Testosterone Pathway Genetic Polymorphisms in Relation to Primary Open-Angle Glaucoma: An Analysis in Two Large Datasets


1Department of Population and Quantitative Health Sciences, Case Western Reserve University School of Medicine, Cleveland, Ohio, United States
2Institute for Computational Biology, Case Western Reserve University School of Medicine, Cleveland, Ohio, United States
3Statistical Genetics, QIMR Berghofer Medical Research Institute, Royal Brisbane Hospital, Brisbane, Australia
4Channing Division of Network Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts, United States
5Schepens Eye Research Institute, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, Massachusetts, United States
6Department of Ophthalmology, Hamilton Glaucoma Center and Shiley Eye Institute, University of California at San Diego, La Jolla, California, United States
7Department of Epidemiology, Harvard T. H. Chan School of Public Health, Harvard Medical School, Boston, Massachusetts, United States
8Department of Ophthalmology, Duke University Medical Center, Durham, North Carolina, United States
9Department of Medicine, Duke University Medical Center, Durham, North Carolina, United States
10Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, Florida, United States
11Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, Michigan, United States
12Center for Human Genetics, Marshfield Clinic Research Institute, Marshfield, Wisconsin, United States
13Department of Ophthalmology, NYU Langone Medical Center, NYU School of Medicine, New York, New York, United States
14Departments of Ophthalmology and Anatomy/Cell Biology, University of Iowa, College of Medicine, Iowa City, Iowa, United States
15Department of Ophthalmology, University of North Carolina, Chapel Hill, North Carolina, United States
16Department of Ophthalmology, WVU Eye Institute, Morgantown, West Virginia, United States
17Scripps Genome Center, University of California at San Diego, San Diego, California, United States
18Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, Florida, United States
19Department of Ophthalmology, Stanford University, Palo Alto, California, United States
20Department of Ophthalmology, Mayo Clinic, Rochester, Minnesota, United States
21Wills Eye Hospital, Glaucoma Research Center, Philadelphia, Pennsylvania, United States
22Einhorn Clinical Research Center, New York Eye and Ear Infirmary of Mount Sinai, New York, New York, United States
23Department of Ophthalmology, Case Western Reserve University School of Medicine, Cleveland, Ohio, United States
24Department of Genetics, Stanford University, Palo Alto, California, United States
25Wills Eye Institute, Johns Hopkins University Hospital, Baltimore, Maryland, United States
26Department of Ophthalmology, University of Illinois College of Medicine at Chicago, Chicago, Illinois, United States
27Division of Preventive Medicine, Brigham and Women’s Hospital, Boston, Massachusetts, United States
28Department of Cellular Biology and Anatomy, Augusta University, Augusta, Georgia, United States
29Department of Biostatistics, Harvard T. H. Chan School of Public Health, Harvard Medical School, Boston, Massachusetts, United States
30Department of Ophthalmology, Flinders University, Adelaide, SA, Australia
31School of Medicine, Menzies Research Institute of Tasmania, Hobart, Australia
32Centre for Ophthalmology and Visual Science, Lions Eye Institute, University of Western Australia, Perth, Australia
33Department of Ophthalmology, Mass Eye and Ear Infirmary, Harvard Medical School, Boston, Massachusetts, United States
Sex hormones may be associated with primary open-angle glaucoma (POAG), although the mechanisms are unclear. We previously observed that gene variants involved with estrogen metabolism were collectively associated with POAG in women but not men; here we assessed gene variants related to testosterone metabolism collectively and POAG risk.

**Methods.** We used two datasets: one from the United States (3853 cases and 33,480 controls) and another from Australia (1155 cases and 1992 controls). Both datasets contained densely called genotypes imputed to the 1000 Genomes reference panel. We used pathway- and gene-based approaches with Pathway Analysis by Randomization Incorporating Structure (PARIS) software to assess the overall association between a panel of single nucleotide polymorphisms (SNPs) in testosterone metabolism genes and POAG. In sex-stratified analyses, we evaluated POAG overall and POAG subtypes defined by maximum IOP (high-tension [HTG] or normal tension glaucoma [NTG]).

**Results.** In the US dataset, the SNP panel was not associated with POAG (permuted \( P = 0.77 \)), although there was an association in the Australian sample (permuted \( P = 0.018 \)). In both datasets, the SNP panel was associated with POAG in men (permuted \( P \leq 0.033 \)) and not women (permuted \( P \geq 0.42 \)), but in gene-based analyses, there was no consistency on the main genes responsible for these findings. In both datasets, the testosterone pathway association with HTG was significant (permuted \( P \leq 0.011 \)), but again, gene-based analyses showed no consistent driver gene associations.

**Conclusions.** Collectively, testosterone metabolism pathway SNPs were consistently associated with the high-tension subtype of POAG in two datasets.

Keywords: primary open-angle glaucoma, testosterone, genetics, pathway analysis

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**Figure.** The testosterone pathway is depicted. Gene names are as follows: AKR1C3-ALDO-KETO reductase family 1, member C3, also known as HSD17B5; HSD17B1; 2, 3, 7, 8, 12, 14-17 beta hydroxysteroid dehydrogenase isoforms; CYP19A1: cytochrome P450, family 19, subfamily A, polypeptide 1; SULT1E1: sulfotransferase family 1E, estrogen-prefering, member 1; SULT1A1: sulfotransferase family 1A, cytosolic, phenol-prefering, member 1; SRD5A1, 2, 3: steroid 5-alpha-reductase 1, 2, 3; HSD3B 1, 2, 3: 3-beta-hydroxysteroid dehydrogenase 1, 2; AR, androgen receptor; ER, estrogen receptor; DHT, dehydroxysterosterone.

Intracrinology refers to the synthesis of sex steroids in these peripheral tissues from adrenal precursors. In fact, intracrine metabolism accounts for essentially all androgen (and estrogen) synthesis in peripheral tissues for postmenopausal women and up to approximately 40% of androgen synthesis in aging men. Thus, biochemical factors that lead to differences in intracrine metabolism may impact the availability of sex hormones and influence disease processes related to declining hormones. For example, various isoforms of 17-beta hydroxysteroid dehydrogenase (17[b] HSD) are essential to the local intracellular generation of testosterone and estradiol from DHEA (Fig.). Interestingly, Costa-Prados and colleagues documented that the neuroendocrine secretory ciliary epithelium expresses 17[b] HSD isoforms 2, 5, and 7, and these cells actively metabolize androgen and other sex hormones. However, genetic variants of 17[b] HSD have been little studied specifically in relation to POAG.

Using a pathway analysis, we previously showed that, collectively, genetic variants in estrogen metabolism enzymes were associated with POAG in women and not men. Here, we formed a custom testosterone metabolism genetic variant panel in case-control datasets from the United States and Australia/New Zealand to further evaluate if there is an association overall, or stratified by sex, with POAG or POAG subtypes defined by IOP. This panel focused on genes related to the intracrine generation of testosterone, as local production of sex steroid may be most relevant to the glaucomatous process.

**Methods**

**Description of the Study Populations**

The US data are derived from the National Eye Institute Glaucoma Human Genetics Collaboration Heritable Overall Operational Database (NEIGHBORHOOD), a genetic consortium that includes the following eight independent datasets: Massachusetts Eye and Ear Infirmary; National Eye Institute Glaucoma Human Genetics Collaboration; Iowa; Marshfield; the Ocular Hypertension Treatment Study; the Women’s
Testosterone Metabolism Gene Variants and Glaucoma

Genome Health Study; and two datasets from the Glaucoma Genes and Environment Study: one genotyped on the Affymetrix platform and the other genotyped on the Illumina HapMap Series. The NEIGHBORHOOD dataset has a total of 3853 POAG cases and 35,480 controls. The Australian and New Zealand data are derived from the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG) and consist of 1115 advanced POAG cases and 1992 controls genotyped on the Illumina Omni 1M or the OmniExpress array. Cases and controls were drawn from Southern Adelaide Health Service/Flinders University, University of Tasmania, Queensland Institute of Medical Research, and the Royal Victorian Eye and Ear Hospital. All participants in both datasets were of European ancestry. The institutional review boards of all participating institutions approved this study.

Ophthalmic Characteristics of Cases and Controls

Across the eight datasets in NEIGHBORHOOD, cases and controls lacked evidence of secondary IOP elevation on slit lamp biomicroscopy, such as exfoliation syndrome, pigment dispersion syndrome, or trauma. For cases and controls, slit lamp examination or gonioscopy did not reveal evidence of significant irido-trabecular meshwork apposition suggestive of angle closure in either eye. For cases, fundus examination revealed a cup-disc ratio (CDR) of at least 0.7 or an intereye difference in CDR of at least 0.2. Each case had at least one eye with visual field (VF) loss consistent with nerve fiber layer damage. For inclusion as a case or a control, controls had a CDR of 0.6 or less in both eyes and a CDR intereye difference of 0.1 or less. IOP at diagnosis was collected and used to categorize POAG as either high-tension glaucoma (HTG with IOP ≥22 mm Hg) and normal tension glaucoma (NTG with IOP <22 mm Hg) subtypes when available. The exact definition of POAG across the eight NEIGHBORHOOD sites can be found in Supplementary Table 2 of Cooke Bailey et al.

Advanced POAG cases in ANZRAG had best-corrected visual acuity worse than 6/60 due to POAG or a reliable 24-2 VF with a mean deviation worse than −22 dB or at least two of four central fixation squares affected with a pattern SD of <0.5%. The less severely affected eye was also required to have signs of glaucomatous disc damage with care taken to exclude secondary glaucomas of all types. Unscreened participants from the Australian Cancer Study (225 esophageal cancer cases, 317 Barrett’s esophagus cases, and 552 controls) and from a study of inflammatory bowel diseases (303 cases and 595 controls) were chosen as controls.

Genotyping Data and Imputation

Details regarding the genotyping of the US and Australian datasets, including information about the genotyping platforms and quality control measures, can be found in the Supplemental Note of Cooke Bailey et al. Estimated genotypic probabilities for the loci in the US and Australian dataset imputed to the 1000 Genomes Project reference panel (March 2012) were analyzed.

Generation of Genetic Data for the Testosterone Pathway Analysis

The genome-wide associations between single nucleotide polymorphism (SNP) allele dosage in relation to POAG were analyzed with ProbABEL (GenABEL project developers; http://www.genabel.org/) for NEIGHBORHOOD and SNPTEST (University of Oxford; http://mathgen.stats.ox.ac.uk) in ANZRAG. Logistic regression models adjusting for age, study-specific eigenvectors, and study-specific covariates for each dataset were evaluated. Using METAL (Center for Statistical Genetics, University of Michigan; http://csg.sph.umich.edu/abecasis/metal/index.html) we performed a meta-analysis to assess SNP dosages in relation to POAG across the US datasets. SNPs with imputation quality score >0.7 and minor allele frequency >0.05 were carried forward for the US and Australian datasets. For this work, we used the P values for association with POAG from the 2974 gene variants in NEIGHBORHOOD and the 2617 gene variants in ANZRAG (with 2609 consensus SNPs between datasets) that were attributable to testosterone metabolism for pathway analyses (see Fig. for genes). The number of gene variants differed slightly for each analysis.

Pathway Analysis by Randomization Incorporating Structure (PARIS) Analysis

As part of the testosterone pathway, we chose to include genes involved in the formation of androstenediol and testosterone, hormone although they are made in the testes, they are also formed from DHEA produced by the adrenal glands and then undergo intracellular conversion to both androgens and estrogens in local tissues. We generated a custom SNP panel derived from 16 genes across 12 chromosomes comprising the testosterone metabolic pathway, as depicted in the Figure. We submitted the P values from SNPs within 50 kb of the start and end sites of these genes to PARIS (v2.4). We have previously described PARIS and used a prior version of this software to assess the estrogen metabolism pathway gene variants in relation to POAG. PARIS derives a P value for association between a given gene variant set and outcome of interest using a permutation procedure. Specifically, it first creates a random collection of SNPs with genomic features that mimic features of the user-defined pathway (in this case, testosterone metabolism), then compares the number of statistically significant (P < 0.05) features within the user-defined pathway to the random pathway. We chose to permute 10,000 times to determine an overall likelihood of the random pathway containing more significant features than the user-defined one. For example, for the testosterone pathway SNP set association with POAG among men in NEIGHBORHOOD, PARIS reported 44 significant features; specifically, 27 of 238 “simple features” (SNPs not in any linkage disequilibrium block [LD block]) and 17 of 45 “complex features” (an LD block with two or more SNPs) had P values less than 0.05 for association with POAG. PARIS calculated a permuted P = 0.0001, indicating that only 1 of 10,000 random pathways with genetic architectures similar to the testosterone pathway had a higher significant feature count (>44 significant features with P < 0.05). Initially, these analyses were carried out in men and women together for the outcomes of overall POAG as well as of the HTG and NTG subtypes. Subsequently, associations between testosterone metabolism SNPs and POAG were repeated in men and women separately. We also used the “-paris-details” flag to investigate specifically which of the genes in the testosterone metabolism pathway were contributing to the significant signal in the overall pathway. Analyses in NEIGHBORHOOD were repeated in ANZRAG using a dataset-specific testosterone SNP set, that is, SNPs in the ANZRAG dataset located within 50 kb of the start and end sites of the 16 genes comprising the testosterone metabolism pathway, because various platforms were used across studies, and different sets of SNPs passed the quality control filters. However, in secondary analysis, we did use the 2609 consensus SNPs between both datasets as the exposure of interest in relation to the various glaucoma phenotypes. Finally,
TABLE 1. The Mean Age Distribution of POAG Cases and Controls in the NEIGHBORHOOD, Stratified by Sex and by IOP (HTG or NTG)

<table>
<thead>
<tr>
<th>Study</th>
<th>Disease Type</th>
<th>NHS/HPFS Affy</th>
<th>NHS/HPFS Illumina</th>
<th>MGHS</th>
<th>OHTS</th>
<th>Marshfield</th>
<th>NEIGHBORHOOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sex</td>
<td>Age (SD)</td>
<td>Age (SD)</td>
<td>Age (SD)</td>
<td>Age (SD)</td>
<td>Age (SD)</td>
<td>Age (SD)</td>
</tr>
<tr>
<td>POAG Male</td>
<td>51</td>
<td>58.7 (8.0)</td>
<td>134</td>
<td>57.7 (7.8)</td>
<td>155</td>
<td>83.9 (11.6)</td>
<td>153</td>
</tr>
<tr>
<td>Controls Male</td>
<td>1520</td>
<td>52.8 (7.9)</td>
<td>583</td>
<td>52.4 (6.8)</td>
<td>0</td>
<td>56.7 (9.6)</td>
<td>782</td>
</tr>
<tr>
<td>HTG Male</td>
<td>35</td>
<td>59.2 (7.8)</td>
<td>98</td>
<td>58.7 (7.7)</td>
<td>0</td>
<td>59.6 (9.7)</td>
<td>165</td>
</tr>
<tr>
<td>Controls Female</td>
<td>2482</td>
<td>53.0 (6.7)</td>
<td>1359</td>
<td>52.8 (6.8)</td>
<td>22,335</td>
<td>54.6 (7.1)</td>
<td>340</td>
</tr>
<tr>
<td>HTG Female</td>
<td>52</td>
<td>55.1 (6.1)</td>
<td>171</td>
<td>55.0 (6.0)</td>
<td>94</td>
<td>58.2 (7.3)</td>
<td>154</td>
</tr>
<tr>
<td>Controls Female</td>
<td>24</td>
<td>56.8 (5.7)</td>
<td>88</td>
<td>56.6 (5.7)</td>
<td>0</td>
<td>57.7 (7.1)</td>
<td>203</td>
</tr>
</tbody>
</table>

Age is mean age in years. –, not applicable or not available; MEEI, Massachusetts Eye and Ear Infirmary; NEIGHBOR, National Eye Institute Glaucoma Human Genetics Collaboration; NHS/HPFS, Nurses Health Study/Health Professional Follow-up Study; OHTS, Ocular Hypertension Treatment Study; WGHS, Women’s Genome Health Study.

* The number of HTG and NTG cases are less than the total number of POAG cases because IOP was not available on all NEIGHBORHOOD cases.

we explored whether our outcomes differed if we excluded SNPs from five genes that coded for overlapping estrogen-metabolizing enzymes (CYP19A1, SULT1E1, HSD17B1, HSD3B1, and SRD5A1). Although unlikely, we also excluded the four small genes with only one feature (HSD17B8, SULT1A1, HSD3B1, and HSD3B2) to minimize any bias they might introduce, as we were primarily interested in collections of genes that worked in biochemical pathways in relation to glaucoma outcomes.

RESULTS

The mean ages of cases and controls stratified by sex and POAG subtype (HTG versus NTG) are provided in Tables 1 and 2 for the US and Australian datasets, respectively. There is a preponderance of female controls in the US dataset due to the large size of the Women’s Genome Health Study, which has a case-cohort design.

In NEIGHBORHOOD, the testosterone pathway was not associated with POAG overall (permutated $P = 0.77$; Table 3) but a significant association was noted in ANZRAG (permutated $P = 0.018$; Table 3). In both datasets, the testosterone pathway was associated with POAG overall among men (permutated $P = 0.033$) but not among women (permutated $P = 0.42$). In both datasets, the testosterone pathway was significantly associated with HTG (permutated $P = 0.011$), but there were inconsistent results with respect to NTG (Table 3). Although the testosterone pathway was associated with NTG in ANZRAG (permutated $P < 0.0001$), it was not associated with NTG in NEIGHBORHOOD (permutated $P = 1.00$). These results were essentially identical when evaluating only the overlapping SNPs (as opposed to the dataset-specific SNP sets) between the US and Australian datasets (see Supplementary Material). Further stratification by sex for the POAG subtypes of HTG and NTG as outcomes was not performed due to the smaller sample sizes in both datasets. In both datasets, the relationship between the testosterone pathway and POAG stratified by sex was similar if the five genes that overlap between the estrogen metabolism pathway (CYP19A1, SULT1E1, HSD17B1, HSD3B1, and SRD5A1) were excluded from analysis (data not shown). Furthermore, we also performed an analysis deleting four small genes with only one feature (HSD3B1, HSD3B2, HSD17B8, and SULT1A1), as such genes will yield a $P < 0.0001$ if there is only one SNP in the feature block with $P < 0.05$; the results of this analysis were the same as the main results (see Supplementary Material).

Similar to the pathway approach, we tested the association between the 16 testosterone pathway genes and our various outcomes to determine the genes that were driving observed associations. When comparing the US and Australian datasets, there was no overlap in the significant driver genes with >1

TABLE 2. The Mean Age and Distribution of POAG Cases and Controls in the ANZRAG, Stratified by Sex and HTG or NTG

<table>
<thead>
<tr>
<th>Sex</th>
<th>n</th>
<th>Age, y (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POAG Males</td>
<td>563</td>
<td>59.9 (14.9)*</td>
</tr>
<tr>
<td>females</td>
<td>592</td>
<td>61.1 (15.8)*</td>
</tr>
<tr>
<td>Controls Males</td>
<td>1270</td>
<td>58.4 (13.2)</td>
</tr>
<tr>
<td>females</td>
<td>722</td>
<td>56.6 (15.1)</td>
</tr>
<tr>
<td>HTG Males</td>
<td>370</td>
<td>59.1 (15.3)*</td>
</tr>
<tr>
<td>females</td>
<td>359</td>
<td>59.0 (13.9)*</td>
</tr>
<tr>
<td>NTG Males</td>
<td>143</td>
<td>62.2 (14.6)*</td>
</tr>
<tr>
<td>females</td>
<td>187</td>
<td>64.1 (14.0)*</td>
</tr>
</tbody>
</table>

* Age and/or maximum known IOP were missing on 116 POAG cases.
feature (genes with permuted $P < 0.05$) responsible for the associations between testosterone pathway and POAG in men and women (Table 4). Furthermore, there were no overlapping significant driver genes with >1 feature responsible for the association between testosterone metabolism SNPs and HTG in both the US and Australian datasets (Table 5). Several genes were responsible for the relationship between the testosterone SNP panel and NTG in ANZRAG, including AKR1C3, HSD17B2, and HSD17B14 (permuted $P$ for gene $\leq 0.056$; Table 5).

We also analyzed individual SNPs in the testosterone metabolic pathway and the various outcomes. As expected, no SNP achieved a $P$ value that passed Bonferroni-corrected significance level ($2609$ consensus SNPs evaluated for five outcomes; thus, the new significance level is $3.8 \times 10^{-5}$). The complete results can be found in the Supplementary Material.

### Discussion

Very little is known about the role of testosterone metabolism in POAG. This work assessed the relationship between gene variants related to the intracrine testosterone metabolism and POAG using two large datasets. In both datasets, we observed that the assembled testosterone pathway SNP set was consistently associated with HTG in two datasets. Furthermore, the pathway was consistently associated with POAG in men but not in women.

Some of the testosterone pathway genetic associations across the US and Australian datasets were not consistent. Specifically, although the testosterone SNPs were not associated with POAG overall in the US dataset (permuted $P = 0.77$), a significant association was found in the Australian dataset (permuted $P = 0.018$). Also, the relationship between the testosterone SNP set and NTG was null in the US dataset, whereas it was significant in the Australian dataset. Various sensitivity analyses using only overlapping SNPs or excluding genes predominately involved in estrogen metabolism did not change the results that were consistent between the US and Australian datasets. We suspect that the inconsistencies between the datasets are due to differing sample size, as the individual genes have very modest effects and no common gene sets emerged as driving the pathway results replicating.

### Table 4. Gene Significance Within the Testosterone Pathway for POAG Patients Stratified by Sex in NEIGHBORHOOD (United States) and ANZRAG (Australia)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chr</th>
<th>No. of Simple Features Based on US Data†</th>
<th>No. of Complex Features Based on US Data†</th>
<th>Gene $P$ Value, POAG</th>
<th>Gene $P$ Value, POAG, Male</th>
<th>Gene $P$ Value, POAG, Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSD3B1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HSD3B2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HSD17B7</td>
<td>1</td>
<td>25</td>
<td>1</td>
<td>0.44</td>
<td>1</td>
<td>1</td>
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<tr>
<td>SRD5A2</td>
<td>2</td>
<td>18</td>
<td>4</td>
<td>0.081</td>
<td>0.36</td>
<td>1</td>
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<tr>
<td>SRD5A3</td>
<td>4</td>
<td>21</td>
<td>2</td>
<td>0.077</td>
<td>0.18</td>
<td>0.22</td>
</tr>
<tr>
<td>SULT1E1</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0.070</td>
<td>1</td>
</tr>
<tr>
<td>SRD5A1</td>
<td>5</td>
<td>22</td>
<td>2</td>
<td>1</td>
<td>0.0035</td>
<td>1</td>
</tr>
<tr>
<td>HSD17B8</td>
<td>6</td>
<td>0</td>
<td>1</td>
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<td>1</td>
</tr>
<tr>
<td>HSD17B3</td>
<td>9</td>
<td>16</td>
<td>4</td>
<td>0.60</td>
<td>0.0088</td>
<td>1</td>
</tr>
<tr>
<td>AKR1C3</td>
<td>10</td>
<td>57</td>
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<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HSD17B12</td>
<td>11</td>
<td>13</td>
<td>3</td>
<td>0.0024</td>
<td>0.42</td>
<td>1</td>
</tr>
<tr>
<td>CYP19A1</td>
<td>15</td>
<td>38</td>
<td>10</td>
<td>1</td>
<td>0.0002</td>
<td>1</td>
</tr>
<tr>
<td>HSD17B2</td>
<td>16</td>
<td>11</td>
<td>5</td>
<td>0.012</td>
<td>0.0037</td>
<td>1</td>
</tr>
<tr>
<td>SULT1A1</td>
<td>16</td>
<td>0</td>
<td>1</td>
<td>$&lt;0.0001$</td>
<td>$&lt;0.0001$</td>
<td>$&lt;0.0001$</td>
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<tr>
<td>HSD17B1</td>
<td>17</td>
<td>15</td>
<td>1</td>
<td>0.40</td>
<td>0.45</td>
<td>0.11</td>
</tr>
<tr>
<td>HSD17B14</td>
<td>19</td>
<td>24</td>
<td>2</td>
<td>0.032</td>
<td>0.41</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

$P$ values $<0.05$ are shown in bold. Simple features refer to SNPs not in any LD block. Complex features refer to LD blocks with two or more types of SNPs. Gene names can be found in the Figure legend. Chr, chromosome.

* All $P$ values are permuted $P$ values as discussed in Methods.

† Genetic architecture is based on the US dataset considering POAG as the outcome. Genetic architecture for the Australian dataset and or different outcomes varied only slightly, and these differences can be seen in the Supplementary Material.
Table 5. Gene Significance Within the Testosterone Pathway for POAG Patients Stratified by IOP in NEIGHBORHOOD (United States) and ANZRAG (Australia)

<table>
<thead>
<tr>
<th>Gene</th>
<th>US</th>
<th>Australia</th>
<th>US</th>
<th>Australia</th>
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</thead>
<tbody>
<tr>
<td>HSD1B1</td>
<td>1</td>
<td>1</td>
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<td>1</td>
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<tr>
<td>HSD1B2</td>
<td>1</td>
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<td>1</td>
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</tr>
<tr>
<td>HSD1B7</td>
<td>0.0002</td>
<td>0.34</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SRD5A2</td>
<td>&lt;0.0001</td>
<td>1</td>
<td>0.18</td>
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<tr>
<td>SRD5A3</td>
<td>&lt;0.0001</td>
<td>0.44</td>
<td>0.49</td>
<td>0.55</td>
</tr>
<tr>
<td>SULT1E1</td>
<td>1</td>
<td>0.026</td>
<td>0.054</td>
<td>0.036</td>
</tr>
<tr>
<td>SULT1A1</td>
<td>1</td>
<td>0.18</td>
<td>0.18</td>
<td>1</td>
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<tr>
<td>HSD1B8</td>
<td>1</td>
<td>&lt;0.0001</td>
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<td>1</td>
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P values <0.05 are shown in bold. Gene names can be found in the Figure legend.

* All P values are permuted P values as discussed in the Methods section.

across the datasets; however, we cannot rule out different disease definitions and environmental influences as the source of the differences we report.

For the associations between the testosterone SNP set and HTG in the US and Australian datasets, there was no common genetic driver of the relationship between testosterone metabolism SNPs and HTG. Different 17\beta HSD isoforms play critical roles in the testosterone pathway and are involved in the intracellular generation of markers that bind both androgen and estrogen receptors (Fig.) via the interconversion of androstenedione and testosterone as well as the interconversion of estrone and estradiol. The literature would suggest that the trabecular meshwork cells\textsuperscript{26} and retinal cells, including retinal ganglion cells,\textsuperscript{9,27} are under estrogenic control, whereas ocular adnexal tissues are predominately under androgenic control.\textsuperscript{28} However, little is known of the relationship between the various 17\beta HSD isoforms and glaucoma, particularly the product of HSD1B14, which was first discovered in the human retina\textsuperscript{29} and predicts a favorable response to tamoxifen in estrogen receptor-positive breast cancer tissue.\textsuperscript{30} As for 17\beta HSD isoforms 7 and 12, which were associated with HTG only in the US dataset, they are involved in local production of estradiol and feature prominently in the endoplasmic reticulum,\textsuperscript{31} whose subcellular organelles are abundant in the trabecular meshwork.\textsuperscript{32} More work is needed to understand if and how testosterone metabolism and functional polymorphisms in the various 17\beta HSD enzymes contribute to endoplasmic reticulum stress found in POAG.\textsuperscript{33}

The association between the testosterone gene variant set and POAG in men but not women in two datasets was a notable finding. There is some evidence that declining estrogen levels are linked to higher IOP in postmenopausal women\textsuperscript{34} and that in women, postmenopausal hormones might lower IOP\textsuperscript{35,36} and be associated with lower glaucoma risk.\textsuperscript{1,36} In contrast, there are scarce data of the impact of sex hormones on health outcomes in aging males.\textsuperscript{37} There are, however, specific case reports of syndromic male hypogonadism associated with elevated IOP.\textsuperscript{38,39} Although specific genes in the testosterone pathway that accounted for this apparent sexual dimorphism were identified in both datasets (for example, HSD17B7 in the US dataset and HSD17B1 in the Australian dataset), no common gene drivers of this relation were found. There are a myriad of reasons why gene drivers common to the US and Australian datasets could not be found, including the dissimilar sample sizes, the slight variation in case-control ascertainment, and differences in genotyping platforms that were used. Furthermore, differences in the quality control and the imputation process generated different SNP sets that conformed to our definition of testosterone gene variants in the US and Australian datasets. Nonetheless, the lack of consistent cross-study driver genes raises questions about whether the relationship between testosterone metabolism SNPs and POAG is truly sex specific. This is in contrast to the relationship between the estrogen pathway SNPs and POAG, where we found COMT to be a driver gene for the sexual dimorphic association with glaucoma (associated with HTG in women but not men) in two separate datasets.\textsuperscript{15}

Study limitations include the fact that none of the individual testosterone pathway SNPs were significantly associated with POAG stratified by sex or with POAG subtypes of HTG/NTG after correcting for multiple comparisons. Nonetheless, it is well known that existing genome-wide association studies of POAG are underpowered to find biologically meaningful gene variants of modest effects due to the need to minimize false discovery rates. In addition, the testosterone metabolic pathway could be construed to extend beyond biochemical pathways focused on intracrine production of sex steroids, such as cholesterol biosynthesis.\textsuperscript{40} Finally, there is a lack of evidence that a genetic signature associated with sex steroid metabolism is related to varying concentrations of estradiol or testosterone in cells relevant to POAG. Nonetheless, there is accumulating evidence that exposures altering estrogen levels modify the risk of POAG.\textsuperscript{11,36,41,42}

Our study does have strengths, including the use of two large datasets, the use of common imputed SNPs across the genome, and the use of updated PARIS software with its enhanced ability to refine gene margins. By including a second dataset to compare findings, we were able to provide a more careful interpretation of the relationship between the testosterone metabolic pathway and POAG and POAG subtypes stratified by sex. By jointly analyzing association signals across a large number of genetic variants, pathway analysis allowed for identification of modest cumulative effects, which could have been missed in standard analyses of individual variants.

In conclusion, in this study involving 40,440 participants from two continents, we observed a significant relationship between the testosterone metabolism SNPs collectively and POAG among men but not among women. We also found that these SNPs were associated with the high-tension subtype of POAG in both the US and Australian dataset, although there was no consensus on driver genes for these pathway associations across the two datasets.

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APPENDIX

Members of the ANZRAG Consortium


Department of Anatomical Pathology, Flinders University, Flinders Medical Centre, Adelaide, South Australia, Australia: Sonja Klebe.

Ophthalmology and Vision Science, Macquarie University, Sydney, New South Wales, Australia: Stuart L. Graham.


Centre for Eye Research Australia (CERA), University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, Victoria, Australia: Jonathan B. Ruddle.

Department of Ophthalmology, University of Sydney, Sydney Eye Hospital, Sydney, New South Wales, Australia: Andrew J. White, Paul R. Healey.

Centre for Vision Research, Westmead Millennium Institute, University of Sydney, Westmead, New South Wales, Australia: Andrew J. White, Paul R. Healey.

QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia: Grant W. Montgomery, Nicholas G. Martin, Graham Radford-Smith, David C. Whiteman, Matthew H. Law.

University of Queensland Diamantina Institute, Brisbane, Queensland, Australia: Matthew A Brown, Katie Cremin.