Homozygous mutations are detected in mozygosity mapping can detect only homozygous mutations. We clearly explained in the Methods and in the Results section, the goal was to identify and map homozygous regions in a large cohort of patients with cone–rod dystrophy (CRD). Genetic technologies are developing at a tremendous pace, allowing us to detect mutations in the entire study group, as also mentioned in the introduction of the article.

We appreciate the critical reading of our article by Drs. Thiadens and Klaver, and we fully agree with their statements that good collaboration among clinicians, molecular geneticists, and epidemiologists is warranted, to establish meaningful genotype-phenotype correlations in patients with retinal dystrophies.

Drs. Thiadens and Klaver question the gene frequencies provided in our study and point out that the genetic testing was not identical for the entire study group. We would like to comment that our study was not an epidemiologic study and has not been presented as such. As stated in the study’s introduction, the goal was to identify and map homozygous regions in a large cohort of patients with cone–rod dystrophy (CRD). We clearly explained in the Methods and in the Results sections that only part of the patients have been screened for known mutations in ABCA4. Moreover, it is obvious that homozygosity mapping can detect only homozygous mutations. Homozygous mutations are detected in ∼35% of Western European patients with autosomal recessive diseases and consequently we did not expect that homozygosity mapping would allow us to detect mutations in the entire study group, as also mentioned in the introduction of the article.

Perhaps we should not have presented the percentages of CRD cases that could be attributed to the CRD genes, since not all patients were screened for the complete genes. On the other hand, genetic testing of entire genes in large patient cohorts has always been expensive and laborious, and only limited articles present an accurate overview of percentages of mutations in all genes associated with a specific retinal dystrophy or in one specific gene. Our study does indicate, however, that mutations in PROM1, CERKL, and EYS are likely to be infrequent causes of CRD.

Furthermore, Drs. Thiadens and Klaver mention that the diagnosis of the patients in this cohort may be uncertain. Such uncertainty may be true to some extent, as sometimes the final diagnosis changes over the years with progression of the disease or with the development of systemic features. In a follow-up study of 75 patients with an initial diagnosis of Leber congenital amaurosis, the diagnosis was revised at a later stage to a different disease in 30 patients. This actually emphasizes the value of molecular genetic testing to aid a correct diagnosis. Genetic technologies are developing at a tremendous pace, and it is currently possible to screen all retinal dystrophy genes in a patient’s DNA in one experiment, by using a resequencing chip or next-generation sequencing (NGS). We are arriving in an era in which it may even be faster to perform genetic screening of all retinal dystrophy genes in a patient’s DNA, rather than to await the results of clinical tests that, particularly in young children, can be challenging or impossible to perform. Besides aiding the diagnosis, we expect that NGS will bring accurate epidemiologic data of mutation frequencies in the near future.

Author Response: Genetic Testing and Clinical Characterization of Patients with Cone–Rod Dystrophy

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References


Effects of Fibroblastic and Endothelial Extracellular Matrices on Corneal Endothelial Cells

In their excellent recent report, published online as a recently accepted paper on July 14, 2010, Gruschwitz et al.1 under-standably omitted a 25-year-old paper of ours.2 In it, Hsieh and I isolated extracellular matrices (ECMs) from chick embryo fibroblasts and human and rabbit corneal stromal cells. These cells induced polarization and elongation of corneal endothelial cells in culture. By indirect immunofluorescence, fibronectin was seen as arrays of long fibers in fibroblastic ECM, whereas in endothelial ECM, fibronectin was found in discrete foci of short fibers. The morphology of endothelial cells in culture was associated with the structure of the ECM that was laid down: short fibers in clusters associated with a typical polygonal shape, with long polarized fibers inducing a fibroblastic appearance.

My coworkers and I applaud the authors and look forward to their future efforts.

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Author Response: Effects of Fibroblastic and Endothelial Extracellular Matrices on Corneal Endothelial Cells

We thank Dr. Baum for his kind, encouraging words and for pointing out his very interesting article on how matrix influences HCEC morphology.1 His work is of great importance to the field of corneal endothelial research. It had a major impact on our earlier work on establishing and optimizing cell cultivation and transplantation of HCECs—namely, his article, “Mass Culture of Human Corneal Endothelial Cells,”2,3 which we cited in one of our most important papers.5

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References

Bevacizumab Suppression of Establishment of Micrometastases in Experimental Ocular Melanoma

We read with great interest the article entitled, “Bevacizumab Suppression of Establishment of Micrometastases in Experimental Ocular Melanoma” by Yang et al.,1 published in the June issue. We congratulate the authors for this informative study. However, we have a few concerns about the study.

1. In Figure 1, the authors demonstrate that bevacizumab reduced VEGF secretion. However, it is more likely that bevacizumab neutralized the secreted VEGF, rather than suppressed it.

2. It is not clear why, even though bevacizumab may have neutralized the secreted VEGF, the neutralized VEGF could not be measured by ELISA. The epitopes for antibodies used in ELISAs (27–191 amino acids) are likely to be against a different region of VEGF than are the functional epitopes bound by bevacizumab.

3. The authors state that VEGF secretion was slightly suppressed with 10 μg/mL bevacizumab (P = 0.0046) and greatly reduced with 100 μg/mL (P = 0.0091) in B16L59 cells, compared with the effect of the IgG1 control treatment. However, they also state that the levels of VEGF after 10 and 100 μg/mL bevacizumab were 8.51 and 15.99 pg/mL, respectively. Thus, the levels were higher in cultures treated with a higher dose of bevacizumab (100 μg/mL). Also, in Figure 1C, it does not appear that the VEGF levels were decreased significantly by bevacizumab.

4. Several earlier studies (e.g., Ferrara et al)2 reported that bevacizumab does not neutralize mouse VEGF, raising the question of how bevacizumab may have reduced the VEGF levels.

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References

Author Response: Bevacizumab Suppression of Establishment of Micrometastases in Experimental Ocular Melanoma

Bevacizumab is a recombinant humanized monoclonal IgG1 antibody that binds to and inhibits the biological activity of human vascular endothelial growth factor (VEGF) in vitro and in vivo assay systems. It contains human framework regions and the complementarity-determining regions of a murine antibody that binds to VEGF.

When comparing the sequence of human VEGF protein with that of mouse VEGF protein, we found that 86% of the protein sequence in mouse VEGF164 is similar to that of human VEGF165.1 We believe that it is possible for bevacizumab to block human and mouse VEGF. A blocking antibody is defined as an antibody that does not have a reaction when combined with an antigen, but prevents other antibodies from combining with that antigen.2,3 We believe that bevacizumab fits this definition and explains why the neutralized VEGF is not detected by ELISA. The epitopes for antibodies used in ELISAs (27–191 amino acids) are likely to be against a different region of VEGF than are the functional epitopes bound by bevacizumab.

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