LETTER

Reply to Chakrabarti et al.: Corneal angiogenesis in patients with null *FOXC1* variants

In the 15 years since transcription factor mutation, and subsequently dosage alterations, were shown to account for a proportion of Axenfeld–Rieger syndrome cases, novel phenotypes continue to be defined. As illustrated by the spectrum of *FOXC1*-attributable CNS anomalies (1, 2), these are frequently present in a subset of cases and only identified by meticulous phenotyping. Our original article in PNAS (3) demonstrated that a proportion of patients with a mutation or altered *FOXC1* gene dosage exhibited corneal angiogenesis, confirming our extensive data from multiple murine mutants regarding Foxc1's role in maintaining corneal avascularity. In keeping with the heterogeneity of transcription factor-induced disease, angiogenesis was observed in two pedigrees with null alleles (a frameshift mutation and a segmental deletion) (4), with milder changes in some 6p25 segmental duplication cases and none with point mutations. Accordingly, one explanation for the discrepant findings was the smaller size of Chakrabarti's cohort ($n = 10$) and the absence of the copy number variants that comprise the majority of our >100 *FOXC1*-attributable cases (4–6).

A second explanation reflects chronology, with angiogenesis most pronounced in infancy (in mice and humans). In patients, angiogenesis is recognized if sought contemporaneously (3), but at later stages, it may be attributed to corneal scarring secondary to elevated intraocular pressure (IOP). The latter is a common feature of pediatric glaucoma, with the effects of surgical IOP correction also confounding retrospective analysis. Phenotyping by a clinician-scientist with interest in forkhead gene biology and developmental genetics may also have assisted the original study. Additional factors potentially include differences in genetic background between the populations, especially modulation of *FOXC1*-induced phenotypes by adjacent genes (*FOXF2* and *FOXQ1*). The 6p25 forkhead triplet's evolutionarily conserved genomic organization supports coordinated gene regulation with loss of common regulatory elements from 6p25 segmental deletions likely explaining more severe angiogenesis phenotypes (3).

A final consideration relates to the increasing number of *FOX* genes shown to possess vascular biological roles. Among these, *Foxc2* shares overlapping developmental functions and similar expression in neural crest-derived periocular mesenchyme with its close paralog *Foxc1*. Our recent finding that neural crest-specific Foxc2 mutant mice also have corneal angiogenesis* demonstrates that corneal avascularity is dependent on multiple *FOX* genes and supports multiallelic inheritance contributing to phenotypic heterogeneity. In conclusion, it is evident that multiple mechanisms account for the well-recognized variability of *FOXC1*-induced phenotypes, and we appreciate the opportunity provided by the letter from Charkabarti et al. (6) to expand our discussion of the results of the study by Seo et al. (3).

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The authors declare no conflict of interest.

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