Supplemental Data

Supplemental Data include two tables and can be found with this article online at http://www.cell.com/AJHG.

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Web Resources

The URLs for data presented herein are as follows:

Nucleotide positions according to the February 2009 human reference sequence (GRCh37), http://genome.ucsc.edu/cgi-bin/hgGateway

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim

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Response to Cruciani et al.

In July 2009 we published in this journal a report containing evidence for gene conversion between the X chromosome and the male-specific region of the Y chromosome[1] at a translocation hotspot (hotspot A; *HSA*) between the *PRKX* (MIM 300083) and *PRKY* (MIM 400008) genes. In this issue, Cruciani and colleagues[2] revisit our data and conclude that we overestimated the per-base-per-generation rate because of a failure to divide the number of conversion events within the sequence under study by the length of the sequence in base-pairs. We agree with Cruciani and colleagues[2] that we made this error, thank them for pointing it out, and apologize to the readers of *The Journal*.

We observed two X-to-Y conversion events, but calculation of an average per-base-per-generation rate for the two events is complicated by the fact that the region sequenced was shorter for one of the events (in hgQ*: 698 bp) than the other (in hgA2c: 1839 bp, excluding primers). In recalculating the conversion rate per base per generation we therefore consider only the twelve Y chromosomes that were sequenced for the entire 1839 bp region and which, measured according to the approach we used previously,[1] encompass between 39,757 and 56,640 generations. Given the spacing of gametologous sequence variants, the single event observed here has a tract length between 4 and 125 bp. Using the equation of Cruciani et al.,[2] we obtain a corrected rate range for X-to-Y gene conversion of between 3.8×10^{-8} and 1.7×10^{-6} per base per generation; this rate is comparable to that found by Cruciani et al.[2] in their own data. The lower bound of the recalculated range is similar to the average base mutation rate (2.3×10^{-8} per base per generation[3]), and the upper bound of the range is two

orders of magnitude slower than the Y-Y conversion rate within palindromes (2.2×10^{-4} per base per generation[4]).

Our report's other conclusions, including the similarity of the events-per-generation rates of crossover (translocation) (~1×10^{-5}; ref.[1,5]) and conversion (~6.6×10^{-6} - ~2.1×10^{-5}; ref.[1]) at *HSA*, remain unchanged. We are gratified to observe that, in their independent resequencing of *HSA*, Cruciani and colleagues[2] also discovered variants indicating conversion from the X chromosome.

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No Evidence of Association of Heterozygous NTF4 Mutations in Patients with Primary Open-Angle Glaucoma

To the Editor: Pasutto et al. recently reported that heterozygous *NTF4* (MIM 162662) sequence variants confer an increased risk of primary open-angle glaucoma (POAG [MIM 137760]).[1] In an effort to replicate these findings, we sequenced the complete *NTF4* coding region in a large dataset of European ancestry. The research was reviewed and approved by the Institutional Review Board of Duke University Medical Center (Durham, NC) and was in accordance with the tenets of the Declaration of Helsinki. Our dataset contained 443 POAG cases and 533 controls. Enrollment criteria for unrelated POAG cases included (1) age of onset greater than 30 years; (2) glaucomatous optic neuropathy affecting both eyes; and (3) glaucomatous visual field loss affecting at least one eye. Intraocular pressure (IOP) was not an enrollment criterion. Eighteen POAG cases with normal IOP were included in our dataset. Exclusion criteria included the presence of any secondary form of glaucoma, including exfoliation syndrome, or a history of ocular trauma. The criteria for unrelated control subjects were (1) IOP less than 21 mmHg; (2) no evidence of glaucomatous optic neuropathy; and (3) normal visual field by either automated perimetry or frequency doubling test (FDT). All clinical examination records for cases and controls were reviewed by a glaucoma subspecialist (RRA). The mean age of onset for POAG cases was 57.6 ± 14.2 yr, and the mean age of examination for controls was 64.7 ± 9.3 yr.

We extracted DNA from peripheral blood by standard methods. We designed primers to avoid amplification of the *NTF4*-like pseudogene (AC008687.5) on chromosome 19. We performed DNA sequencing on a 3730 DNA analyzer from Applied Biosystems by using Sanger sequencing of genomic PCR products from the *NTF4* coding exons. All DNA samples were sequenced successfully in both directions. Sequences were analyzed with Sequencher 4.8 software by at least two people independently. Each of the identified variants was also confirmed by a second independent PCR reaction and sequencing analysis.

We identified five POAG cases and 12 controls with nonsynonymous coding changes in the *NTF4* gene (Table 1). The overall frequency of coding changes (5/443 versus 12/533) was not significantly different between cases and controls. The most frequent sequence variants were A88V (six subjects) and R206W (three subjects). Although Pasutto et al. reported these variants as risk alleles, we observed a higher frequency of these variants in controls than cases (the difference was not significantly different according to Fisher's exact test). The A88V variant was found in one case and five controls, and the R206W variant was found in one case and two controls. We identified seven novel coding variants, including S29X, S89N, R90C, R114G, R133H, R140C, and T207I, in the *NTF4* gene. None of these variants was observed at a significantly