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A Journey from Blood Cells to Genes and Back

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Abstract

I was attracted to hematology because by combining clinical findings with the use of a microscope and simple laboratory tests, one could often make a diagnosis. I was attracted to genetics when I learned about inherited blood disorders, at a time when we had only hints that somatic mutations were also important. It seemed clear that if we understood not only what genetic changes caused what diseases but also the mechanisms through which those genetic changes contribute to cause disease, we could improve management. Thus, I investigated many aspects of the glucose-6-phosphate dehydrogenase system, including cloning of the gene, and in the study of paroxysmal nocturnal hemoglobinuria (PNH), I found that it is a clonal disorder; subsequently, we were able to explain how a nonmalignant clone can expand, and I was involved in the first trial of PNH treatment by complement inhibition. I was fortunate to do clinical and research hematology in five countries; in all of them, I learned from mentors, from colleagues, and from patients.

INTRODUCTION

I started my training in medicine in 1953 at the University of Genova, Italy. It was a good medical school, especially in some areas, but genetics was not one of them.[1] Medical students were given a chance to do laboratory work as interns, subject to being selected by one of the professors. I managed to get into the Department of Biochemistry, headed by Arturo Bonsignore, whose research was on the enzymology of carbohydrate metabolism. I owe him a lot, because when I was not yet 20, he made me understand that research was about asking a question and designing an experiment that has a good chance to answer that question. At the same time, I noticed early on that in my medical school, the theoretical clinical teaching was good, but the bedside teaching was minimal—the clinical teachers were keeping medical students at arm's length. Therefore, I joined the nonacademic Division of Infectious Diseases, whose chief, Felice Geriola, was a highly experienced and dedicated clinician. I owe him a lot as well, because he made me understand that in order to confirm a diagnosis, you must gather evidence, and if you don't stick your neck out, you may not make any diagnosis at all. In the morning, I was in the hospital,[2] and in the afternoon and evening, I was in the biochemistry lab; combining clinical work and research work became a habit throughout my life.

For my medical degree, I had to write a short thesis. My project was about the action of insulin on the transport of sugars into white blood cells (85), which required fairly large amounts of blood, usually my own. At the same time, I was trying to learn as much as possible about what others were doing in the biochemistry lab, and in 1957 I was fortunate to witness a remarkable event in medical genetics. In Chicago, Carson et al. (28) had recently shown that people who developed acute hemolytic anemia when taking primaquine for the prophylaxis of malaria had a deficiency of glucose-6-phosphate dehydrogenase (G6PD) in their red cells. Bonsignore had taught us that G6PD is the first enzyme of the pentose phosphate pathway, and he was proud of his Beckmann UV spectrophotometer (model DU, which few labs had at the time)—the ideal instrument for measuring G6PD activity. Gennaro Sansone, an experienced pediatrician in Genova, suspected that primaquine-induced acute hemolytic anemia might be related to favism, known for decades as a life-threatening complication that occurs, mostly in children, after eating fava beans (148). Sansone brought with him his young colleague Giuseppe Segni,[3] as well as blood samples from two boys who had had favism; enzyme assays were carried out, and compared with control samples, the G6PD in the red cells of the two boys was all but undetectable. I still remember the excitement of the day when it was thus discovered that the genetic factor underlying favism was indeed G6PD deficiency (149).

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[1] In fact, genetics did not appear in the curriculum at all. The closest relative was a course by the strange name "Science of Orthogenesis"; in that course, I was taught that people, depending on their "constitution," could be short or tall and slim or fat, in various combinations, and that this depended mainly on the endocrine system. The person who taught that course had been a student of the endocrinologist Nicola Pende, whose command of genetics was questionable, to say the least—he had been one of the signatories of the Manifesto della Razza, which was used by Mussolini's government as the basis for the 1938 law on "purity of race" that overnight turned Italian Jews into outcasts.

[2] I also took turns on emergency duty. One night, I admitted a young girl, Rosanna, who had polio and was developing respiratory failure, and I had to use an iron lung machine that had just recently been installed in the Division of Infectious Diseases. Rosanna did not recover, but she lived in an iron lung for the next 28 years, during which she became a militant activist for the rights of people with disabilities, for which she became a prominent figure in Genova. Today, the square in front of San Martino Hospital bears her name.

[3] Giuseppe was originally from Sardinia, where favism is common; in 1962, his father, Antonio Segni, became the president of the Italian Republic.

POLYRIBOSOMES IN ERYTHROID CELLS (NEW YORK)

After specialty training in hematology at the University of Pavia, I was fortunate to become, on January 1, 1963, a clinical research fellow at Columbia–Presbyterian in New York under Paul Marks, who was then chief of hematology. My assignment was to investigate how ribosomes from rabbit reticulocytes made hemoglobin, and I realized that I had to give myself a crash course in molecular biology, of which I knew nothing, and of which at the time there were no textbooks. In the lab I seemed to do well, because within a few weeks, Paul—known as a demanding boss—was pleased with my results. On the educational front, I was reading avidly and aiming to benefit as much as I could from the high-level intellectual environment provided by both the medical school—whose staff included legendary figures such as David Shemin, David Rittenberg, Ruth and Reinhold Benesch, and Erwin Chargaff—and the basic biology departments on the 116th Street Columbia main campus.[4] I learned a lot from Paul Marks about planning hard experiments and rigorous vetting of data; a special bonus was weekly visits by Vernon Ingram,[5] who had discovered the molecular basis of sickle cell anemia (73). Later, I was fortunate to spend a few days in his lab at the Massachusetts Institute of Technology in Cambridge, Massachusetts, to learn from Luigi Bernini how to perform fingerprint analysis of proteins by trypsin digestion, followed by two-dimensional resolution of the resulting peptides.

On the clinical side, I was enthusiastic about working with the Presbyterian Hospital residents, who were first class. They were up to date with all the latest in clinical medicine, and I could tell them a bit about pathophysiology, but I had to do it almost behind Paul's back, because he wanted me in the lab 101% of the time. Some of my work was essentially a refinement of what was known already (96), but we also wished to provide direct proof in a mammalian system that ribosomes were workbenches ready to make any protein mRNA would dictate. We set out to do this by reprogramming guinea pig reticulocyte ribosomes with mRNA from rabbit reticulocytes, and we obtained radioactive rabbit hemoglobin, but the counts were so low that the results were reported only in preliminary form (95). In retrospect, our technique was simply not good enough to preserve the minute amount of mRNA from nucleases.[6] It took another few years for a group in Ohio to prove the point by using almost exactly the same approach (83).

BIOCHEMICAL GENETICS (IBADAN)

During my second year in New York, I had applied successfully for the position of lecturer in hematology at the University College Hospital in Ibadan, Nigeria, regarded as a premier institution in anglophone West Africa. My main motivation for this move was that, if there was a role at all for a young hematologist, the need was certainly greater in Nigeria than in Italy or the United States.

I arrived in Ibadan on October 1, 1964, on the fourth anniversary of Nigeria's independence. The clinical and laboratory work was heavy, but I was determined to pursue research as well. It was already known that G6PD deficiency was highly prevalent in Nigeria (64), and this seemed an interesting problem to tackle. Within the genetic polymorphism of G6PD, the wild type had (by definition) normal activity, and it was designated as B by electrophoretic mobility. A nondeficient variant common in Africa, with faster electrophoretic mobility, was therefore called A, and a

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[4]Suffice it to mention the presence of Theodosius Dobzhansky (originally from Ukraine), who had just published his book *Mankind Evolving* (42) and who became known to a wide public through his famous aphorism that "nothing in biology makes sense except in the light of evolution" (43).

[5]I cherish an autographed copy of Ingram's 1963 book *The Hemoglobins in Genetics and Evolution* (74).

[6]I was washing all of my glassware myself with chromosulfuric acid, then rinsing it three times in deionized water and three times in quartz-distilled water; only later did I realize that we should have used autoclaved glassware and sterile reagents. In those days, "nuclease-free water" was not in the suppliers' catalogs.

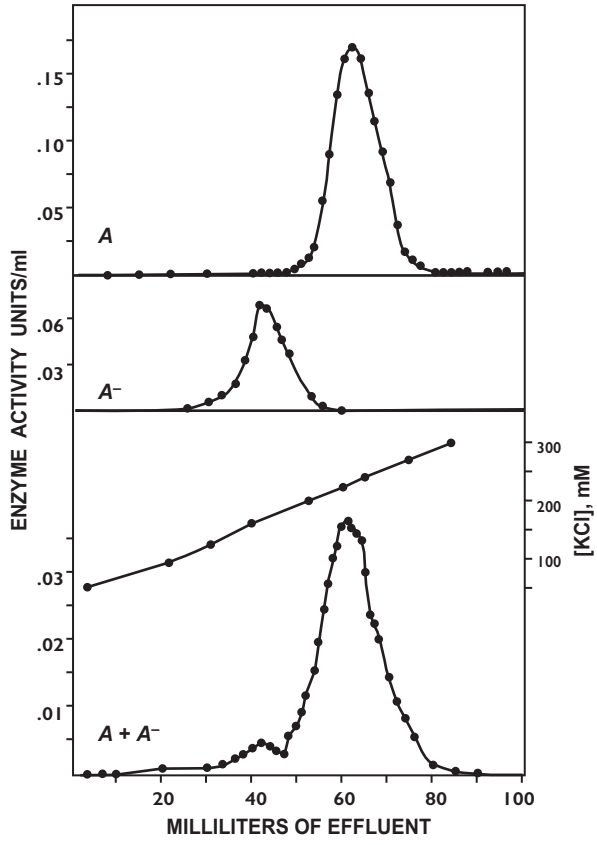

Figure 1

Resolution of G6PD A and G6PD A$^-$ by column chromatography on DEAE-Sephadex. This was the first evidence that G6PD deficiency was due to a structural change in the protein. Abbreviation: G6PD, glucose-6-phosphate dehydrogenase. Figure reproduced from Reference 91 with permission from Elsevier.

deficient polymorphic variant that had the same electrophoretic mobility was called A$^-$; by contrast, a polymorphic G6PD variant in the Mediterranean area had B-like electrophoretic mobility. To work out the relationships among these genetic variants, hemoglobinopathies were the obvious reference framework: Was G6PD A$^-$ a structural variant [like, for instance, hemoglobin S (Hb S)], or was it instead a G6PD A with a deficit in biosynthesis (like a thalassemia)? It proved easy to obtain partially purified preparations of the enzyme from blood units that had expired in the blood bank, of which I was in charge, and we were able to resolve G6PD A from G6PD A$^-$ by column chromatography (see **Figure 1**). This finding ruled out the thalassemia-like model: G6PD A$^-$ must be structurally different from G6PD A. We obtained further evidence from melting curves (91), a technique still used today (55), through which we showed that the thermal inactivation of G6PD A$^-$ was distinctly different from that of G6PD A.

Norman Allan was in charge of the hematology subdepartment within the Department of Pathology, headed by George Edington,[7] and my teaching assignments were mainly for

[7] *Pathology in the Tropics* (44), written by Edington and Herbert M. Gilles, was for many years and through two editions a very popular textbook. Norman Allan, born in Nigeria from Scottish parents, later was for years in charge of hematology at the Royal Infirmary in Edinburgh.

third-year medical students. At a faculty board meeting, I was approached by the professor of anatomy Adesanya Grillo, one of the first Nigerians to obtain a PhD in Cambridge, England; he deplored that there was no teaching of genetics and asked me to give some lectures on this subject to second-year medical students, within the anatomy course. I had no academic credentials in genetics (which he knew) and had never had any formal training in the subject (which he did not know), but I remembered reading in the preface to a major textbook that the best way to learn a subject was to teach about it, and I accepted the challenge. Of course, I had to work hard in the library, and I decided that, rather than starting from Mendel's laws, I would start from DNA and the Meselson–Stahl experiment on semiconservative replication, and then derive Mendel's laws therefrom. Within a few weeks, we also derived the Hardy–Weinberg equilibrium in population genetics, and we outlined the basics of biochemical genetics.[8] I will never be grateful enough to Professor Grillo for forcing me to learn genetics, at least enough to teach it.

The G6PD story was far from finished. G6PD deficiency was at the time regarded as a kind of honorary hemoglobinopathy, and hemoglobin remained my beacon. If Hb S had a single amino acid replacement, it seemed likely that the same might apply to G6PD variants; and if, by the technique I had learned in Ingram's lab, a good fingerprint could be obtained from 3 mg of hemoglobin, I was hoping the same might be true for G6PD, even though the molecule was certainly much larger. We had no funds in the Department of Hematology for research, but luckily, we had a visit from the chief of the World Health Organization (WHO) Human Genetics Unit, Dr. Italo Barrai. He seemed to appreciate that we had created in the blood bank a microresearch lab, where we had done electrophoretic typing of G6PD on hundreds of blood samples (92). When he was back in Geneva, Italo decided to establish in Ibadan a WHO Collaborating Center for G6PD, with an annual budget of about $3,000. This seed money[9] enabled us to generate results and several publications, on the strength of which we applied to the US National Institutes of Health for an R01 project. When, in 1972, we got this grant, I thought we were rich! I was able to offer positions and buy reagents, and even a Gilford recording spectrophotometer. After my first Nigerian graduate student, Adeyinka Afolayan (1), got his PhD, I was fortunate to further recruit Olaniyi Babalola, who was extremely hardworking and a perfectionist.[10] Together with a postdoc from Italy, Ranieri Cancedda, we managed to purify to near homogeneity both G6PD B (the normal G6PD) and G6PD A$^-$, but the yield of the latter, from 20 units (10 L) of expired blood, was barely 1.5 mg (11). We therefore attempted fingerprinting on thin-layer silica plates rather than on paper, but obtaining reliable amino acid composition from the minuscule spots proved hopeless. However, in collaboration with the hemoglobin biochemist John Beetlestone, we did carry out a comprehensive comparative analysis of the enzyme kinetics of G6PD B, A, and A$^-$, including the pH dependence of the K_m^{G6P} (a task never done before for any enzyme

[8]Our library at the University College Hospital had acquired what was at the time a classic by the British scientist Harry Harris: *Human Biochemical Genetics* (63). The medical section was run by an exceptionally able librarian, Margaret Amosu; we were neighbors on the campus, and her daughter, Akwe, was like another daughter to us.

[9]Half a century later—today—this would be impossible. Nowadays, the WHO has no funds with which to sow seeds for promising projects in developing countries; on the contrary, it has to do fundraising to support many of its own activities, the costs of which are not met by the annual sums that member countries are obligated to provide. I am confident that the WHO knows very well that they should never allow external funders to influence their policies, but one wonders whether it might be better if they preferred to confine their activities to what institutional funding permits. Surprisingly, in the era of genomics, the WHO no longer has a Human Genetics Unit, as they did before.

[10]Olaniyi sadly died in a boat accident and was awarded his PhD posthumously.

variant), and we did dare surmising in our discussion that there must be one amino acid difference between G6PD B and A and a second one between G6PD A and A$^-$ (10).[11]

BACTERIAL GENETICS AND MOLECULAR BIOLOGY (ST. LOUIS)

After three years in Ibadan, I took advantage of the generous study leave policy of the university and accepted an invitation from David Schlessinger, whom I had met since my time in New York, to do a short sabbatical in his lab in the Department of Microbiology at Washington University in St. Louis, Missouri. I was well aware that molecular biology had developed from the study of bacteria and bacteriophage, and I had read with alacrity a recent textbook on the subject,[12] but I was intimidated by the idea of working in yet a new area.

David was a most welcoming host. Both he and David Apirion were excellent teachers; they gave me a crash course in the basic techniques of microbiology and said that I was to investigate how exactly ribosomes were involved in the bactericidal action of streptomycin. Within a couple of weeks, it came home to me that obtaining ribosomes from an overnight culture of *Escherichia coli* was much more convenient than having to inject a rabbit with phenylhydrazine for five days to induce a reticulocytosis, obtaining blood by a cardiac puncture on day 8, and finally preparing a lysate from the reticulocytes. Also, doing genetics work in a haploid organism for which there was already a genetic map, and whose generation time was 30 minutes, seemed much easier than doing so in humans, whose generation time is about 25 years, whose genome is about 1,000 times larger, and in which at the time only a handful of genes had been mapped[13] (most of them on the X chromosome, because sex was an easy marker to link them to).

Luigi Gorini's group in Boston had discovered that streptomycin can cause misreading during mRNA translation, with consequent production of faulty protein (41). This finding had caused a lot of excitement, especially since at the same time the genetic code was being cracked. David shared the excitement, but he had reasons to doubt this was the molecular explanation of how streptomycin killed bacteria. The notion of the ribosome cycle—i.e., the assembly of the 50S and 30S ribosomal subunits with mRNA to form polyribosomes, followed by their dissociation once translation is completed, followed by reassembly with the same or with another mRNA species—owed much to David's work over those past few years (116). I learned how to pour agar plates and spread bacteria onto them, pick colonies and grow them, perform replica plating, and do phage-mediated gene transfer; we did dozens of experiments in intact cells and in cell-free systems, with dozens of sucrose gradients, and came to the conclusion that the critical action of streptomycin is to block the ribosome cycle at the initiation step of protein synthesis, when the 50S–30S assembly takes place (94).[14]

What I learned during my sabbatical in hard-core microbiology turned out to be most valuable only a few years later, when recombinant DNA technology came into its own, and David Schlessinger became a lifelong close friend. At Washington University there was continuous

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[11] Some 10 years later, it was established that G6PD A differs from G6PD B by a change from asparagine to aspartic acid in amino acid position 126; G6PD A$^-$ has the same difference from G6PD B, plus a replacement of methionine for valine in amino acid position 68 (70) (see **Figure 4** later in this article).

[12] *The Genetics of Bacteria and Their Viruses* (65), a book published in 1964 that was both authoritative and readable, had been written by the distinguished Irish microbiologist William Hayes.

[13] In Ogbomosho, Nigeria, we had located a man who had two abnormal hemoglobins (S and C), elliptocytosis, and an abnormal haptoglobin. We obtained blood samples from 43 members of his family, and we determined blood groups and several other genetic traits that we were able to test for. Our quixotic idea was to find linkage between at least two autosomal loci, but of course we got nothing.

[14] Google Scholar has alerted me that this paper was still cited eight times in 2019–2021, half a century after it was published.

intellectual stimulation, and a special bonus was to meet Rita Levi-Montalcini, who, thanks to her discovery of the nerve growth factor, was already a legendary figure; we were honored by her friendship long before she received her Nobel Prize in 1986.[15]

MALARIA SELECTION HAS PLAYED A ROLE IN SHAPING THE HUMAN GENOME

In malariology, geographic areas are classified as hypo-, meso-, hyper-, or holoendemic, and transmission may be seasonal or year-round; in Ibadan, *Plasmodium falciparum* was holoendemic year-round.[16] The most regular sign of malaria is of course fever, and on the clinical floors of the University College Hospital, I learned soon enough that when a patient had a fever, the question was not whether the cause was malaria, but whether it was only malaria or also something else on top. J.B.S. Haldane and Giuseppe Montalenti[17] had first formulated the hypothesis that malaria was the selective force that had caused the increase of hemoglobinopathy genes in certain populations (61, 124). Only a few years later, A.C. (Tony) Allison had provided direct evidence in the field, by studying heterozygotes for Hb S in East Africa (4), and both he (5) and Arno Motulsky (127) suggested that the same may be true for the *G6PD* alleles causing enzyme deficiency.

I was somewhat hesitant about venturing into the area of human evolution, but I was very fortunate to meet Tony when he visited Ibadan. We talked about balanced polymorphism. In the case of Hb S, it seemed clear that, of the three genotypes, A/A individuals have high mortality from malaria, A/S individuals are highly protected, and S/S individuals suffer from sickle cell anemia. I kept telling students that we had a dual obligation toward these patients, because not only did they have a serious disease, but they were also carrying the burden of a genetic trait that was helping the entire population to survive. I was unsure how this could work for an X-linked gene, since in males there are only two genotypes; to this, Tony responded, "But there are still three in females." I was keen on understanding not only equilibrium gene frequencies but also possible mechanisms of selective advantage, and I had only recently learned about Mary Lyon's groundbreaking work, from which she inferred X-chromosome inactivation in the mouse (111); Ernie Beutler had shown, using G6PD itself, that this applied to humans as well (16). Thus, it is true that in females there are always three genotypes—whether a locus is autosomal or X-linked—but there is also an important difference, because only in the latter case is there somatic cell mosaicism.

Prompted by Tony's remark, I suddenly realized that we were in a position to ask a simple question: When *P. falciparum* infects a female heterozygous for G6PD deficiency, would it prefer the G6PD-normal red cells or the G6PD-deficient red cells? I was fortunate to interest in this problem Essien Usanga,[18] the youngest among our excellent medical technologists at the blood bank. George Brewer had told me about a cytochemical test developed in his lab, based on methemoglobin elution, whereby the two types of red cells could be stained differentially (51). Within

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[15] Rita was a member of several academies, including the Pontifical Academy of Sciences. She later put me up for the Pius XI Medal, which I received from the hands of Pope Paul VI in 1976.

[16] Unfortunately, malaria was and is often lethal, especially in children, but adults living in a holoendemic area develop immunity. Since we had moved to Nigeria as adults, I and my wife, Paola, as well as our children, Stefano and Fatima, took antimalarial prophylaxis regularly, but even so, we all had one or two attacks of malaria. Thus I learned that *P. falciparum* malaria is an utterly unpleasant experience but, if promptly treated, hardly ever lethal.

[17] Montalenti, for many years a professor of genetics in Rome, was in Italy a founding father of *Drosophila* genetics and human genetics. In 2005, I was honored to speak in his former lecture theater when I received the Montalenti Medal.

[18] Essien subsequently worked for two years in my lab in London, obtained a PhD from the University of London, and became a senior lecturer at the University of Calabar, Nigeria, where I visited him in 2017.

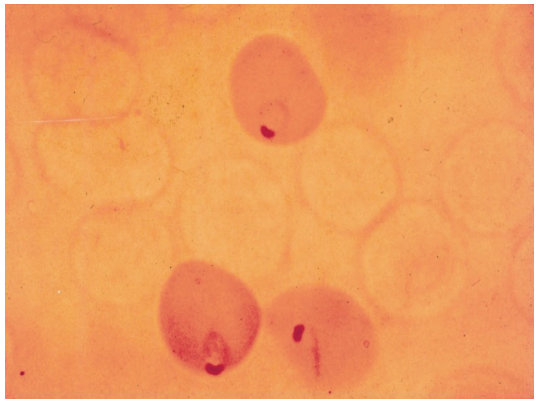

Figure 2

Red cell mosaicism in a young girl who had *Plasmodium falciparum* malaria and was heterozygous for G6PD deficiency. The blood smear was stained by a cytochemical method whereby G6PD-normal red cells retain hemoglobin, whereas in G6PD-deficient red cells hemoglobin is eluted. The parasite rate was consistently higher in G6PD-normal red cells. Abbreviation: G6PD, glucose-6-phosphate dehydrogenase. Figure reproduced with permission from Reference 98; copyright 2011 Blackwell Publishing Ltd.

months, *P. falciparum* gave us the answer: In every single case, we found more parasites in G6PD-normal red cells than in G6PD-deficient red cells (see **Figure 2**), and the statistics were good. Of course, there are many other genetic and immune factors that affect parasitization, but since the two cell types were in the same heterozygous mosaic girl, all those factors were the same, and therefore the experiment was internally controlled. We dared send a manuscript from Ibadan to *Science*, and it was promptly published (110).

We could not tell whether there were more parasites in G6PD-normal red cells because of increased invasion, more successful maturation, or decreased clearance by macrophages; we called the last suicidal infection, and many years later this was found to be the case (27). Our work did not by itself prove that heterozygotes had significant clinical protection. To this end, I was fortunate to collaborate with Adetokunbo Lucas[19] in the Department of Public Health. We set up a substantial field study of malaria in the rural area of Abeokuta, where a young German pediatrician, Ulrich (Uli) Bienzle,[20] collected quantitative data and determined the *G6PD* genotype of 700 children; it emerged that heterozygotes for G6PD deficiency with the A^{-}/B genotype had lower parasite counts than all other groups (17).

Some years later, I met Mary Lyon at a symposium in Venice. I was astounded by her unassuming narration of how, by using a natural science observational approach with domestic cats, followed by deliberate genetic work in mice, she came to formulate the X-chromosome inactivation hypothesis (111), which can be regarded perhaps as the most spectacular epigenetic event in the physiology of mammalian development. This symposium also provided an opportunity to get to know other distinguished geneticists, including Stanley Gartler, with whom I wrote a brief report of that meeting (100). Mosaicism resulting from X inactivation had been key to understanding G6PD and protection from lethal malaria, and I always remained intrigued by how it

[19]Professor Lucas was subsequently appointed director of the WHO Tropical Diseases Research Program, which he ran very successfully for over 10 years; indeed, in tropical medicine and epidemiology he became one of the most highly respected figures in the world. He died in 2020 in Ibadan on the eve of his 90th birthday.
[20]Bienzle later became director of the Nocht Institute of Tropical Medicine in Berlin, Germany, where I visited him.

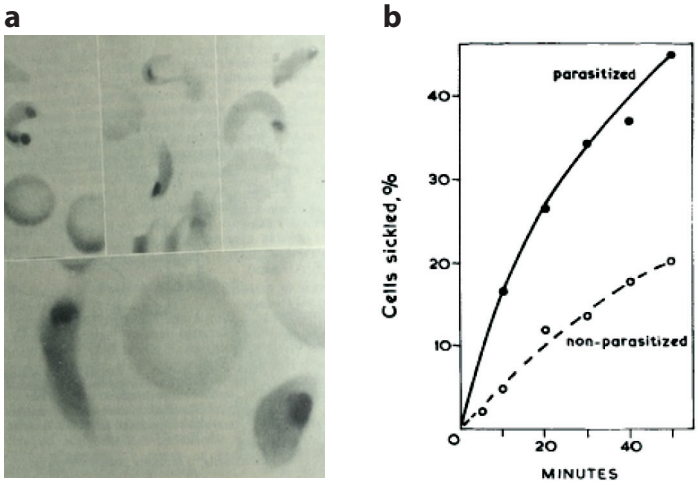

Figure 3

Rate of sickling of *Plasmodium falciparum*–infected red cells and of uninfected red cells in blood from an A/S heterozygote who had malaria. (*a*) Images of parasitized red cells at various stages of sickling. (*b*) Time course of sickling. Figure reproduced from Reference 105 with permission from Elsevier.

provides, in heterozygous females, a built-in potential for somatic cell selection (47, 109).[21] Understanding X inactivation at the molecular level started with the discovery by Andrea Ballabio[22] and colleagues (22) of the *XIST* gene, to date one of the best studied among the genes that generate a long noncoding RNA.

In the case of the HBB^S gene, selection of AS heterozygotes by malaria must balance the lethality of homozygotes, and therefore the selective action must be more powerful than in the case of G6PD deficiency. Although at the time the mechanism was not known, it was known that A/S individuals were not immune to infection; rather, they would not die from it. I thought that here too we could ask a simple question: Does infection accelerate sickling? It was easy in Ibadan to find A/S heterozygotes who had asymptomatic parasitemia, and when we exposed their blood to slow deoxygenation, we got the answer: The first red cells to sickle were those infected by *P. falciparum* (105) (see **Figure 3**). Since in vivo the spleen is a powerful filter for parasitized red cells (66), and presumably even more so if the parasitized red cells are sickled, our finding suggested that, once again, the mechanism of protection was suicidal infection. Indeed, it was proven later that in vitro parasite growth was not impaired in A/S cells (49), whereas removal by macrophages of parasitized A/S red cells, compared with that of parasitized A/A red cells, occurs at a much earlier stage in the parasite cycle (9). Since that time, many studies have confirmed that selection for the HBB^S gene

[21] I find it a bit embarrassing that several textbooks designate G6PD deficiency as X-linked recessive. X-linked is of course correct, but recessive is wrong, since in heterozygotes half of the cells (on average) are G6PD deficient, and at the clinical level, many cases of neonatal jaundice, favism, and drug-induced hemolytic anemia occur in heterozygotes.

[22] Andrea had started research work with Graziella Persico at the International Institute of Genetics and Biophysics when I was there. He subsequently became director of the Telethon Institute of Genetic Medicine in Naples and a leading figure in the biology of lysosomes. Research grants from Telethon, a major charitable agency promoting the study of genetic diseases under the able leadership of Francesca Pasinelli, became a sign of distinction in Italy because of their rigorous peer-review policy (consonant with the one we had previously enforced at the institute); its scientific board (on which I served when I was in London) consisted exclusively of non-Italians and Italians working abroad.

is ongoing (45) (see **Table 1**) and that heterozygotes are protected (23) not from infection but from lethality (2). Many factors play a role, including acquired immunity (172), but the primary mechanism remains that *P. falciparum* in an A/S red cell accelerates sickling and ultimately the disposal of parasitized sickled red cells (7, 90).[23]

CLONING THE *G6PD* GENE (NAPLES–LONDON)

In 1974, I moved from Ibadan to Naples, where I had been appointed director of the International Institute of Genetics and Biophysics (IIGB) to succeed, after an interim period, the distinguished Italian geneticist Adriano Buzzati-Traverso, who had founded the institute. Although my administrative duties were heavy, I did not give up on G6PD. In collaboration with colleagues in Nigeria and in Italy, we discovered new genetic variants of G6PD (156, 161), but I was still obsessed with working out the primary structure. When I discussed this with Graziella Persico, who joined my lab on her return from a stint at the National Institutes of Health, we agreed that, in the trail of the recent success by the Maniatis group with the globin genes (117), we must clone the human *G6PD* gene.

This was an ambitious goal at the time, but we had two assets: First, IIGB was one of the few places in Italy where we already had hard-core molecular biology based on molecular genetics of bacteria (58), phage (38), and *Drosophila* (142),[24] and second, Antonio De Flora, whom I knew from my time in Genova, had been able to produce an anti-G6PD antibody (125) and was kind enough to let us have an aliquot. Graziella got hold of and produced herself several cDNA libraries from HeLa cells and human placenta, and she worked out a highly demanding protocol whereby, after discarding clones from abundant RNA species (we knew G6PD could not be one of them), the remainder were tested, in pools of eight, for their ability to inhibit, by cDNA–RNA hybridization, the cell-free translation of human fibroblast RNA into a protein that, on a sodium dodecyl sulfate (SDS) gel, was in a position corresponding to 52 kDa, and precipitated by the anti-G6PD rabbit antibody. From one of the eight-some pools, two promising clones were identified, and one of them was found to be clearly X-linked, based on the signal intensity after Southern blotting on DNA samples from XY males, XX females, and XXX females (136).

The first cloning of a sequence related to a human enzyme had a significant echo in the Italian national press. Subsequently, when I was in London, with incisive contributions by Tom Vulliamy, Philip Mason, and others, we obtained the full cDNA sequence (137) and the structure of the genomic gene (118).[25] One could now identify point mutations in G6PD variants (93, 164) encountered in different parts of the world (see **Figures 4** and **5**).[26]

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[23] There have been several theories about malaria protection. It is true that our paper (105) has been cited only 153 times in 52 years, but there has never been a shred of evidence against it.

[24] The leader of the *Drosophila* group was at the time Edoardo Boncinelli (Dado to his friends), who made major contributions to the biology of *Hox* genes in mammalian developmental biology and pathology (see 19). He later became a highly successful science writer for the general public, with more than 100 books published on topics ranging from neurobiology to evolution to philosophy, in addition to publishing his own translations of classic Greek poetry.

[25] Graziella Persico became an internationally renowned scientist at IIGB, where, among other things, she discovered placental growth factor and an epidermal growth factor–related protein, encoded by *TDGF1*, that became known as cripto. Cripto is a membrane-bound signaling protein that plays an essential role in embryonic development and tumor growth, and mutations in this gene are associated with forebrain defects. Sadly, Graziella died in 2007 while still in her prime.

[26] For decades, G6PD variants were classified on the basis of biochemical and clinical criteria according to a scheme originally suggested by Yoshida et al. (171). As more data became available, some aspects of this classification have come into question, and for some time I have been urging the WHO to revise the classification, which has now been done (see 168).

Table 1 Population genetics of organisms and of somatic cells

Feature	Property	In a population of organisms		In a population of somatic cells	
		Consequence	Example(s)	Consequence	Example(s)
Genetic drift	Mutant is neutral (by definition)	Mutant gene frequency may become high when the founder population is small (bottleneck)	Inherited polycythemia due to a founder *VHL* mutation[a]	Mutant clone expands when there are only a few normal cells	Mutant clones in aplastic anemia
Selection	Mutant has a growth advantage	Mutant progeny will gradually take over	Fixation of species-specific genes	Mutant clone will expand and may become dominant	Leukemogenic mutations
	Mutant has a conditional growth advantage	Mutant progeny will increase in a particular environment	HBB^S gene in malaria-endemic area	Mutant clone will expand in a particular environment	*PIGA* mutant clone in the presence of GPI-specific T cells (PNH)
Convergent evolution	Mutation may involve different genes	Independently arisen mutant genes will increase in frequency	Several genes related to melanogenesis in tropical areas	Mutant clones with mutations in different genes yield similar disease	Clones with *JAK2*, *CALR*, and *MPL* mutations in myeloproliferative disorders
	Mutation involves the same gene	Mutant alleles will increase in frequency	Different *G6PD* mutant alleles in malaria-endemic areas[b] (see **Figure 5**)	Mutant clones with different mutant alleles may concurrently expand	Clones with different *PIGA* mutations that may coexist in PNH

Abbreviations: GPI, glycosylphosphatidylinositol; PNH, paroxysmal nocturnal hemoglobinuria. Table adapted with permission from Reference 108; copyright 2018 British Society for Haemotology and John Wiley & Sons Ltd.

[a]The first example has been found in the Turkic people in Chuvashia, Russia (150).

[b]This has been thought to apply also to the HBB^S gene in Africa, where its presence in the context of different haplotypes has been regarded as evidence of recurring independent mutations; however, Shriner & Rotimi (151) have obtained data more consistent with a single origin.

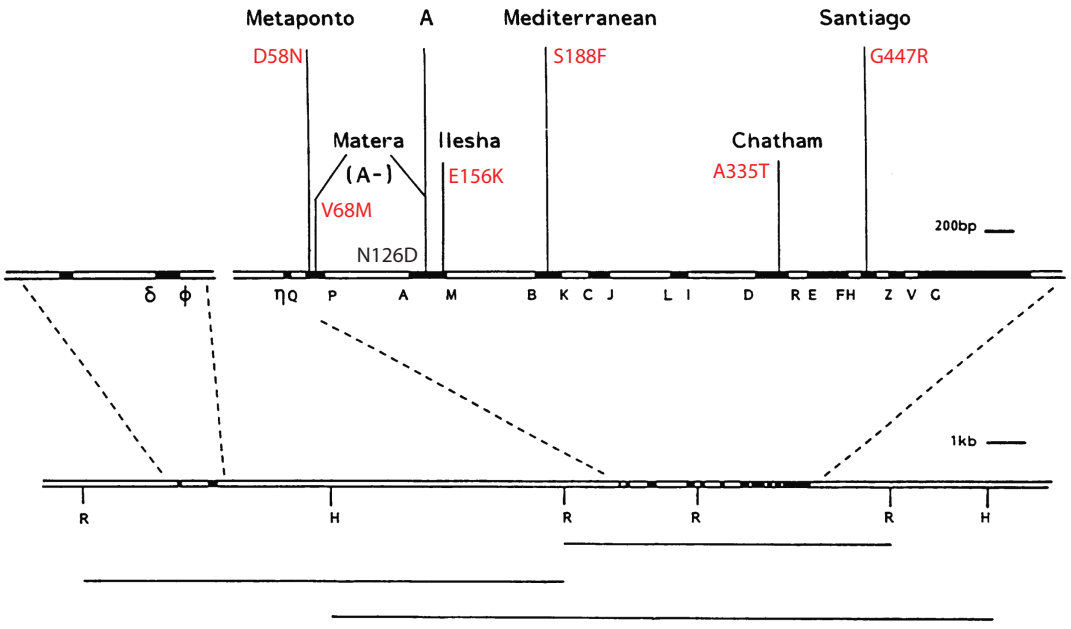

Figure 4

Examples of common and rare G6PD-deficient variants, which are caused mostly by missense point mutations. The three lines at the bottom represent the three genomic clones used to identify the mutations (in the pre-PCR era, we needed to prepare a phage library for each patient to be investigated). The line diagram above shows 26 kb of DNA on Xq28 encompassing the *G6PD* gene, with an expanded view above it (in two sections, because intron 2 is large). The six names above are those of the first set of *G6PD* variants for which the molecular basis was elucidated. G6PD Mediterranean and G6PD A^{-} (a sample of which had been found in Matera, Italy) turned out to be two of the polymorphic G6PD variants that are most widely distributed in the world. G6PD Chatham is also polymorphic; G6PD Ilesha and G6PD Santiago are very rare. Amino acid replacements in red are those responsible for G6PD deficiency. Abbreviation: G6PD, glucose-6-phosphate dehydrogenase. Figure adapted from Reference 164.

While some of the IIGB staff also had a university position, IIGB itself was a research institute that belonged to the Italian Consiglio Nazionale delle Ricerche (CNR). Accordingly, CNR appointed a scientific council that had the mandate to review and approve—or not approve—the research projects of the institute and monitor their progress. Funding decisions for each year took into account the assessment made by the scientific council, even though the amount was always less than requested. The scientific council held three statutory meetings each year and consisted of four internal members, plus myself as director, along with several external members representing universities and international bodies, including the WHO and the European Molecular Biology Organization (EMBO). EMBO nominated Sydney Brenner, who was promptly elected chairman.

I think that, coming from the famous Medical Research Council (MRC) Laboratory of Molecular Biology in Cambridge, Sydney initially had doubts about our laboratory, which, although it had rapidly acquired some notoriety, was still housed in prefab huts near the Naples football stadium, but he took his assignment most seriously, and his contribution proved tremendous. I was responsible for sending to him (as to all scientific council members) voluminous packets of research reports and proposals, and I formed the impression that, before boarding his flight in London, he had not even looked at them, but by the time the meeting started the next morning in Naples, he had clear ideas about what was important and what was not, and he had pointed questions for everybody. Sydney also came more than once to our house as a dinner guest; I was fortunate to get to know him rather well and to see him again at his apartment in Cambridge after

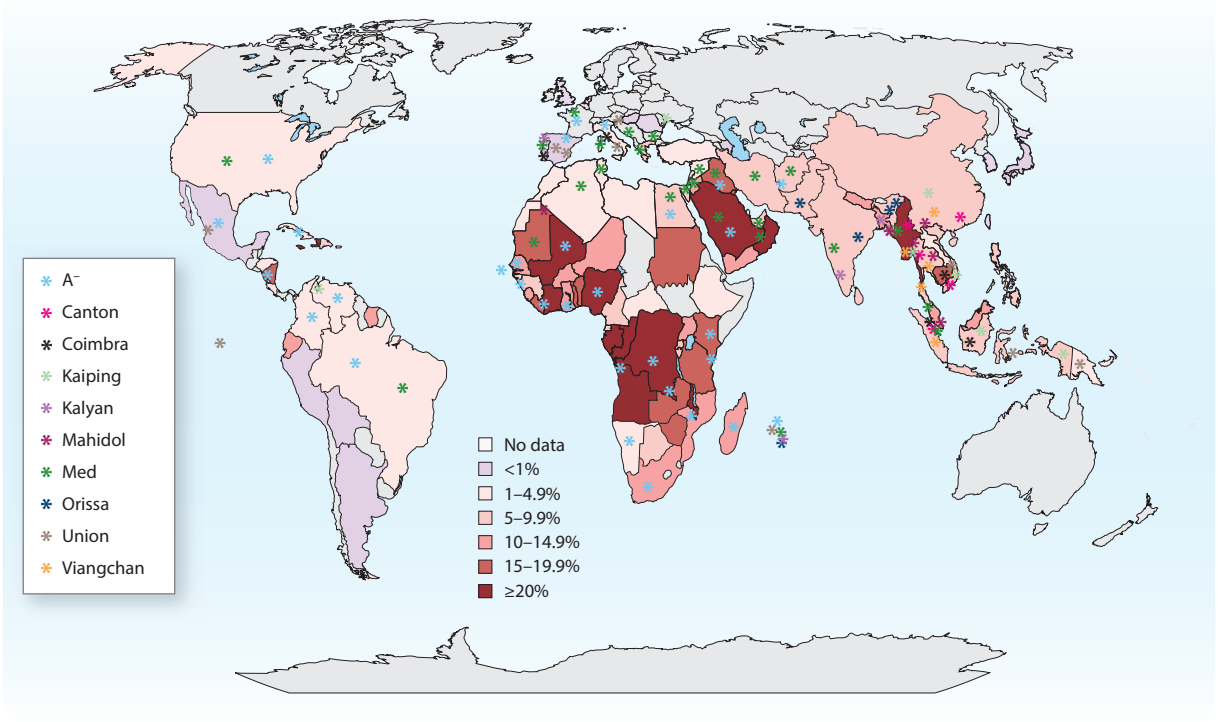

Figure 5

World map of G6PD deficiency. The population frequencies of polymorphic G6PD alleles that cause enzyme deficiency were gathered from the world literature. Each country was assigned a color based on the best estimate of the average frequency of G6PD deficiency. Colored asterisks indicate 10 of the most common polymorphic variants (as indicated in the key on the left), and some but not all of the countries where they have been found are indicated on the map. Abbreviation: G6PD, glucose-6-phosphate dehydrogenase. Figure adapted from Reference 93 with permission from Elsevier.

I had moved to London. While I tried to follow as much as possible his thoughts about biology in general, I had not imagined that I could ever interest Sydney in G6PD, but eventually he did find it useful in order to illustrate how the fish *Fugu rubripes* manages to have an atypically small genome. The amino acid sequence of G6PD is highly conserved in evolution (130), and the *G6PD* gene consists of 13 exons and 12 introns, one of which is very large (**Figure 4**); in *Fugu*, the genomic structure is the same, and the sequence of each exon is conserved, with intron 2 still being the largest, but all the introns are drastically reduced in size, as though the gene had been subjected to a concertina-like squeeze (119). On so many subjects, Sydney's conversation was interesting and often fun, but when discussing science, I would simply listen, and more than once I felt not just that he was leaps ahead, but like he was actually on another planet.

GETTING TO KNOW THE GURUS

For someone who had learned genetics by teaching medical students and had not belonged to any major genetics institution or academic enclave,[27] I was fortunate to get to know not only

[27]I admit I was elected to EMBO membership in 1978, to the Human Genome Organisation in 1985, to the American Academy of Arts and Sciences as a Foreign Member in 2004, and to the Accademia Nazionale dei Lincei as a Foreign Member in 2022.

Sydney Brenner—a holistic biologist—but also other highly distinguished geneticists. One of the first in my lifetime was Ruggero Ceppellini, who was a professor of genetics at the University of Turin and then at the University of Milan, in Italy, but much of his scientific work was based at the then famous Basel Institute for Immunology in Switzerland. Ruggero was often impatient in discussions with colleagues; he told me that this was because, compared to others, he was "tachypsychic" (his own Greek rendition of quick-witted), and a conversation with him was enough to keep me stimulated for a week. After working out a genetic model for both the ABO and the Rhesus blood group systems (subsequently validated by molecular analysis), Ruggero devoted himself to studying the human leukocyte antigen (HLA) loci, work for which he is rightly regarded as one of the founders of immunogenetics (18). Few people may remember that it was Ceppellini who coined the term haplotype (138),[28] now commonplace in genomics and essential in genome-wide association studies. I cherish the memory of when we were both involved in a short course in Oxford, only a few months before he died, in 1988.

As for Luigi Cavalli-Sforza (known by all as Luca), he was already an internationally renowned human geneticist when he wrote to me—an unknown entity—that on his way to the Central African Republic for fieldwork with the Babinga Pygmies, he was planning a detour through Ibadan so that we could meet. I was over the moon when he and his team visited; an autographed copy of his book *The Genetics of Human Populations* (31), written jointly with Walter Bodmer at about the time of his visit, remains for me a fundamental reference, and I was honored by his friendship since that first meeting. In an encyclopedic book written with Alberto Piazza and Paolo Menozzi (122), Luca and his colleagues introduced principal component analysis (extensively used since in many areas of biological research) in order to quantitatively describe the genetic structure of individual populations. This analysis showed that, based on the frequencies of alleles at multiple genetic loci, human populations are essentially a continuum, and therefore within the human species the term race is scientifically void. (The word race, unfortunately, is still often used in the medical literature; we have not yet won that battle.) Luca also wrote with Marc Feldman another innovative book exploring the relationship between biological evolution and cultural evolution (32).

Luca's scientific prowess was based on a strong mathematical background; in addition, before turning to human populations, he had made a name for himself in bacterial genetics through pioneering work that led to the isolation of a high frequency of recombination (*Hfr*) strain of *Escherichia coli*. This work had been carried out in part with Esther and Joshua Lederberg (29), and a few decades later, when I lived in New York, I was fortunate to be befriended by Joshua, who, though a declared agnostic, presided upon and invited us for a seder at his home near Rockefeller University. He told us that it was part of the Passover tradition to share the festive dinner with foreign visitors (a Thai colleague had also been invited). We later invited Joshua, with his second wife, Marguerite, to our little log cabin in New Jersey. He was in a talkative mood, and he confided that he felt, in a way, that he had had it too easy in life because, unlike other famous scientists, he had been awarded a Nobel Prize at the early age of 33; "from then on," he said, "it was a downhill ride." He also told us that the award ought to have been shared by Luca, and it was Luca who, when Joshua died in 2008, wrote a wonderful paper in his memory (30).

When I was in London, I also came to know Luca's coauthor and friend Walter Bodmer. He was one of the few born-and-bred geneticists who later became most prominent also as a cancer researcher (for many years, he was the director of the famous Imperial Cancer Research Fund

[28]The first paper with the word haplotype in it was "Genetica delle Immunoglobuline," published by Ceppellini in 1967 in *Atti Associazione Genetica Italiana* (34). The Associazione Genetica Italiana was a scientific society founded in 1954; while I was at IIGB in Naples, I had the honor of being elected its president for 1979–1980.

laboratory at Lincoln's Inn Fields in central London). Walter is not only a talented scientist but also an unusually gifted speaker, who can tailor his talk to any audience (I will never forget a Christmas lecture for the lay public he gave at the Royal Institution), and he supported the work of another renowned Italian geneticist, Marcello Siniscalco, with whom we were good friends for decades. In London I was also lucky to meet Guido Pontecorvo,[29] one of the founding fathers of somatic cell genetics. He was officially retired but still very active in providing mentorship to young people.

A much younger and (unlike me) card-carrying human geneticist in Italy was Guido Modiano. I learned a lot from him starting in 1974, when he came to help me as a teacher in Ibadan and in Enugu, Nigeria. We subsequently worked together in Nepal, where we found for the first time that in the Tharu people, malaria selection has not only balanced a polymorphism but has actually led to fixation of an α-thalassemia deletion mutation (155).

As for medical genetics, I wish to pay tribute to two great figures I have known well. Antonio Cao, a Sardinian pediatrician, devoted his professional life to thalassemia research; he and his team made prenatal diagnosis accessible to all, and as a result, the number of births in Sardinia with severe β-thalassemia plummeted from about 90 to about 5 per year (26).[30] Gianni Mastella, a pediatrician in Verona, devoted his professional life to cystic fibrosis;[31] he conducted and promoted basic and clinical research on this serious disease (147) for enough years to see the transition from the management of microbial complications to the introduction of ivacaftor (141), the first targeted drug for a subset of cystic fibrosis patients.

SICKLE CELL DISEASE

I never tire of reminding students that it was the discovery by Harvey Itano of a structural abnormality in the hemoglobin of patients with sickle cell disease (SCD)[32] that led Linus Pauling to coin the term molecular disease (135); thus, SCD is at the very source of the development of molecular medicine.[33] Since that time, the study of SCD has fostered our understanding of the allosteric properties of hemoglobin (24), the pathophysiology of hemolysis (14, 81), and malaria selection in human populations (4). Notably, unlike A/S heterozygotes, SCD patients are not protected from severe malaria (123); in fact, they are at high risk of dying from it (121). Within hematology, SCD is by far the most prevalent genetically determined anemia, with a worldwide distribution, although the majority of patients are in sub-Saharan Africa and India (139), which

[29] One of Guido's brothers, Gillo, was a famous film director, and another brother, Bruno, was a nuclear physicist (from Enrico Fermi's group) who "defected" to the Soviet Union. All three brothers had left Italy before World War II as a consequence of the anti-Jewish laws issued in 1938 by Mussolini's government.

[30] I may have helped in a small way for Antonio to undertake, with my friend David Schlessinger, a collaboration that turned into a most productive multifaceted investigation of the genetics of an island population (the SardiNIA program funded by the US National Institute on Aging, where David had moved). The program was most ably continued by David, with Francesco Cucca as principal investigator (see 40, among many other works).

[31] I served for several years, first as a member and then as chair, of the scientific advisory board of the Fondazione Fibrosi Cistica in Verona. In return, at the board's summer meetings, Gianni regularly took us to the opera at the famous Verona Arena.

[32] For a hematologist passionate about genetics, SCD has naturally been an important constant. My earliest paper on the subject was published in 1968 (92), and the latest was published in 2022 (123).

[33] As far as I know, the first Institute of Molecular Medicine—thus named from the start—was opened in 1991 by the MRC in Oxford. It has much to do with hemoglobin, because it was the brainchild of David Weatherall, who for 30 years had studied and greatly advanced the understanding of the thalassemia syndromes. David, by whose friendship I felt honored, was of course the first director of the institute; after he retired in 2001, the building was renamed the Weatherall Institute of Molecular Medicine, and his successors as head of the institute were first Andrew McMichael and then Douglas Higgs. I served for several years on the institute's scientific advisory board and attended David's memorial service after he died in 2018.

Table 2 An old protocol for gene therapy

Step	Description
1	Obtain gene with appropriate control regions
2	Make retroviral construct
3	Obtain bone marrow stem cells
4	Transfect stem cells in vitro and grow up in selective medium
4a	Replace defective gene by homologous recombination (a labor of love)[a]
5	Ablate remaining bone marrow
6	Autograft

Table adapted with permission from Reference 87; copyright 1992 Blackwell Publishing Ltd.
[a]This line reflects an addition I made during the INSERM Colloque held in 1991 at the Château de Montvillargenne (37). Today, the defective cell could be edited instead of being replaced.

is a major challenge with respect to clinical management (166) and public health (113). I have seen patients with SCD in Nigeria and Tanzania, in persons from the West Indies in London, in African Americans in New York, and in blue-eyed Sicilians in Italy. Thus, the spectrum of patients' cultural and socioeconomic background has been wide; yet they all have the same biallelic genotype, and the leitmotif of their clinical phenotype is the pain crisis.[34] For a large part of my hematologist's life I felt humbled by the fact that we were able to offer these patients only symptomatic treatment, and a blood transfusion when indicated. Certainly I was not the only one who perceived the striking contrast between how much we knew about SCD and how inadequate was our therapeutic armamentarium; ad hoc meetings were held to discuss this (see 101). The potential therapeutic approaches in a review paper I published in 1981 (86) included gene therapy, but at the time, that was no more than part of a wish list. Anyone who had touched on recombinant DNA technology since the 1970s must have thought of gene therapy; we were hopeful from the outset, but we were also well aware of the hurdles (see **Table 2**).

Martin J. Cline, a qualified hematologist who at the University of California, Los Angeles, had pioneered the study of hematopoietic growth factors (36), was certainly a fan of gene therapy—so much so that he was misled into thinking that hurdles could be ignored or sidestepped. He sought permission from his institutional review board to inject a pBR322 plasmid with a β-globin cDNA insert into the bone marrow of patients with β-thalassemia—even though he must have wondered himself about (*a*) how the plasmid would enter and become integrated into hematopoietic stem cells and (*b*) how gene expression would be promoted. Since the institutional review board said no, Cline enlisted the collaboration of two colleagues in Israel and in Italy; I know the details because one of them was in Naples, where one patient with thalassemia received the plasmid injection in her left femur, after the area had been irradiated "in order to make room for transduced stem cells" (as a daily newspaper put it the next day). The resonance in the Italian media was so great[35] that some of us were forced to declare publicly that we had nothing to do with this unauthorized

[34]A painful episode in a patient with SCD can be very severe, and it can be appropriately called a pain crisis. Although in most cases we do not have direct evidence that it is caused by vascular occlusion, this is a reasonable presumption, and therefore the more scientifically sounding term vaso-occlusive crisis is often used. The impact on the patient's quality of life of recurrent pain crises, and of the awareness that a new one may be impending, can be awful, such that some of us have referred to this as the paradox of sickle cell anemia: The anemia is always there, but the clinical picture is dominated by pain crises.
[35]The human experiment was also reported by the *Washington Post* on October 8, 1980, under the title "Doctor Tried Gene Therapy on 2 Humans" (77).

human experimentation. The patients had no benefit from the procedure, although fortunately, as far as I know, they did not come to any harm.[36]

A major limiting factor in improving management of SCD was that an animal model did not exist. We discussed this with Frank Grosveld, especially after his groundbreaking discovery of enhancer elements that he called the locus control region (57); by juxtaposing this region to the β-globin gene, he had been able to obtain high-level expression of the normal human hemoglobin in transgenic mice, and there was no reason why the same should not be the case with an Hb S construct. I must admit I became excited when I saw typical sickle cells in the blood of a mouse, and the presence of underlying deoxy-Hb S polymers was confirmed by electron microscopy (56). Unfortunately, our transgenic mouse was not able to reproduce; it was left for others to establish several sickle mouse models that have been most helpful in studying the pathophysiology of SCD (132, 146) and have become crucial in the preclinical trials that are now imperative for any potential new drug. Thus, it is gratifying that the 1981 wish list has become reality. We have hydroxyurea, which was initially found serendipitously to increase the level of Hb F and is now the best-established and most widely used SCD-ameliorating agent in all parts of the world, including Africa (159); we have voxelotor (163), which reduces deoxy-Hb S polymerization by shifting its O_2 dissociation curve; and we have crizanlizumab (8), which reduces the red cell–endothelial cell interaction that may be involved in initiating a vaso-occlusive crisis. As for a definitive cure, one can be effected by allogeneic bone marrow transplantation (162). In the meantime, obtaining appropriate gene expression was essentially solved by the discovery of the locus control region (57), whereas the issue of efficient gene transfer into stem cells required the development of retroviral and then lentiviral vectors (129). In fact, since what I have to call the cowboy attempt of 1980, it took 20 years to achieve gene therapy of β-thalassemia in mice (120), and even longer for gene therapy of hemoglobinopathies to prove successful in human patients (33) and become a clinical procedure (157) through the use of a lentiviral vector (20, 79) or CRISPR-Cas technology (48).[37]

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA AND SOMATIC CELL GENETICS (IBADAN–LONDON–NEW YORK)

I was offered the chair of hematology at the University of London's Royal Postgraduate Medical School (RPMS), at Hammersmith Hospital, where in 1981 I had the honor to succeed Sir John Dacie. It was not an easy move, because our children were teenagers, and the environment was very different from either Ibadan or Naples.

Hammersmith was a referral center for difficult clinical problems of almost any sort, including patients with paroxysmal nocturnal hemoglobinuria (PNH), who had been studied for years by Dacie with his colleague Mitch Lewis and with others. Dacie had manifested interest when, in Ibadan, we had first provided evidence, using G6PD as the best marker then available [as Philip Fialkow and colleagues (46) had recently shown], that PNH was a clonal disorder (see **Figure 6**) that must be caused by a somatic mutation (133), as Dacie had hypothesized (39). We had assumed the mutation was dominant; however, since it was discovered that blood cells from PNH patients were deficient in the complement decay-accelerating factor (now known as CD55) (82), increasing evidence had suggested that the defect in PNH cells might be failure in

[36]The two hematologists who persuaded their patients to undergo the experiment were—surprisingly—not sanctioned, and in fact received increased visibility; Martin Cline's scientific career, by contrast, was irretrievably compromised.

[37]The dire limitations to the use of many therapies in Africa, mostly for economic reasons, have been discussed elsewhere (103), and some corrective measures have been proposed (112).

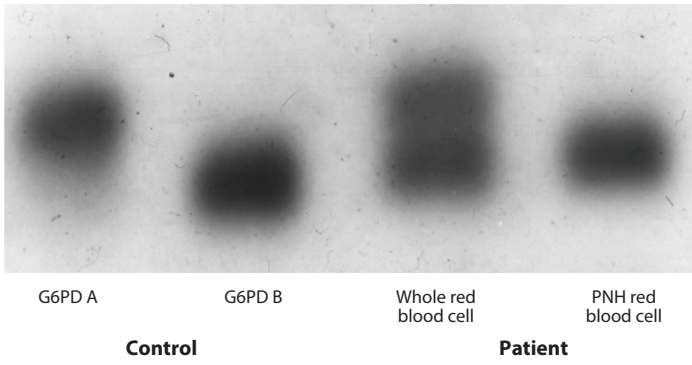

Figure 6

Clonal origin of abnormal red cells in PNH. In a patient with PNH, who was heterozygous (lane 3) for the electrophoretic variants G6PD A (lane 1) and G6PD B (lane 2), the red cells lysed in acidified serum—the PNH red cells (lane 4)—displayed only the B variant. These cells must have arisen from a single hematopoietic stem cell in which the X chromosome with the *G6PD* A allele was inactive. Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; PNH, paroxysmal nocturnal hemoglobinuria. Figure adapted from Reference 133 with permission from Elsevier.

the biosynthetic pathway of the glycosylphosphatidylinositol (GPI) molecule, the anchor to the cell membrane for many proteins; such defects had been already demonstrated in Thy-1$^-$ mouse lymphoma cell mutants (152). We felt it had become a high priority to obtain human material on which we could do biochemistry, and we established from PNH patients Epstein–Barr virus–transformed lymphoblastoid cell lines (68). In these cell lines, we were able to pinpoint a metabolic block in the GPI biosynthesis, at the early step of the UDP-GlcNAc-phosphatidylinositol-α-1,6-*N*-acetylglucosaminyl-transferase (69).[38] A loss of function of an enzyme would be expected to be recessive, not dominant, and we confirmed this by selection of PNH/normal somatic cell hybrids, in which the defect was corrected. If the mutation in PNH was recessive, it would need both alleles at the locus concerned to be somatically mutated in the PNH clone, and this seemed a very remote possibility; hence, we hypothesized and then proved that the locus involved must be on the X chromosome (67), whereby one mutation would be enough to make mutant cells deficient. Monica Bessler, a highly competent and hardworking Research Fellow who had joined our lab at the RPMS[39] after her training in Zurich,[40] set out to identify the PNH gene by transfecting our PNH cell lines with an expression library, and she had some preliminary results. However, we were stopped in our tracks when we learned that Taroh Kinoshita and colleagues (153), using the

[38]Having worked in some places that were more privileged and others that were less privileged, I am keenly aware of the enormous advantage for research in being surrounded by a variety of good science. At the RPMS, at the time, we were fortunate that, among many other distinguished scientists, Dorothy Crawford, an Epstein–Barr virus expert, was just one floor above us, and Winifred Watkins, an authority on blood cell surface glycoproteins, having retired, still in her prime, from the MRC Immunochemical Genetics division at Northwick Park, was now hosted in our department.

[39]We were located in the Commonwealth Building, and training of doctors from the entire commonwealth was part of the RPMS's original mission. Every year we ran a one-week course on advances in hematology, which was attended by juniors and seniors from all over the world, and we also ran a one-year MSc hematology course, which was attended by young trainees, many of whom are now professors of hematology in their respective countries.

[40]Monica Bessler subsequently became a highly successful physician–scientist, particularly in the area of congenital bone marrow failure syndromes, on the faculty of the Children's Hospital of Philadelphia.

same approach in Osaka, Japan, had elegantly scooped us when they identified the phosphatidylinositol glycan anchor biosynthesis class A (*PIGA*) gene.[41] The icing on the cake was that *PIGA* indeed mapped to the X chromosome, and somatic mutations of *PIGA* were found in nearly all PNH patients (15).

Even in otherwise sound literature, I have been surprised more than once in coming across the statement that Darwinian selection "drives evolution." It transpires from the writings of Darwin himself that, while he was unable to call allelic genes by their name, in his obsessive-compulsive study of variation he had the intuition that there must be innovative events (which we now know are mutations) that make evolution possible. What Darwin could not anticipate is how clearly the binomial mutation–selection operates not only in populations of organisms but also in populations of somatic cells; in fact, there is a close parallel between the genetics of one and of the other (see **Table 1**). In oncogenesis, the way selection works is often rather obvious, like when a *KRAS* gain-of-function mutation gives a cell a substantial growth advantage. In the case of PNH, it was not equally obvious how a mutation that disables red cells from withstanding activated complement was compatible with clonal expansion. But who more than Darwin had realized that selection was a function of the environment? For a cell population, that would be the tissue microenvironment, and in our case, it was the bone marrow. Work from the lab of Neal Young (173) had suggested that in aplastic anemia, hematopoietic stem cells were suffering, in the bone marrow, from a T cell–mediated autoimmune attack, and it was known that PNH may evolve from aplastic anemia. If the target of the attack was the GPI molecule, then only *PIGA* mutant cells would be spared, and the *PIGA* mutant clone would expand. With Bruno Rotoli, a wonderful colleague and friend for years, we outlined the notion of PNH evolving from aplastic anemia through a conditional growth advantage (97, 145) of the mutant clone (see **Figure 7**); this notion also became known as

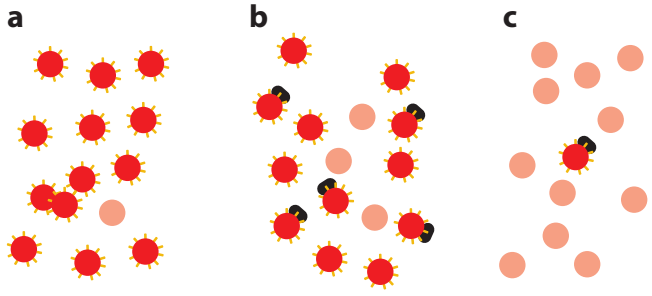

Figure 7

A model for the pathogenesis of PNH. The dark red circles represent normal hematopoietic stem cells, on which the yellow prongs represent GPI-linked surface molecules. The pink prongless circles represent stem cells in which a *PIGA* mutation prevents insertion in the membrane of any of the protein molecules that are normally GPI-linked. (*a*) In normal bone marrow, a rare *PIGA* mutant stem cell may exist. (*b*) Normal (nonmutant) stem cells are suffering damage from an autoimmune attack by CD1d-restricted T cells (*black brackets*) that targets GPI; PNH cells lacking GPI-linked molecules are not affected and may grow. At this stage, the patient may manifest signs of bone marrow failure, and aplastic anemia may be diagnosed. (*c*) As a result of targeted selection exerted by the autoimmune attack, the majority of blood cells are now from the *PIGA* mutant stem cell. The patient now has overt PNH but has been rescued from aplastic anemia. Abbreviations: GPI, glycosylphosphatidylinositol; PNH, paroxysmal nocturnal hemoglobinuria. Figure adapted from Reference 97 with permission from Elsevier.

[41] I am glad to say that Taroh and I became not only collaborators (see 15) but also friends, with mutual visits to our respective labs.

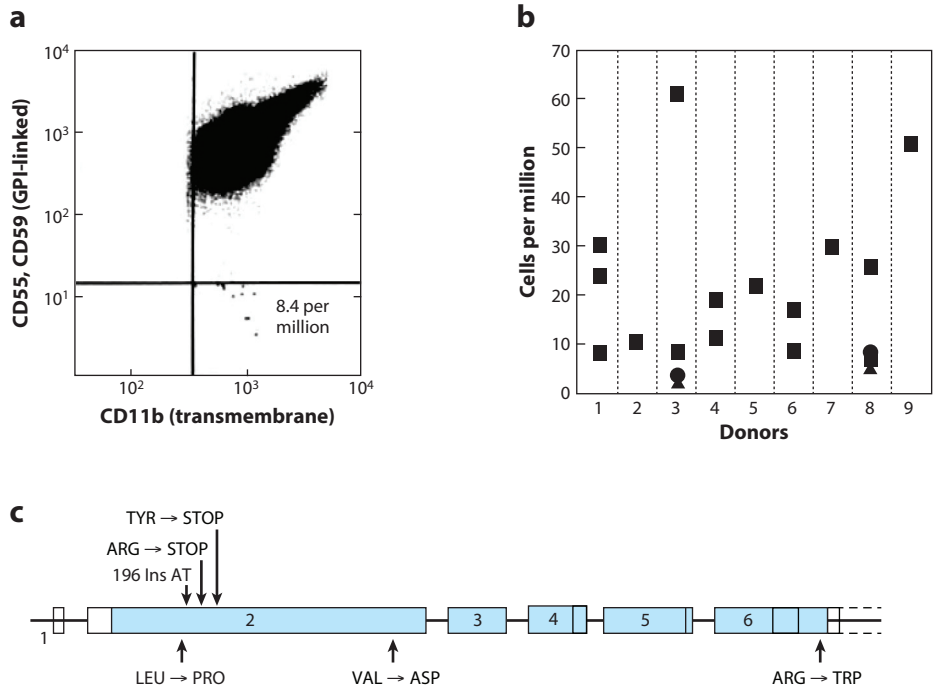

Figure 8

Presence of blood cells deficient in GPI (i.e., PNH-like blood cells) in all normal individuals. (*a*) Flow cytometry analysis of peripheral blood granulocytes (CD11b-positive), showing a clear distinction between the large majority that are normal and a small minority (about 8 per million) that are deficient in two GPI-linked surface proteins, i.e., that have the PNH phenotype. (*b*) PNH-like granulocytes found on repeated sampling in all of nine randomly chosen healthy individuals; these mutant granulocytes may derive from mutant hematopoietic stem cells or from mutant downstream myeloid progenitor cells. (*c*) Structure of the *PIGA* gene on Xp22, with inactivating mutations detected upon analysis of physically flow-sorted PNH-like granulocytes from normal individuals. Two of these mutations were identical to those previously observed in bona fide PNH patients. Abbreviations: GPI, glycosylphosphatidylinositol; PNH, paroxysmal nocturnal hemoglobinuria. Figure adapted from Reference 6; copyright 1999 National Academy of Sciences.

the escape mechanism (104), and it has been widely accepted, with (35) or without (12) reference to the original source. This model postulated the coexistence of two components in the causation of PNH: a *PIGA* mutation and GPI-specific T cells. Over the next few years, we provided evidence for both: *PIGA* mutations are seen in blood cells of all normal people (6) (see **Figure 8**), and as for T cells, Anastasios Karadimitris[42] and colleagues provided the first evidence that T cell receptor distributions were skewed (80), and Luciana Gargiulo and colleagues found GPI-specific CD1d-restricted T cells in PNH patients (52) as well as in a majority of patients with aplastic anemia (53).

CANCER GENETICS (NEW YORK AND TUSCANY)

My former chief at Columbia University, Paul Marks, had become in 1980 the president of the Memorial Sloan Kettering Cancer Center (MSKCC) in New York, and over more than a decade

[42] Anastasios (Tassos) Karadimitris was the last clinical registrar I was fortunate to recruit in London, and he was then a research fellow in my lab in New York. Now, back in London, he is a professor of hematology at Imperial College London (Hammersmith Hospital).

he was able to greatly strengthen basic science in that institution, to match its clinical excellence. Although it was not trivial to leave London after 13 years, in 1994 I accepted Paul's specific mandate to set up de novo and to run a human genetics department in a major cancer center. At the time, the hunt for genes that increase the risk of breast cancer, pioneered by Mary-Claire King (62), had led to the identification of *BRCA1* (50) and *BRCA2* (169), as well as of other genes predisposing to cancer. The principles and practice of genetic counseling for cancer susceptibility were being developed, and Joan Marks was the director, at Sarah Lawrence College, of one of the first training programs for genetic counselors. At MSKCC itself, Raju Chaganti and Suresh Jhanwar had perfected cytogenetic techniques, including chromosome-specific fluorescent staining (72), and Ken Offit was very active in cancer genetics (131).

I was certainly not an oncologist, but for some years I had directed the MRC Leukaemia Unit at the RPMS, and Terry Rabbitts, Letizia Foroni, and I had been among the first to use immunoglobulin and T cell receptor rearrangements to identify and monitor lymphoid malignancies (99, 140). At any rate, I did a lot of studying, and once again I found the subject very interesting. I also realized that, as chair of a new department, my own research was taken for granted; I would be judged by the caliber of the people I was able to recruit. I had immediately offered a faculty position to Pier Paolo Pandolfi, who had already worked with me in London (84); within a few years, with his extraordinary ability to painstakingly construct mouse models of human cancer (165), he became a star. So did Michel Sadelain, whom I recruited with the aim to develop gene therapy; he is now an acknowledged leader in the field (21).[43] I also promoted the creation of the New York Human Genetics Club (modeled on the Red Cell Club that I had run in the UK). With the benefit of having so many research institutions active in genetics (Columbia, Mount Sinai, Albert Einstein, New York University, Cornell–MSKCC, the New York Blood Center, and others) within subway reach, we were able to meet twice a year for half a day of short primary papers, usually presented by postdocs and PhD students.

We also arranged, at MSKCC itself, short courses in human cancer genetics, at a time when it was becoming clear that understanding inherited predisposition to cancer and identifying the somatic mutations that drive tumor growth were the two comparably important components of research in oncogenesis. In this respect, I found it difficult to improve on the model of cancer development that had been put forward since 1975 by John Cairns (25) (see **Figure 9**). Because of my work on PNH, I felt at home with somatic mutations (89), and thanks to my son, a mathematician doing research in dynamic systems, I knew a bit about stochastic phenomena (107). We have provided evidence that the somatic mutation rate has a log-normal distribution in the normal population, just like many other quantitative traits (**Figure 10**).

I had applied for my first academic job in Ibadan, but I was lucky that from then on I was invited to jobs. In 2001 I left MSKCC, and in 2004, after a short time in Genova, at the time when I should have retired, I was confronted with a proposal that was a real challenge. In Italy, where a National Health Service system has been in operation since 1978, the responsibility of running the service belongs to individual regions, of which there are 20. I was approached by the *assessore*

[43] In 1996, both Michel and I attended in Minneapolis the first meeting of the American Society of Gene Therapy [ASGT, now the American Society of Gene and Cell Therapy (ASGCT)], founded by George Stamatoyannopoulos. Stam had the charisma needed to start and lead a new society in a rapidly developing area; he was well adapted to Seattle, Washington, but the books in his home library were largely in Greek. He asked me to serve on the ASGT Ethics Committee, which I chaired for four years. The duties associated with that role gave me glimpses into the complex relationship between research and business and into researchers turned businesspeople.

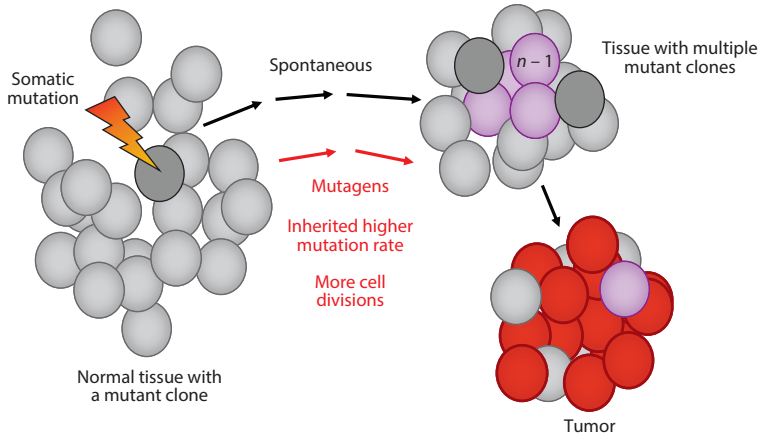

Figure 9

How somatic mutations and Darwinian selection can produce cancer, illustrating the model originally formulated by Cairns (25). Somatic mutations produce mosaicism in all tissues; most mutations are neutral, but those that increase growth rate or chance of survival will favor clonal expansion, and clones with specific sets of mutations can become a tumor. Most somatic mutations are spontaneous; however, several inherited or environmental factors can increase the absolute number of mutations, and thus favor/accelerate oncogenesis. Figure adapted from Reference 89 (CC BY 2.0).

(regional minister) for health of the Tuscany region, Enrico Rossi,[44] about how best to organize cancer services; in this respect, he regarded my experience at MSKCC as a valid credential. An institution that combines clinical care of cancer patients with basic and clinical research had long

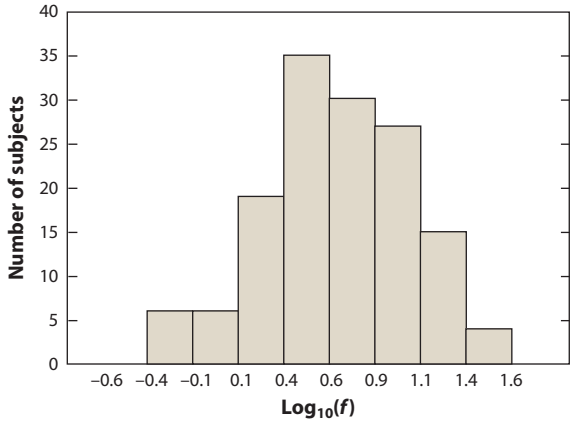

Figure 10

The intrinsic somatic mutation rate has a log-normal distribution in a population of healthy persons. Figure adapted from Reference 143 (CC BY 4.0).

[44]Enrico Rossi had already joined the Partito Comunista Italiano as a student at the University of Pisa, where he read philosophy; at a very young age, he became the mayor of Pontedera, an industrial town where Piaggio manufactured the Vespa (the motor scooter that became an icon of the Italian postwar economic boom) and where today one can visit the Vespa Museum. Rossi subsequently became the president of the Tuscany region (2010–2020).

been regarded as an ideal model not only in the United States but also in Europe. The Royal Marsden Free Cancer Hospital in London had pioneered that model in 1851, and the Istituto Nazionale Tumori had been founded in Milan in 1925; having a similar institution, a self-standing cancer center, had become an ambition—in some cases almost an obsession—in several regions in Italy. Enrico Rossi was very dedicated to his role and to the ideal of providing quality care to all citizens, and with respect to cancer services, he was facing a political–academic challenge. There were no fewer than three major universities in Tuscany: the University of Siena (founded in 1240); the University of Florence (founded in 1321), in the region's capital city; and the University of Pisa (founded in 1343). Needless to say, if a cancer institute was to be created, each of the three would claim title to it.

Over several months, we discussed several alternatives between us and with experts from elsewhere in Italy, and outside Italy. So-called cancer networks were becoming fashionable, but most of them were exercises in paperwork rather than reality. The challenge was to reconcile the fact that foci of excellence for individual tumors existed in different sites with our objective—namely, to make optimal care available to all those who had cancer, in a population of some 4 million people. We agreed that an institute was better than a network; in the digital era, an institute does not need to be all in one physical place, and I agreed to be the scientific director of the Istituto Toscano Tumori (ITT).

This was the beginning of a very busy decade (2005–2015). I came to know in person all the oncology activities in a dozen hospitals, we set up multicenter disease management teams, and—in pre-COVID times—we had weekly ITT-wide case discussions online. As part of my conditions for accepting the job, I was able to recruit young people from Cambridge, Geneva, and elsewhere[45] and offer them a reasonable startup package to work in the ITT Core Research Laboratory, which was housed in a new building that had been constructed ad hoc and headed by Rosario Notaro, who had already been an invaluable collaborator in New York. At the same time, small ITT grants helped to support research projects throughout ITT.[46] We had a rigorous peer review system in place, supported by an international scientific council, with Sydney Brenner as honorary chair.[47]

We also took part, on behalf of the Italian Ministry of Health, in the European Union Joint Action on Cancer (2014–2017), which had as one of its specific aims to develop comprehensive cancer care networks,[48] for which ITT served as a model. A network was set up in the South Moravia and Vysocina regions of the Czech Republic; the coordinator was Ladislav Dušek of the Masaryk Memorial Cancer Institute in Brno, where he organized a meeting to which members of the Joint Action on Cancer were invited. The meeting went well; for me, an extra bonus was the venue. I deserted one of the sessions in order to visit the Gregor Johann Mendel Museum. From a historical point of view, it remains puzzling why Mendel and Darwin, though contemporaries, did not interact, but for me, just reading some of the original records and walking in the charming garden of the monastery was an unforgettable experience that I recalled with elation on July 20, 2022, the day of Mendel's 200th birthday (115).

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[45]Rosario Notaro, Mario Chiariello, Silvo Conticello, Laura Poliseno, and Barbara Stecca are now well-established cancer scientists.

[46]These activities were documented in successive triennial reports (75, 76). While at ITT, I also wrote a book for the general public, *Capire il Cancro* (88), which was published by Rizzoli in 2006 and again in paperback in 2009.

[47]At the International Conference on Cancer Genotypes and Cancer Phenotypes, held by ITT in Florence on July 4 and 5, 2008, Sydney gave a riveting talk on somatic and germline genetic diseases.

[48]A report on the European Union Joint Action on Cancer, *European Guide on Quality Improvement in Comprehensive Cancer Control* (3), was published in 2017.

BACK IN THE CRADLE OF HUMANKIND (DAR-ES-SALAAM)

After a decade in Florence, I was proud of the multicenter ITT, and particularly of its Core Research Laboratory; now I wished to return to the continent from which the human species evolved. I had been at the Muhimbili Hospital in Dar-es-Salaam as a visiting faculty member for a few weeks in 1974, and again in 2009, upon request of the UK Wellcome Trust, on a site visit of the research activities they were supporting in Kenya and Tanzania. I had been particularly impressed by the Sickle Cell Program, headed by Julie Makani at Muhimbili, and I moved there in November 2015. I feel fortunate that, toward the end of my career, I could work with a person, Julie, who has a single-minded dedication to improve in every possible way the lot of patients with SCD in Africa (167).

My job was again in hematology, and one of my main assignments was to take part in the training of an excellent group of residents and fellow; thinking back to when I started in Ibadan, I was gratified by being able to perceive the progress in specialized medical education in Africa over the past two generations. As for genetics, highly significant developments have been the founding in 2003 of the African Society of Human Genetics[49] and the launch in 2010 of the Human Heredity and Health in Africa (H3Africa) initiative, funded by the National Institutes of Health and the Wellcome Trust, with the specific intent to promote the study of the genomes of African populations (60). Since there is a large body of evidence that *Homo sapiens* originated in Africa and that for most of its time in existence the species evolved on this continent (158), more genetic diversity was to be expected in African populations than in others, and this has been abundantly confirmed (144). With greater diversity, linkage disequilibrium is less, or conserved haplotypes are shorter, which impacts the way genome-wide association studies must be conducted and the results they may generate (see, e.g., 59, 128, 160).[50] Progress in hematology as well as in genetics was highlighted in a course, Advances in Haematology in Africa, that Julie Makani and I organized in Dar-es-Salaam in 2018; we had speakers from 11 countries and more than 80 participants from 17 African countries.

CONCLUSION

As I belong to a generation that has spanned from the discovery of the DNA double helix in 1953 to the official completion of the Human Genome Project in 2001, I feel that one of the most significant trends has been the reunification of different sectors in biology and medicine. At the onset of molecular biology, there was a genetic strand and a structural strand (114); these strands have now become vigorously intertwined, as the one-dimensional information in the DNA sequence encodes a developmental program that cannot be understood without taking into account the three-dimensional arrangement of DNA in chromatin, as well as thousands of DNA–protein and protein–protein interactions. When I was young, the relationship between genetics and biochemistry ranged from desultory to antagonistic; now, protein chemistry cannot do without recombinant DNA technology issued from molecular genetics, while understanding

[49]The founding president of the African Society of Human Genetics was the distinguished Nigerian geneticist Charles Rotimi (see 144, 151). In 2007, I was invited to the society's fifth conference, held in Cairo, Egypt. In 2022, Rotimi and Francis Collins cochaired the Presidential Symposium of the American Society of Human Genetics, which, significantly, was devoted to African genomics; Julie Makani was one of the speakers. Notably, the 14th International Congress of Human Genetics took place in 2023 in Cape Town, South Africa.

[50]It is interesting that in genome-wide association studies, the X chromosome is sometimes not included, and when it is included, data are often analyzed irrespective of gender. By separating genders, we spotted a long-sought X-linked locus that influences the level of Hb F in patients with SCD (160).

gene regulation is impossible without good biochemistry. It is natural that there are still separate academic departments of genetics and biochemistry, but there is only one biology.

As for medical genetics, one of my teachers in medical school was peremptory in telling us that a disease must be either inherited or acquired, though he admitted that an inherited disease could be affected by acquired factors. Today, if we search properly for genes affecting susceptibility, there is hardly any acquired disease for which they are not found, and I tell my students that, on a line traced from genes to environment, every disease is somewhere within the spectrum. Whole-exome sequencing is currently being marketed in ways that some of us find overaggressive, but it can solve a difficult diagnosis in many patients with rare disorders (170). At the same time, the advances that, as a whole, I find conceptually the most relevant to medicine are in the genetics of somatic cells (106, 107) (see **Table 1**). Somatic mutations occur from early human embryogenesis onward (estimated 3 mutations on average per cell division) (78); therefore, somatic cell mosaicism in our bodies is not the exception but the rule. There is an increasing interest in nonmalignant pathology that may arise from mosaicism (13), but PNH remains the prototype, as in this case we have identified a plausible agent for Darwinian selection (102). As for malignant pathology, although Rudolf Virchow (1821–1902) did not know about somatic mutations, his hypothesis that cancer originates from normal cells was spot on. It is now clear that the ultimate nosographic identity of a tumor is the set of somatic mutations that have generated the tumor. The implications for clinical oncology are stunning. For decades, chemotherapy has used nonspecific targets, such as DNA replication or the mitotic spindle, but now we have a catalog of tumor-specific somatic mutations (154) that can be targeted such that, in principle, non-tumor cells might be unaffected.[51] In a recent database of licensed anticancer drugs, those deliberately developed against a molecular target are the majority (134). I had not been told, when I was a student, that the key to cancer therapy would be genetic analysis.

Unity in biology and medicine signifies progress; to me, having done genetics and hematology in five countries, so does the unity of efforts in research, education, and health services in different parts of the world. As stated by Craig Cameron Mello,[52] "Science is a unifier. Science values questions rather than beliefs. . . . There are so many forces that divide people; barriers of language, custom, ideology, and belief . . . [S]cience tells us that we share a deep common history as a species, and that we will very likely share a common destiny as inhabitants of a small and fragile planet" (71, p. 1432). Applying these concepts to genetics, we can observe that an individual germline or somatic mutation is the same wherever it occurs. Applying these concepts to genetic medicine, we can say that it is a priority to redress inequalities; once a gene therapy or gene editing procedure proves safe and effective, we must strive to make it accessible to patients throughout the world (112, 126). This may sound utopian, but drawing from my experience in medical science in five countries over more than 60 years, I believe it is possible.

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[51]As a hematologist, I cannot refrain from noting that the trailblazing work in this area came from the treatment of chronic myeloid leukemia, with the targeting of the BCR–ABL fusion protein by the tyrosine kinase inhibitor imatinib, for which Brian Druker, Nicholas Lydon, and Charles Sawyers received the Lasker–DeBakey Clinical Medical Research Award in 2009. In Europe, a foremost promoter and pioneer of imatinib was my former colleague at Hammersmith, John Goldman, a towering figure in chronic myeloid leukemia management and research (54). In 2008, I was part of celebrating John's 70th birthday in a villa near Florence; sadly, he departed in 2013, and he is remembered by regular events in his honor.

[52]Mello was awarded the 2006 Nobel Prize in Chemistry for his role in discovering the function of RNA interference in development.

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Since I am constantly reminded that there are space limitations, I will not mention here mentors and colleagues who were already named in the main text. With respect to genetics and hematology, here is an inevitably incomplete list of those with whom I have had the pleasure to work, for which I wish to thank them:

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