PERSPECTIVES: MALARIA

Protecting Against Bad Air

Lucio Luzzatto and Rosario Notaro

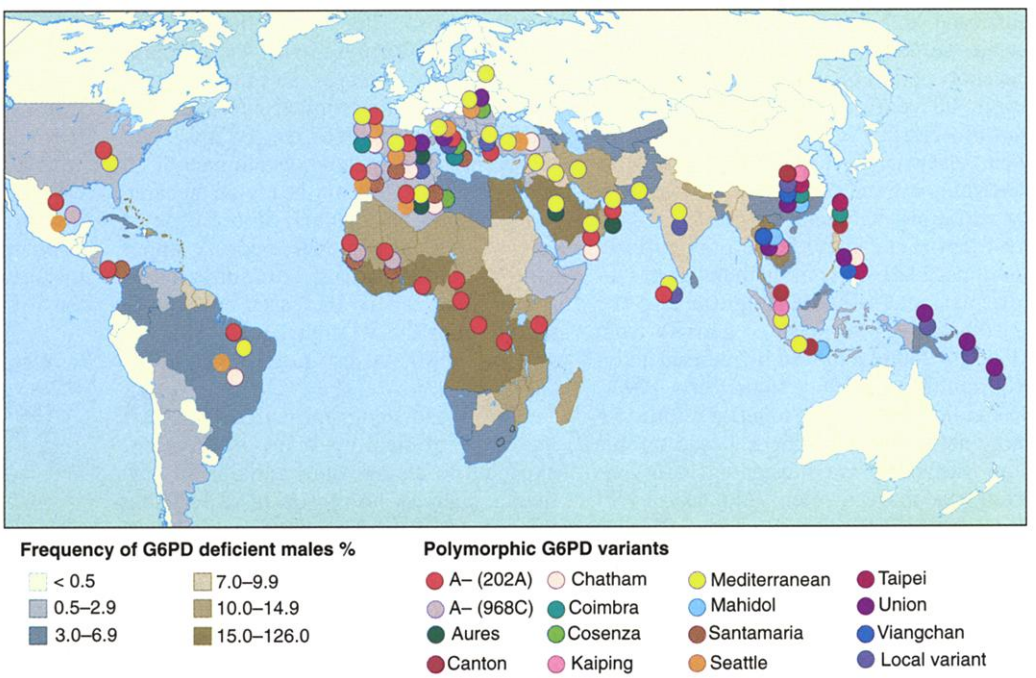
It was J. B. S. Haldane who first pointed out that an infectious disease causing high mortality among children could be important in shaping human evolution by exerting selective pressure on mutant genes protecting against that infection (*1*). Evidence is steadily accumulating that a fatal form of malaria caused by *Plasmodium falciparum* (an intracellular protozoan parasite of red cells) fits Haldane's description of such an infectious disease. Named by the Romans who thought that it was caused by bad air (*mala aria*), malaria is believed to have exerted selective pressure on variant forms of genes expressed in red blood cells, such as glucose-6-phosphate dehydrogenase (*G6PD*) (*2*). On page 455 of this issue, Tishkoff *et al.* (*3*) use molecular analyses and mathematical modeling to estimate when two of the most common G6PD variants (A– and Med) originated in humans.

The evolutionary history of G6PD is a strong reminder that this enzyme is crucial for protecting cells against oxidative stress. In contrast to all other free-living organisms, obligate anaerobic Archaea (nonbacterial prokaryotes) do not have G6PD (*4*). G6PD maintains a balance between reduced and oxidized glutathione, a molecule that protects against oxygen free radicals. Mouse embryonic stem cells engineered to have a defective *G6PD* gene are exquisitely sensitive to oxygen and give rise to embryos that die in utero (*5, 6*). It is puzzling why G6PD deficiency is lethal in mice but not in humans. The answer is simple: All human *G6PD* variants discovered so far are missense mutations or small in-frame deletions that do not result in a complete loss of enzyme activity. Individuals with an inherited G6PD deficiency are at risk of developing anemia if they are exposed to fava beans or certain drugs.

G6PD deficiency, the most common enzyme deficiency in humans, is thought to protect against *P. falciparum* infection. There is remarkable overlap between geographical regions with populations that have a high frequency of G6PD deficiency and where malaria is or was endemic (see the figure). In clinical field studies, fewer *P. falciparum* parasites were found in children heterozygous for G6PD deficiency (that is with one variant and one normal copy of the *G6PD* gene) than in children with normal copies of the gene (*7*). Furthermore, G6PD-deficient children had fewer episodes of life-threatening malaria than did children in whom G6PD activity was normal (*8*). In vitro experiments reveal that *P. falciparum* is able to invade G6PD-deficient red blood cells but does not mature normally (*9*). Parasitized G6PD-deficient red blood cells are phagocytosed more readily by macrophages than are parasitized red cells with normal G6PD activity (*10*).

There is yet another and perhaps even stronger argument in support of selection for G6PD deficiency by malaria. There are more than 130 known G6PD variants, and at least 34 of these are polymorphic, that is, they are present at high frequencies in some human populations (*11*). All of the 34 polymorphic variants are found in populations who live in malarially endemic areas. If only one G6PD variant resulted in G6PD deficiency, then it could be argued that the geographical correlation between the frequency of G6PD deficiency and malaria endemicity arose by chance. For example, a G6PD variant of ancient origin could have spread throughout tropical and subtropical regions, in the absence of any selective pressure, following the marked increase in population density associated with the introduction of agriculture. However, given that there are 34 common G6PD variants, it is unlikely that all of them spread throughout malarial areas by chance, and that none of them spread to areas where there was agriculture but no malaria.

The study of human evolution has been aided by the discovery of minute DNA changes—called restriction fragment length polymorphisms (RFLPs)—that alter where restriction enzymes cleave DNA (*12*). A set of closely linked RFLPs or other DNA changes is called a haplotype (*13*). Assuming that most of these DNA changes are in noncoding regions of the genome and so are not subject to selection, haplotypes can be used to track the origins of gene mutations. This strategy was pioneered by Orkin to track the β-globin



The global village. Global distribution of *G6PD* gene variants causing G6PD deficiency. A deficiency in this enzyme may protect against infection with *P. falciparum*, the parasite causing a fatal form of malaria. Shaded regions indicate the prevalence of G6PD deficiency: The % values are the frequencies of G6PD-deficient males (because the G6PD gene is X-linked, these values are identical to gene frequencies). The colored dots depict the distribution of 14 of the most common G6PD variants, including A– (found in sub-Saharan Africa) and Med (found in Mediterranean countries, the Middle East, and Asia). The blue circles represent "local" variants that have been found only in a single population.

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gene mutations that cause thalassemia (*14*). Analysis of seven RFLPs linked to the *G6PD* gene led to the identification of several haplotypes and a possible genealogy for the most common G6PD variants (*15–17*). It has been estimated that the A– variant, based on the fact that it has a selective advantage, could have arisen between 5000 and 10,000 years ago (*17*). Building on this work, Tishkoff *et al.* (*3*) have analyzed RFLPs associated with the normal forms of *G6PD* (B and A) and with the A– and Med G6PD deficiency variants in 591 individuals from 14 different populations worldwide. In addition to four of the seven known RFLPs, these investigators analyzed three new microsatellites (DNA containing variable numbers of tandem repeats) located in a noncoding region within 19 kilobases of the *G6PD* gene.

With mathematical modeling of the RFLP and microsatellite analysis, the authors were able to estimate when the A– and Med variants arose. They propose that the A– variant is more ancient, originating 6357 years ago (very close to the previous estimate) (*17*), whereas the Med variant is more recent, originating 3330 years ago. This is somewhat surprising as the A– variant is limited to Africa, whereas the Med variant ranges from Portugal to Indonesia, hinting that Med should be the more ancient variant (see the figure). However, there is considerable overlap in the range of the estimates: 3840 to 11,760 years for A– and 1600 to 6640 years for Med. We eagerly await analyses of other G6PD variants, such as Seattle and Union, which have broader geographical distributions than either A– or Med, suggesting that they may be even more ancient (see the figure).

It is intriguing that estimates for the origin of the A– and Med G6PD variants are consistent with the introduction of agriculture in the Middle East and Africa about 10,000 years ago, which provided conditions conducive to the spread of malaria. Volkman *et al.* (*18*), reporting on page 482 of this issue, have narrowed down previous calculations which estimate that ancestral *P. falciparum* arose between 5000 and 50,000 years ago (*19*). By analyzing sequence variations in the noncoding regions of some *P. falciparum* genes, they calculate that the ancestral strain of *P. falciparum* emerged 3200 to 7700 years ago. This estimate overlaps nicely with the Tishkoff *et al.* estimate for the age of the A– and Med G6PD variants. These molecular analyses provide further evidence for a close connection between malaria and G6PD deficiency, and yield a glimpse into the complexity of the co-evolution of a parasite and its host.

The Tishkoff *et al.* findings, however, do not answer two crucial questions: How does G6PD deficiency protect against *P. falciparum*, and is it only heterozygotes that are protected? (There has been much debate about whether hemizygotes, males with only one variant copy of the X chromosome–linked *G6PD* gene, are also protected.) Considering that malaria remains a devastating public health problem, these questions are not merely of academic interest. Malaria has been credited with contributing to the fall of the Roman Empire—it would be a vindication of human cultural evolution, if we could learn from natural selection how to defeat this terrible disease.

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PERSPECTIVES: NEUROSCIENCE

The Meaning of a Mini

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Nerve cells communicate with one another by releasing neurotransmitter molecules at specialized junctions between neurons called synapses. In response to electrical impulses (action potentials) transmitted along the nerve axon, synaptic vesicles fuse with the neuron's presynaptic membrane, releasing their neurotransmitter contents into the synaptic cleft (see the figure). The neurotransmitter then diffuses across the synaptic cleft, activating receptors in the postsynaptic membrane. Usually, synaptic vesicles fuse with the presynaptic membrane in response to action potentials, but spontaneous fusion resulting in the release of a single vesicle's contents has been recorded in the absence of action potentials (*1*). When a single synaptic vesicle spontaneously fuses with the presynaptic membrane, neurotransmitter diffuses across the synaptic cleft and activates postsynaptic receptors, eliciting a miniature endplate potential or mini. Spontaneous fusion usurps some (but not all) of the molecular machinery required for action potential–dependent fusion. Indeed, genetic manipulation or toxin treatment that blocks neurotransmitter release evoked by action potentials also blocks spontaneous fusion (*2*, *3*).

Spontaneous fusion events and the minis they generate were reported more than 50 years ago by Fatt and Katz (*1*). Yet it is still not clear whether spontaneous fusion is important in neuronal communication or merely represents leakage from an otherwise efficient synaptic machinery. Saitoe *et al.* (*4*) have tackled this problem by examining the organization of developing neuromuscular junctions (NMJs)—specialized synapses between neurons and muscle cells—in a variety of fly mutants that have defects in different steps of neurotransmitter release. They report on page 514 of this issue that spontaneous neurotransmitter release is crucial for organizing sites of neuronal communication in the NMJ during *Drosophila* development.

In the NMJ of the developing fly, alignment of the presynaptic active zone and the postsynaptic density (which contains a high concentration of glutamate receptors) is essential for fast and reliable transmission of action potentials. Clustering of glutamate receptors in the postsynaptic density takes place in several stages. Initially, glutamate receptors are spread diffusely throughout the muscle membrane but are somewhat enriched in the vicinity of muscle nuclei (*5*, *6*). They then start to cluster at sites where the neuron and muscle cell will eventually make contact (*6*, *7*). Later, these broad receptor clusters are reduced (refined) still further when the innervating neuron makes contact with the muscle cell. Neuronal activity is required for the final clustering of receptors in the postsynaptic density, but action potentials do not appear to be involved. Tetrodotoxin (TTX), a drug that inhibits action potentials (but not minis) by inactivating sodium channels, does not prevent glutamate receptor clustering (*8*). However, postsynaptic gluta-

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