14 Signposts for Common Disease: Focusing on Macular Degeneration

The life of Henry Anatole Grunwald revolved around words. Grunwald left Nazi-occupied Vienna in 1938 as a teenager and made his way to New York by way of France, Morocco, and Portugal. He joined *Time* magazine as a copy boy and rose to become managing editor by the age of forty-five. He ran the magazine for the next nine years, and then moved on to become editor in chief of all Time Inc. publications for another eight years. During Grunwald's managing editorship, *Time* helped set society's agenda by adding regular sections to the magazine such as "The Sexes," "Behavior," and "The Environment," and by featuring controversial articles such as the famous 1966 cover story, "Is God Dead?". Editing was so engrained in Grunwald's blood that he usually read with a pencil poised to make revisions. He would correct any mistakes of grammar or usage he spotted, even those in published articles or books. No piece was safe from his editorial eye—once at a funeral he caught himself fixing a typo in a hymnal.

In 1973 *Time* was one of many national publications that helped solve a mystery then gripping the country, uncovering hidden clues, revealing suspicious connections, and setting puzzle pieces into place. This story involved no ordinary individuals—a break-in and its subsequent cover-up went to the highest level of the American government. As the Watergate saga played out, the case against Richard Nixon strengthened each day. Should *Time* take a stand and demand his resignation? The magazine had traditionally eschewed editorials, but this was no ordinary matter. Grunwald decided that an exception was warranted.

"How strange, I thought, that three decades ago I had arrived in this country as a young refugee, in whose eyes the figure of the president of the Unites States was, if not God-like, certainly exalted," Grunwald later

wrote in his autobiography. "And here I was now arguing for a president's resignation." After going through what seemed like endless drafts, the editorial appeared in the November 12, 1973, issue. Grunwald told America that Richard Nixon "has irredeemably lost his moral authority, the confidence of most of the country, and therefore his ability to govern effectively. . . . The wise and patriotic course is for Richard Nixon to resign." The editorial drew wide public attention and provoked twenty-five hundred letters, most of them critical. Even Clare Booth Luce, the widow of *Time's* founder, wrote to complain. Nine months later Nixon resigned.

Grunwald had the courage to confront the president of the United States. A few years later he would call on his courage to confront a debilitating disease that for him was particularly poignant—a disease that likely had its roots in his personal DNA code.

Chances are you don't worry about contracting phenylketonuria, cystic fibrosis, or Huntington's disease. Statistically there's little chance that there is a history of any of these in your family, and even though mutations in the cystic fibrosis gene are prevalent as mutations go, it's still the case that such diseases are rare. Most likely you don't worry about suddenly coming down with any of the thousand-plus other genetic diseases whose simple inheritance patterns we understand and whose underlying genes have been identified, because they are also rare, some of them affecting fewer than one in one hundred thousand newborns.

As you age, what *are* the diseases that most concern you? Probably they include heart disease, cancer, diabetes, Alzheimer's, Parkinson's, and stroke—one of which is likely eventually to kill you—and macular degeneration, an eye disorder that makes it difficult to see fine details. The condition affects the macula, the part of the retina responsible for central vision, and its prevalence increases steeply with age. Ultimately it robs many elderly of their sight. And what medical conditions are you concerned about for your children? Probably common disorders such as attention deficit disorder and learning disabilities, depression and dyslexia, autism and asthma.

Does your personal DNA code have anything to do with common diseases like these? Unquestionably it does. But unlike traits with simple (Mendelian) inheritance patterns, these common afflictions have a complex genetic basis, for several reasons.

First, the diseases themselves are heterogeneous, meaning that they take many forms. For example, there are "wet" and "dry" forms of macular degeneration. There are also many different types of cancer and heart disease. Unlike the case with the simple hereditary diseases such as PKU, cystic fibrosis, or Huntington's, which make their presence clearly known, it may be difficult to determine whether you even have one of the common ailments. For example, blood pressure and blood-sugar levels range along a continuum from too low to too high. Where on that continuum is the cut-off for saying one has diabetes or hypertension?

Second, several different genes contribute to your overall risk of these diseases, not just one. This feature of common diseases means that there will be no simple inheritance pattern that can be determined from a pedigree such that one out of two, or one out of four, children can expect to be affected when one of the parents carries a mutation.

Third, each susceptibility gene usually makes only a minor contribution to the condition, with the extent of these contributions differing from gene to gene, and from mutation to mutation within that gene. A DNA sequence variant in one gene could make you 2.5 times as likely to get diabetes, a variant in another could make you 1.6 times as likely, and a variant in a third could make you 2.1 times as likely. Hardly the all-or-nothing effect of the defective or normal *PAH* or *CFTR* or *HD* genes. When it comes to common diseases, many individuals may have the disease but not carry a particular variant of a contributing gene; many others will carry the variant of that gene but not show the symptoms of the disease.

Fourth, genes involved in the disease may interact with one another in ways that make certain combinations of variants much worse than if their effects were simply additive; other combinations may attenuate the disease risk.

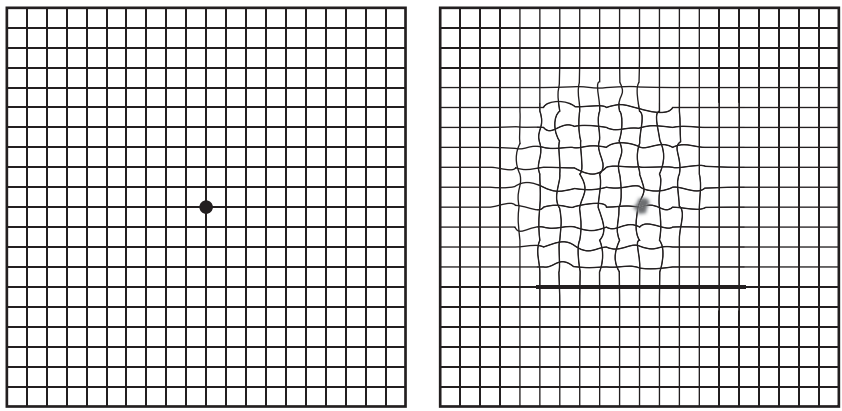
Finally, genes that contribute to complex diseases interact with the environment. For example, the effect of a variant that increases your risk of cancer may be hastened if you smoke. Conversely, the effect of a variant that increases your risk of heart disease may be diminished by a healthy diet and a cholesterol-lowering drug.

At the age of sixty-nine Grunwald had retired from *Time* and had completed a stint as ambassador to his native Austria. He was still vigorous and in the midst of writing his autobiography. While vacationing with his wife

in a villa outside Florence, he picked up a carafe to pour himself a glass of water but instead poured a puddle on the table, missing the glass entirely. Returning to New York to get his vision checked for what he thought would simply lead to a prescription for new glasses, he realized that he could see virtually nothing through his left eye. The diagnosis: age-related macular degeneration. The prognosis: a continuing but unpredictable decline in sight. Such progressive loss of sight is a disturbing and disheartening occurrence for anyone; for a man whose very identity depends on reading and writing, it can be devastating.

Macular degeneration, the most common cause of blindness in the United States, takes an enormous toll: nearly one in three Americans over the age of seventy-five are afflicted with it. The disease gradually destroys the central vision needed for seeing objects in fine detail. It affects the macula, a site in the center of the thin layer of tissue at the back of the eye called the retina, which is responsible for converting light into electrical signals that are relayed to the brain, where they are interpreted as images. The "dry" form of the disease—accounting for 85 percent of cases—occurs as cells in the macula break down, leading to blurred vision. But this form can progress to the more serious "wet" form, caused by abnormal blood vessels that leak, resulting in severe damage to the macula. The wet form, the kind Henry Grunwald suffered from, can progress rapidly and lead to significant vision loss.

To track the progress of macular degeneration, ophthalmologists have patients peer at a card with an Amsler Grid (see figure), a grid of lines like those on a sheet of graph paper but with a dot in the center. Vision loss

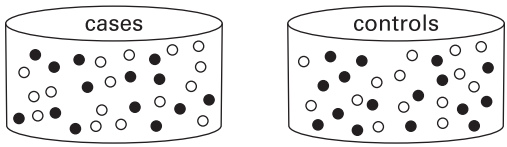


is indicated if the patient sees lines that look curvy or cannot see the dot. Grunwald described his experience with the disease in his book *Twilight: Losing Sight, Gaining Insight*: "For several months, the lines did not move, but then, just as I was becoming confident that my right eye was safe, the lines bent as if seen through heat waves. I rushed to my doctors, who spotted some bleeding in my right eye."

How do geneticists find genes that have only small effects on disease risk, but that are still critically important for our health? Family pedigrees generally won't help, because the likelihood that children will display the same disease patterns as their parents is low. The effects of the gene variants is simply too small for that approach to work.

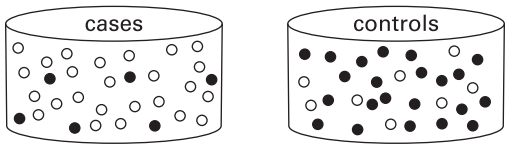
Instead of analyzing pedigrees, geneticists compare the personal DNA codes of two groups of unrelated individuals: those who clearly have the disease, called cases, a group in which DNA sequence variants that contribute to the disease are likely to be overrepresented, and individuals who clearly do not have the disease, called controls, a group in which these sequence variants are less likely to be found. Because these kinds of DNA sequence variants do not have an all-or-nothing effect, statistical tests are needed to reveal an association between particular DNA markers and a disease.

In the figure, the white balls and black balls represent two different DNA bases at one position in one DNA strand of a chromosome—say, white balls are A bases and black balls are G bases. If we examine thirty cases and controls for some disease and ask what base they have at this position, imagine we see the distribution shown here:



Because there are about the same number of black and white balls in each group, we conclude that the particular base variant that any individual has at this position is unrelated to whether or not he or she has the disease.

Now let's move along the genome to another position, and carry out the same analysis. In this position a white ball means a G and a black ball a T:



Here we see a strikingly different result: in the cases there are twenty-four white balls and six black ones; in the controls, there are eight white balls and twenty-two black ones. What these two distributions indicate is that someone with the disease is more likely than chance to have a G at this position; someone without the disease is more likely to have a T there. But note that the association is not absolute: some individuals have the disease even though they have a T (cases showing black balls), and some have a G and don't get the disease (controls showing white balls).

Robert Klein and Jürg Ott at Rockefeller University and their colleagues at Yale University School of Public Health and the National Eye Institute identified ninety-six people with cases of age-related macular degeneration and fifty control individuals with normal vision who were older than the cases (to increase the likelihood that they were truly free of the disease). Importantly, only individuals who identified themselves as "white, not of Hispanic origin" were chosen for the study, to reduce the chance that DNA differences between the groups due to different ancestries would be mistakenly linked to the risk of macular degeneration.

Then came the hard part. For both the cases and the controls, the researchers looked at 103,611 different positions in their personal DNA codes where they knew that most people have one of two or sometimes three bases on one of the strands of a chromosome—say, a G in some people and a C or sometimes a T in others. In more than fifteen million tests, they determined which base is at each of the 103,611 positions in each person, an experiment that only recently became possible with the determination of the complete sequence of the human genome, along with remarkable advances in gene-testing technology.

They posed the simple question: Is the identity of the base at any position in the genome associated with the disease state? Do people with

macular degeneration have a significantly higher likelihood of having a certain base in a certain position? Looking through that haystack of 103,611 positions in the genome, the researchers found the needle they were looking for: one position, which goes by the name rs380390, was "significantly" associated with the disease state, meaning that the likelihood of this association occurring by chance is less than one in two hundred.

When Klein and his coworkers looked closely at the consequence for the health of their subjects of this single variation in the six billion base-pairs that make up the personal DNA code, they found something remarkable: people with a C at that position in one of the strands of both of their chromosomes were 7.5 times more likely to have age-related macular degeneration than someone with a G at that position in the same strand of both chromosomes. Those who had inherited only one chromosome with a C at this position had 4.5 times the risk of getting macular degeneration. Based on how often the C occurs at that position in the population, the researchers estimated that about half of the risk of developing macular degeneration comes from having inherited DNA with that base at that single position! That C does not *cause* the disease but it is a marker—a signpost—that indicates that a disease-related gene is nearby.

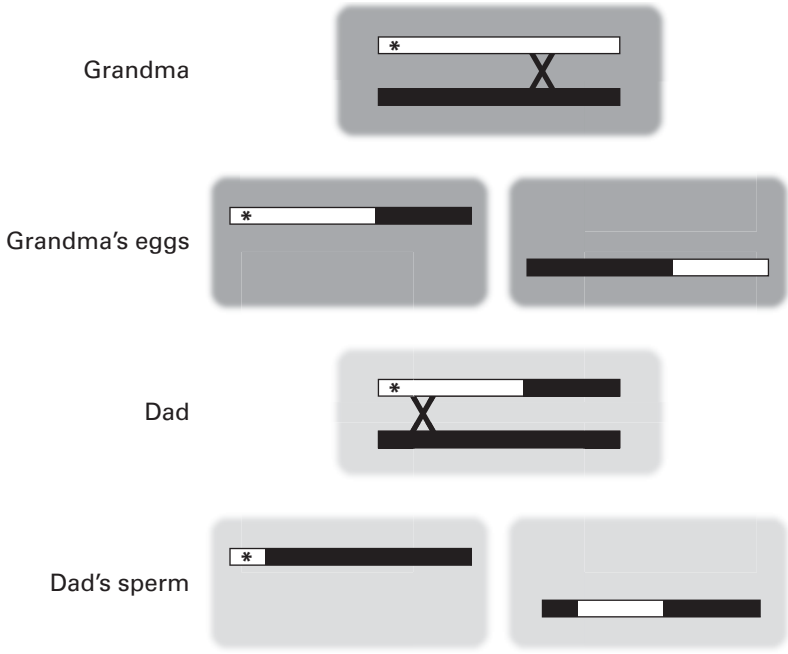
Grunwald went under the laser knife and emerged from the operation with stabilized vision, but five months later his sight again deteriorated. More laser treatments followed, but his macular degeneration relentlessly reduced his vision until he lived "in a half-veiled world" in which everything is seen "as if through a scrim." Grunwald eventually adapted to that world, but he chafed against its limitations. He had to resort to asking for help to cross streets, find airport gates, and press elevator buttons.

He purchased all of the various lenses and cameras and magnifiers that promised to enable some semblance of reading. In the James Bond movies, "Q" is the inventor who provides 007 with the latest fancy devices. Grunwald recalled a physician at the Lighthouse, an organization whose mission is to provide services for the blind and partially sighted, who was "Q" for the vision-impaired. The physician always had "something new to show you," proudly demonstrating to Grunwald his latest monoculars, binoculars, and extra-thick glasses. But even with such help, the erstwhile editor rued his inability to perceive subtle indicators of emotion in people's

expressions, to appreciate works of art, and simply to distinguish his food in a poorly lit restaurant. Arriving at a Chinese restaurant, he attempted to shake hands with a statue of a monkey that he mistook for the maître d', provoking his son to ask, "Who's your friend, Dad?"

Just as with pedigree studies, the key assumption of association studies is that an individual with a DNA sequence variant that contributes to his disease will have inherited not just that variant, but also DNA sequence variation in the chromosomal neighborhood. We assume that more or less everyone who has some DNA sequence variant that disposes them to get a disease inherited it from the same common ancestor who lived in the distant past. The variant in our DNA was passed down through the generations, and is now common to a large number of people, at least 1 percent of us.

Klein and his colleagues reasoned that with a pedigree, because we're looking at only a few generations, the amount of DNA reshuffling that has occurred is limited. In the figure, the white rectangle is a chromosome your paternal grandmother inherited from her mom, and the black one



represents the equivalent chromosome she inherited from her dad. Now, let's say Grandma had a variant in one of her genes, marked by an asterisk. This variant, because it's on the white chromosome, came from her mother.

When Grandma went through the process of generating her egg cells, a reshuffling event, as described in chapter 12, occurred on this chromosome at the location marked by the X. This event resulted in egg cells with chromosomes that are a mixture of Grandma's maternal and paternal DNA, as shown.

Your dad resulted from fertilization of the egg on the left, so he inherited from Grandma the chromosome whose left half is white and right half is black. He would have inherited not only the variant gene but also about half of that chromosome's worth of flanking DNA, about fifty million base-pairs, intact from his mother. From his father he inherited the all black chromosome.

When your father produced his sperm cells, another reshuffling event occurred, again designated by an X. If you resulted from the sperm cell on the left, you inherited from him the chromosome still containing that very same variant from his mother. Now, however, the variant is in the midst of a smaller block of Grandma's chromosome, shown in white, of about 20 million base-pairs.

Children and grandchildren tend to inherit from their parents and grandparents very large blocks of DNA, amounting to millions of contiguous base-pairs. But individuals plucked from the general population are much more distantly related than that, because there were many more generations back to their common ancestor, who, as we'll learn in chapter 18, lived in Africa about fifty thousand years ago.

Each of the approximately twenty-five hundred generations that gave rise to the people in Klein's study provided an opportunity for the chromosomes to reshuffle. And reshuffle they did. Promiscuously. The reshuffling hatchet fell with each generation, and each time had the chance to separate a base-pair that varied in the population from the base-pair that caused the disease.

Each reshuffling event reduced the size of the block of base-pairs that were co-inherited. But twenty-five hundred generations is actually not all that many—taking us about fifty thousand years into the past—so the blocks of DNA that are inherited together are long enough, usually thou-

sands of contiguous base-pairs, to include in one block both the rs380390 variant and the nearby mutation that disposes carriers to macular degeneration. This number of generations is too few to completely scramble the blocks of DNA sequence, and thus a block of co-inherited DNA—a segment in which the reshuffling hatchet never landed in those 2500 generations—remains surrounding each common DNA sequence variant. The figure shows the results of this process in this chromosome:

Although smaller and smaller segments of DNA flanking the variant (with the asterisk) are co-inherited in each generation, a block of thousands of base-pairs will remain intact, passing down to each subsequent generation the same DNA sequence variants in that region that were present in the ancestor in whom the potential macular degeneration–causing mutation arose long ago. The rs380390 DNA sequence variant, whose identity the researchers know, serves as a signpost of the disease-causing mutation because both of the affected base-pairs are in the same co-inherited block of sequence. The rs380390 DNA sequence variant serves as a surrogate for the mutation that actually contributes to the disease, indicating its presence and thereby warning of the risk of disease.

How do geneticists find such a DNA sequence variant that predicts disease risk? They use hundreds of thousands of signposts along the chromosomes that are the sites in the human genome where the base-pair sequence has been found to vary. They read what is written (A or T or G or C) in one strand on each of those signposts along all forty-six chromosomes in each of the people in the case and control groups. There are so many signposts that one is likely to lie within several thousand base-pairs of every disease-disposing mutation. An enormous set of data is produced for each individual—say, a G at base 5,268 of a certain strand of chromosome 1 in some individuals, a T at that location in others, and so on for hundreds of thousands of other locations—which is then statistically analyzed to see if any particular bases at any of the chromosomal positions appear more often than one would expect by chance in people with the disease. If one of them does, it is a signpost that signals the presence of a nearby mutation that increases the risk of disease.

Henry Grunwald lived with macular degeneration for more than twelve years, until his death of heart failure in February 2005, at the age of eighty-two. He confronted and mostly overcame the denial, the anger, and the depression that are typical of many people facing personal misfortune. But he occasionally hurled a magazine to the floor, "when, for the thousandth time, I realized that I could not read print without magnification, and I have cursed my various magnifiers as clumsy and inadequate." He turned increasingly to recorded books, to a computer that read back to him in a synthetic voice the pages he fed into a scanner, and to his wife, who read to him in a natural voice. To continue writing, he had to rely on dictation.

As his vision faded he took some comfort in mental pictures. Indeed, early in the disease he wrote, "I became a visual glutton, devouring the images around me in order somehow to hold on to them before they grew even dimmer. The faces of people I loved—my wife, my children, my grandchildren, many friends. . . . The Manhattan skyline in cold, pure autumn light. . . . Videos of favorite films. . . . Old photo albums illustrating my life. The red sunset over Vineyard Sound seen from my summer house. A Thanksgiving turkey ceremoniously displayed on a platter, a plate of pasta sprinkled with flakes of white truffles." And he came to realize that more than raging against his declining vision, he was raging against aging and his ever-nearing encounter with death. Geneticists will likely find DNA signposts for longevity, but a cure for aging will probably remain elusive.