotypic inclusion criteria as for the Scottish sample. Positive family history was recorded in 11.3% of cases from the London recruitment, 7.1% of Croatian cases and 5.6% of a subset of 180 Dutch cases had an affected first degree relative (14). Family history information for cases collected in Cambridge was not available.

**Controls**

Control groups were readily available population-matched samples unselected with regard to RRD; i.e. unselected controls, a small fraction of which would have RRD. The SOCCS was used as a control group for the Scottish samples in the first stage of the discovery. This is a prospective population-based study in Scotland (1999–2006), with colorectal cancer cases drawn from all over Scotland, matched to healthy controls by age, sex and area of residence (19). One thousand and nine hundred and sixty-seven genotyped individuals were used in the analysis, aged 18–62 (mean = 50.25). Two sets of control groups from the WTCCC were also used in subsequent stages: 2592 samples from the 1958 UK birth cohort (1958BC) was used in stage two of the discovery phase and 2737 individuals from National Blood Service (aged 17–69, mean = 43.5) in the replication phase (43). Controls for the Dutch samples were 320 control individuals from an independent schizophrenia study that were locally recruited, aged 36–74 (mean = 50.22).

Finally, Croatian participants to a colorectal cases and controls on-going study were used as a control data set for the Croatian RRD cases (269 individuals; aged 24–92, mean = 66.1).

**Genotyping**

**Genome-wide scan**

Nine hundred and twelve Scottish RRD cases were genotyped using the Illumina CNV370v3-Quad array, and compared with population-matched Scottish controls from the SOCCS study previously genotyped on the Illumina-300 and 240S HumanHap arrays. Raw data analysis was done using the Illumina genotyping analysis software GenomeStudio with a GenCall scoring cut-off
of 0.15 and solely on the new data itself (not using external references). A final clustering was done using only the 892 samples with an overall genotype call >90%. Further quality control was performed separately for cases and controls using the check marker function of the ‘GenABEL’ R package. This step removed SNPs with a low call rate (<97%), those in a high Hardy–Weinberg disequilibrium (P < 1 x 10^{-6}), as well as samples identified as duplicates or highly related, mixed (high heterozygosity), those with a low call rate (<97%), sex discrepancies, and outliers based on whole-genome identity-by-state sharing. In total, 870 cases, 1968 controls and 299,789 common SNPs (minor allele frequency >0.02) remained for analysis. When cases and controls were analyzed jointly, three additional samples failed quality control. Deeper relatedness check based on genomic kinship coefficients derived from the whole-genome identity-by-state sharing identified 15 pairs of individuals related to the first-cousins or greater level (π-hat > 0.125 in PLINKv1.07, Φ > 0.0625 in ‘GenABEL’ (44)). Removing one individual of each related pair left 867 cases and 1953 controls for the final analysis. Plots using principal components derived from the identity-by-state sharing measures showed good overlap of ancestry between the Scottish RRD cases and controls (Supplementary Material, Fig. S1).

Further to these standard QC steps, SNP showing strong signals in association analysis were examined more closely. Several were removed due to poor clustering in either cases or controls or both after visualization of individual clusters (e.g. presence of four rather than three clusters) and two SNPs, rs4862110 and rs4957798, were removed due to discordance between SNP arrays (36). In the final analysis presented here, 299 737 SNPs, genotyped in both cases and controls and QCed, were used.

Imputation of the genotyped SNPs and the subsequent analysis was not performed since different genotyping platforms had been used in cases and controls. It is well documented that in this scenario imputation is tricky and often leads to type I error inflation (45).

**iSelect platform**

Five thousand and nine hundred and eighty-one SNPs were selected for the second stage of the discovery phase to be assayed on an iSelect Illumina platform, 4706 selected from the genome-wide scan results complemented by 1275 from 18 candidate gene regions (listed in Supplementary Material, Table S1). Tagging SNPs in the candidate gene regions were selected with Tagger program within the Haploview v4.0 software using Centre d’Etude du Polymorphisme Humain from Utah (CEU) population of the HapMap project (International HapMap consortium) (46). A threshold for r^2 was set at 0.8. Region of 100 kb upstream and downstream from each gene was used in the selection process. Eight hundred and thirteen assays failed design, including 183 of the candidate genes set (in agreement with failure rate to be expected from the Illumina Infinium technology). The list of the candidate genes tagging SNPs that were tested are listed Supplementary Material, Table S1. Clustering was done using Illumina Genome Studio software and QC steps (call rate, HWE, sex discrepancies) were carried as described for the genome scan analysis. Difference in genotype missingness rates between cases and control was also tested separately for each new study using the function—test-missing in PLINKv1.07 (47). No SNPs displayed significant missingness difference (P < 0.00001) between cases and controls. To ensure that signals from all of the SNPs with best ranks in the discovery stage 1 were taken forward in stage 2 (i.e. among the best 200 of any stage 1 analysis), we investigated the representativity by proxy (LD r^2 > 0.2) of those which failed on the iSelect chip or QC. Eight SNPs with initial P-values < 10^{-4} or in possible functional gene, FRMD4A and GRM7 were thus additionally typed using the Taqman technology. The final number of SNPs carried on for the second stage of the discovery phase was 4347.

**Single SNP typing**

Seven SNPs in total were genotyped in the replication phase using the Taqman technology. The six top associated SNPs, which displayed consistent direction of effect across all cohorts and one additional SNP (rs913444) based on the pathway analysis results. Quality control tests were carried out as described above. Call rates for SNPs genotyped using the TaqMan technology were set to 90%. The highest in the London replication samples was 99.8% (rs8132771) and the lowest 93.5% (rs955943). Only one marker (rs12960119; call rate = 93.6%) passed this threshold in the Croatian samples.

**Statistical analysis**

**Association testing**

The genome-wide scan was performed using a regression model with age and sex as covariates and fitting an additive SNP effect, using the egscore function of the ‘GenABEL’ R package (44) which takes into account possible population stratification (48). QQ plots of observed versus expected ordered test statistics were drawn using the ‘snpMatrix’ R package (49). The grey area represents the area between the 2.5 and 97.5% probability bounds of non-departure to the null hypothesis. Five analyses were performed, all RRD Scottish cases versus all SOCCS Scottish controls, RRD cases with known high myopes (spherical equivalent refraction ≤ -5) removed, SOCCS controls with cancer cases removed and gender separate analysis (QQ plots presented in Fig. 2 and Supplementary Material, Fig. S2). The top ranking associated SNPs from each analysis (P-value < 10^{-3}) were listed to be taken forward in the second stage of the discovery.

In the second stage of the discovery phase, association between RRD with SNP genotypes was tested using logistic regression including sex and age as covariates and using the PLINKv1.07 software (47). Both additive and dominant models were tested. Association analyses were done for each cohort separately and the results from all cohorts (with the Scottish discovery step 1 rerun using the PLINKv1.07 implementation of the logistic regression adjusting for age sex and three ancestry principal components) combined in an inverse variance-weighted fixed effect meta-analysis using METAL (50). We calculated empirical significance levels for the association in all of the cohorts by permutation as implemented in PLINKv1.07, and used these empirical significance levels for further meta-analysis.

Given the low sample size of our replication cohorts and that inclusion of covariates in case–control association studies has been shown to reduce their power in this scenario and with low
disease prevalence, we carried out analysis in the replication phase without the inclusion of covariates. Results from both discovery stages and the replication phase were also combined in a meta-analysis (unadjusted for covariates). Forrest plots and heterogeneity measures for the most significant SNPs were obtained using the ‘meta’ R package (51).

**P-value threshold**

To correct for multiple testing in the initial genome-wide scan, we used a multiple testing correction method that takes into account the correlation structure due to linkage disequilibrium (LD) among the GWAS genotyped SNPs tested, using the ‘SimpleM’ R library (52). Imputation of the missing genotypes was carried out using k Nearest Neighbors using the ‘scrine’ R package (53). The calculated effective number of independent SNPs, \( n = 197,628 \), was then used to correct for multiple testing using the standard Bonferroni procedure. Discovery stage significance threshold was additionally corrected for the testing of two genetic models (additive and dominant). The genome-wide significance threshold was therefore set at \( 1.27 \times 10^{-7} \) \( (=0.05/(197,628 \times 2)) \). Despite following up only a subset of SNPs used in the first step of the discovery phase into the second step, the same genome-wide significance threshold was used for the meta-analysis of the two steps to be conservative. The overall meta-analysis (combining discovery stage and replication stage data) used the conventional \( 5 \times 10^{-8} \) significance threshold, additionally corrected for the use of two genetic models, hence set at \( 2.5 \times 10^{-8} \).

**Genetic risk score**

The multilocus genetic risk score was calculated for each individual using five risk variants (the most significant homogeneous results from the discovery phase). A simple allele count was used to calculate the genetic risk score using PLINK’s v1.07 ‘score’ option. Individuals with one or more missing genotypes were not included in the analysis. Odds ratios were calculated relative to the median number of risk alleles among controls using SPSS v16.0 (54) (subjects with five or more risk alleles were collapsed together due to the low number of individuals with high-risk allele numbers).

**Genetic variance of disease liability explained**

The GCTA software (55) was used to estimate the proportion of variation in disease liability captured by the genome-wide SNP array (discovery stage 1). The combined-SNP effect was fitted as a random effect in a mixed linear model predicting the transformed trait (probit transformation) and its variance estimated by restricted maximum likelihood. This estimation is then corrected for the incomplete LD between the tag SNP and the causal variant and for the ascertainment of cases (compared with the disease prevalence in general population). Stringent QC procedures (PLINK v1.07) (47) were implemented on the genotyped data to avoid experimental biases causing false case–control differences in relatedness (56). Closely related individuals were removed to make sure only distant relationships were taken into account (kinship coefficient threshold was set at 0.05 and implemented within the GCTA software). As a population structure control, the control cohort (SOCCS without colorectal cases) was split into two groups and the procedure repeated in order to check that the detected explained variance there was negligible.

**Pathway analysis**

The top six SNPs from the discovery phase were selected in a first pathway analysis. Identification of all possible genes underlying the SNPs’ association signals was done using the ‘seed’ output from the GRAIL (20) (Gene Relationships Among Implicated Loci) software. All genes located in the interval were considered in the subsequent pathway analysis using the IPA software (Ingenuity Systems, www.ingenuity.com). IPA uses an internal manually maintained database to identify networks that maximize connectivity. When running the analysis, endogenous chemicals were excluded from the search and only the direct interactions were taken into account. An extended analysis, including all SNPs from the discovery phase with associated \( P \)-value \( < 10^{-4} \) (23 SNPs in total, Table 1), was also performed using the same parameters.

**SUPPLEMENTARY MATERIAL**

Supplementary Material is available at HMG online.

**ACKNOWLEDGEMENTS**

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*Conflict of Interest statement.* None declared.

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