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

Table 1. *NTF4* Sequence Variants Identified in 443 POAG Patients and 533 Controls

Nucleotide Alteration	Amino Acid Change	POAG	Age at Onset (yrs)	Control	Age at Exam (yrs)	Reported Previously
c.263C > T	A88V	1	73	5	67[a]	Yes
c.616C > T	R206W	1	63	2	70[a]	Yes
c.86C > A	S29X	0		1	56	No
c.266G > A	S89N	1	62	1	56	No
c.268C > T	R90C	0		1	56	No
c.340C > G	R114G	0		1	74	No
c.398G > A	R133H	1	40	0		No
c.418C > T	R140C	0		1	70	No
c.620C > T	T207I	1	40	0		No
Total		5		12		

[a] Age at exam for these two variants is the mean age at exam.

different frequency in cases than in controls. We found no instance of a single subject harboring more than one *NTF4* coding variant. Interestingly, a nonsense mutation (S29X) was found in one control but no cases. This control subject was 56 years old and had hyperopia and presbyopia. Because of the small number of controls with *NTF4* variants, genotype-phenotype correlations cannot be detected.

In summary, our data indicate that coding variants in the *NTF4* gene are not associated with an elevated risk of POAG in individuals of European ancestry from the southeastern United States. *NTF4* is less conserved evolutionarily than other neurotrophins (Nerve growth factor, brain-derived neurotrophic factor (*BDNF*), and neurotrophin 3).[2] *NTF4*-deficient mice only show minor cellular deficits and develop normally to adulthood, whereas knockouts of other neurotrophins prove lethal during early postnatal development.[3] This might partially explain why we identified a number of different coding variants in the *NTF4* gene. Although our study population is individuals of European ancestry, its more recent evolutionary history is not identical to the original study population of Pasutto et al.[1] In addition, the mean age of our cases (57.6 years) and controls (64.7 years) is lower than that of the original study population (66.9 years for cases and 73.9 years for controls, discovery dataset). Our study contained only 18 (4%) normal tension glaucoma (NTG) patients, whereas the original study had 82 (21%) NTG patients in the discovery dataset.[1] The two replication groups in the original study identified *NTF4* mutations only in NTG patients, and not in POAG patients.[1] Therefore, the discrepant findings could be due to population differences, to glaucoma subtype-specific associations, or to associations that change with age.[4] However, our study demonstrates that heterozygous coding changes in *NTF4* do not play a significant role in the pathogenesis of POAG in a representative clinic-based population of European ancestry in the southeastern United States.

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Web Resources

The URL for data presented herein is as follows:

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

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