

Divide and conquer:

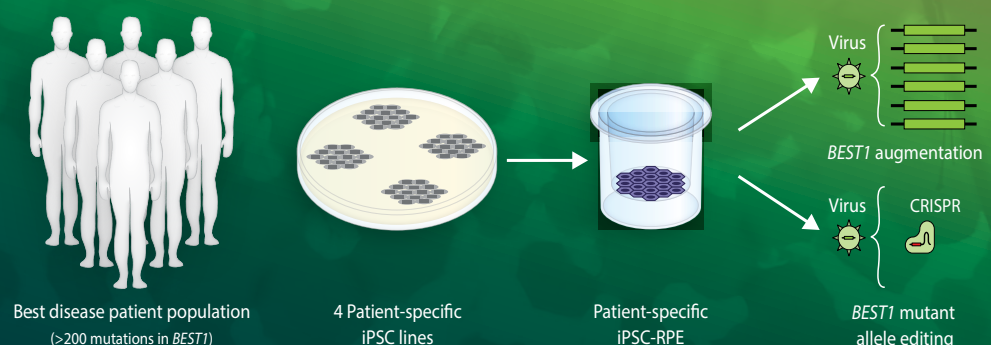
Developing a systematic approach to gene therapy for an inherited form of macular degeneration

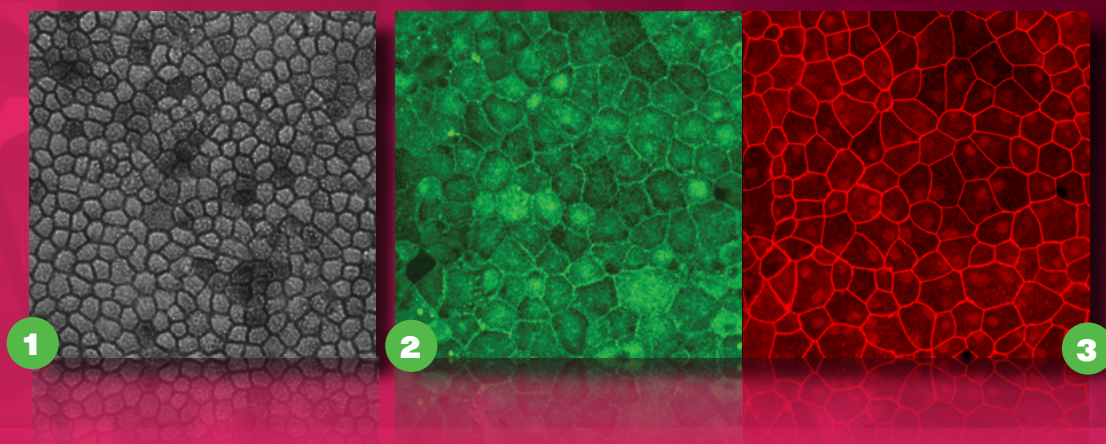
Best disease and gene therapies

Best vitelliform macular degeneration, or Best disease, is a currently untreatable blinding disorder that results in the loss of central vision in thousands of affected individuals. The disease is caused by one of several hundred small mutations in the *BEST1* gene, which codes for a protein that regulates the movement of chloride molecules across a vital layer of the retina called the retinal pigment epithelium (RPE). In a new and groundbreaking study just published in the *American Journal of Human Genetics*, a group of MERI researchers led by Divya Sinha, an Assistant Scientist in David Gamm's lab, and Ben Steyer, an MD-PhD student in Kris Saha's lab, demonstrated that a two-tiered gene therapy strategy may be able to treat all Best disease mutations in a highly effective manner.

Inherited retinal disorders (IRDs) such as Best disease, as well as the many forms of retinitis pigmentosa and Leber's congenital amaurosis (LCA), are good candidates for gene-based therapies as these disorders are caused by mutations in a single gene. Gene therapy is not a one-size-fits-all fix, though, as it must be customized for each gene and, sometimes, for each specific mutation in a particular gene. Thus, to treat all of the Best disease mutations, hundreds of therapies might need to be developed, and some might only be relevant for a handful of people worldwide. To better understand the challenges in developing gene therapies, it is important to understand the concept of dominant and recessive genes, so fasten your seat belts....

McPherson ERI researchers made induced pluripotent stem cells from multiple individuals with Best disease and directed those stem cells to produce RPE, the cell type affected by each of the *BEST1* gene mutations. They then tested the cells to determine what type of gene therapy—gene augmentation or gene editing—was best suited for that particular individual. In all cases, at least one of these two treatment strategies succeeded in eliminating the cellular defects caused by the gene mutation.





1 A microscopic image of a layer of retinal pigment epithelium (RPE) cells grown in a laboratory dish from induced pluripotent stem cells of an individual with Best disease (left panel). Two panels show fluorescent microscopic images of this person's RPE cells highlighting the distribution of *BEST1* protein (green) **2** and another protein (zonula occludens-1) involved in tightly connecting RPE cells together (red) **3**.

Dominant vs. recessive genetics

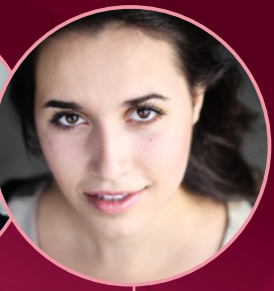
To understand this difference, you first need to remember that every gene in your body comes as a pair. Dominant IRDs are caused by mutations in only one of the two genes coding for a specific type of protein, which means that the other gene (and the protein it makes) is normal. Unfortunately, the “bad” protein (think of it as the evil twin) overpowers the good twin, causing disease. To fix a dominant IRD, the bad gene needs to be specifically identified and removed or fixed without inadvertently harming the good twin (and remember, as twins, they are very hard to distinguish). This requires the customized gene editing noted above.

Recessive IRDs, on the other hand, are caused by mutations in both genes. These mutations result in no functional protein being made from either one, so there are no twins at all. As a result, recessive IRDs are less picky in comparison; fixing them requires “only” a new source of the protein. Of course, none of this is truly easy, but the latter scenario involves a much more well-established process known as “gene augmentation.” It is also more cost-effective and faster to replace an entire gene than deal with the hundreds of individual mutations that can occur in a gene like *BEST1*.

To use another analogy, dominant “evil” mutations produce workers that actively look to sabotage the efforts of their capable coworkers, whereas recessive mutations produce proteins that never show up for work at all. As it turns out, the latter situation is usually simpler to treat than the former. But the vast majority of cases of Best disease are caused by dominant mutations, so are these people out of luck? The McPherson ERI team aimed for a better answer.

Rethinking the status quo

The McPherson ERI team hypothesized that it may be possible to adequately dilute the influence of the dysfunctional BEST twin by ignoring it entirely and adding many more good *BEST1* twins through gene augmentation. Testing this novel idea required the expertise and resources of four different labs:



- **In the Gamm Lab** (Dept. of Ophthalmology and Visual Sciences), Divya Sinha led the stem cell portion of the study. She used stem cell lines from four Best disease patients with four different *BEST1* mutations to generate, study, and treat their RPE cells in the laboratory. Using a specialized viral delivery system, Dr. Sinha performed gene augmentation by introducing a healthy copy of the *BEST1* gene into the RPE cells.
- **Once the Best disease patients' RPE cells** were treated with a boost of "normal" *BEST1* gene, their functional response was evaluated by Pawan Shahi in the Pattnaik lab (Dept. of Pediatrics). Shahi used electrophysiology to record the *BEST1* protein activity in the RPE cells and found that three of the four patients showed complete reversal of the cellular dysfunction.
- **What about the remaining patient** whose RPE function hadn't been restored? Ben Steyer in Kris Saha's lab (Dept. of Biomedical Engineering) was able to devise a way to rub out the bad twin without touching the good twin. This was done using CRISPR-Cas9 gene editing, a method that has garnered much attention but is just now being tested in humans. Steyer and colleagues found that the patient's RPE cells that failed to improve after gene replacement showed full reversal of the disease after gene editing.
- **One of the potential problems** associated with gene editing is the threat of randomly introducing unintended gene mutations into the patient's genetic code (so-called "off-target effects"). So how does one go about searching for unknown needles in giant genetic haystacks? Sushmita Roy (Dept. of Biostatistics and Medical Informatics) uses powerful computational methods to address this problem. Dr. Roy's lab, working with Katie Mueller (a PhD student in the Saha lab), found that all other genes besides the bad *BEST1* twin were essentially unaffected by the gene-editing process, a critical step in determining the safety of that therapeutic approach.

Together, these researchers succeeded in showing that a single-gene replacement strategy could be an effective treatment for a large subset of individuals with Best disease, and that gene editing could work for the rest, leaving no one behind. **From a broader perspective, they showed that gene augmentation could be a viable therapy for at least some dominant genetic diseases.** This revolutionary concept could greatly reduce the complexity and cost of developing gene therapies for dominant IRDs and shorten the timeline for clinical trials. And that's cause for optimism. "I'm very excited about these findings," Dr. Sinha noted. "This is what we work for."