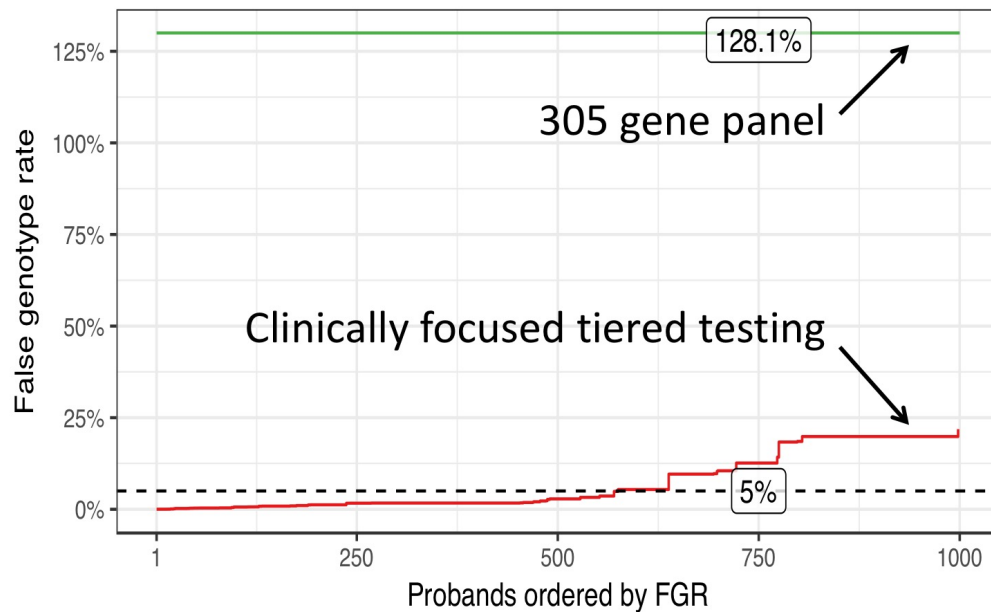


The False Genotype Rate



a tutorial from

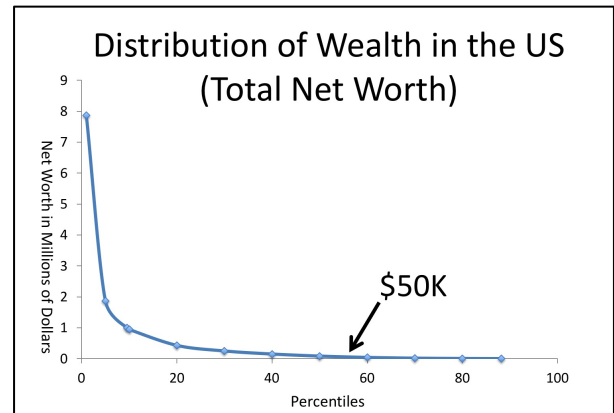
StoneRounds.org

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As you have seen in the other tutorials, there are dozens of single-gene disorders that affect the structure and function of the retina. The most common of them affects only one in ten thousand people while the rarest ones affect fewer than 50 people in the United States. We need to be able to treat all of these patients, regardless of how rare or how advanced their disease is. And if we are going to sustainably deliver these treatments to the tens of thousands of people who need them, we will need to be able to do it for less than \$50K per patient. This is because without some type of very significant governmental or actuarial wealth redistribution, the vast majority of people in the US will not be able to afford treatments at the \$850,000 price point that some commercial entities are proposing for their products.



To help design a strategy for treating everyone with one of these conditions, my lab studied 1000 consecutive families diagnosed with inherited retinal disease in my clinic at the University of Iowa. This study was published in the journal *Ophthalmology* in 2017.

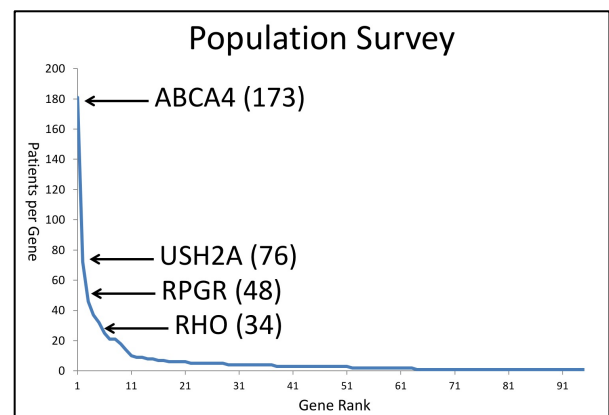
One of the objectives of this study was to find out how sensitive our genetic testing strategy was in 2017, and to determine how common each of the different clinical disorders are and the relative contribution of each gene to the total. However, the most important goal of the project was to devise a way to use clinical information to overcome the huge amount of noise in the genome. As part of the battle against this under-appreciated noise, we wanted to come up with a statistic that would make it more understandable to practicing clinicians. We call this statistic the false genotype rate.

We began by reviewing the literature and devising specific genetic tests for each of 62 different clinical categories based on the genes and mutations known to cause these diseases. These focused investigations included some special tests of non-exonic regions, mitochondrial DNA and repetitive regions that are not covered by whole exome sequencing. If this focused testing failed to identify the patient's genotype, whole exome sequencing was performed, and the analysis was tailored to the specific clinical entity.

Using this tiered approach, we identified mutations in 760 of the 1000 families. 576 of these genotypes – well over half -- were found with the focused test alone and 61 of these were mutations that would be missed by whole exome sequencing.

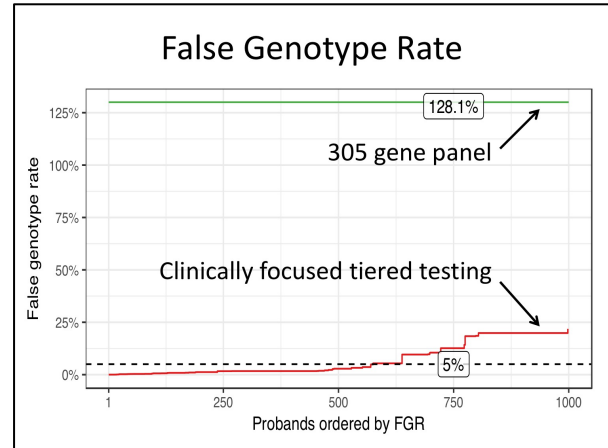
The average cost of the testing was \$980 per family despite the fact that some families had multiple tests performed on them. This is because many of the clinically focused tests are much less expensive than a whole exome sequence.

The mutations we found were distributed across 104 different genes. Some genes were much more common than others – *ABCA4* caused disease in 173 families while 91 of the genes each caused disease in fewer than 1% of the cohort.



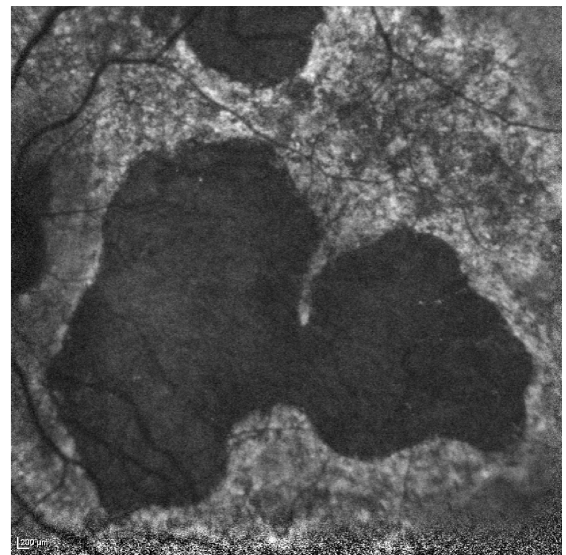
Most people are relatively unaware that every person's genome is riddled with variants that are rare enough and damaging enough to be a plausible explanation for their disease. The opportunity to observe one or more of these "false genotypes" by chance increases as the number of genes tested goes up. We used data from the Exome Aggregation Consortium to estimate the number of false genotypes one would observe using either a clinically-focused testing approach, or a 305 gene exome sequencing panel.

The 305 gene panel is over ten times more likely to yield one or more false genotypes than the clinically focused approach. More than half of the families in our 1000 family cohort were assigned to a clinical category whose focused test had a false genotype rate of less than 5% while the average false genotype rate for a 305 gene retinal disease panel is 128% -- meaning an average patient tested in this way will be found to have 1.28 false genotypes in addition to their true disease-causing genotype.



Here is a real life clinical example that will further illustrate the false genotype problem.

This 48 year old woman first had some atrophic macular lesions noticed on a routine eye exam at age 33. She is now 20/20 in the right eye and 20/60 in the left. She was diagnosed with diabetes at age 32 and started wearing hearing aids at age 46. Her fundus exam reveals a very blonde fundus with circular areas of RPE atrophy in the macula of both eyes, which is strikingly evident in the fundus autofluorescence images.



Next generation sequencing of 305 genes was performed and plausible disease-causing genotypes were identified in two retinitis pigmentosa genes (*RGR* Gly91Ser and *SPP2* Tyr179Cys). Both of these variants are very rare in normal individuals.

One of the weaknesses of whole exome sequencing is that it doesn't assess the mitochondrial genome. So, to get the correct molecular diagnosis for this lady, you have to know that bilateral atrophic macular lesions in a patient with diabetes and hearing loss suggests a mitochondrial condition caused by a specific point mutation at position 3243 of the mitochondrial genome. In this case, the 305 gene panel yielded only confusing false genotypes.

Note that this false genotype rate can only be reduced by narrowing the pre-test hypothesis – there is no technical improvement in the sequencing itself that can reduce this dangerous noise.

If you encounter multiple possibilities despite a narrow pre-test hypothesis, you can usually identify the correct one by examining and testing other family members.

A clinically focused strategy is more sensitive and less expensive than performing a very broad next generation panel in every patient. A focused strategy also results in a much smaller false genotype rate, which will be critically important as clinical trials of gene replacement therapy become more common.

Clinical expertise and the methodical examination of your patients' relatives are both very important factors in arriving at a correct molecular diagnosis and will remain so even as the cost of next generation sequencing continues to fall.