Review

The origin of Behçet's disease geoepidemiology: possible role of a dual microbial-driven genetic selection

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ABSTRACT

It is recognised that the genetic profiles that give rise to chronic inflammatory diseases, under the influence of environmental agents, might have been implicated in the host defence mechanism against lethal infections in the past. Behçet's disease (BD) is an immune-mediated inflammatory disease, expressed as vasculitis, triggered by environmental factors in genetically susceptible individuals. We carried out a review of published data to draw up an evolutionary adaptation model, as Author's perspective, for genetic susceptibility factors and inflammatory immune response involved in BD pathogenesis. Two lethal infectious agents, Plasmodium Falciparum and Yersinia Pestis, are proposed as the putative driving forces that favoured the fixing of the major genetic susceptibility factors to BD, thus determining its geoepidemiology. Further studies are needed to confirm the validity of this evolutionary model which includes and integrates the key insights of previous hypotheses.

Evidence of a close link between microbial exposure and onset of diseases with an inflammatory immune component is increasing. Humans have lived in an environment where infectious diseases have been mostly endemic (1). It has been postulated that such a pressure by infective agents led to genetic selection aimed at a more effective pro-inflammatory response thus boosting resistance to specific infections. This positive effect can however be counterbalanced, under given circumstances, by a proneness to lethal complications or to immune-mediated inflammatory diseases (1-4). Here, a review of published data was carried out to draw up an evolutionary adaptation model, according to the Authors' perspective, for genetic susceptibility factors and inflammatory immune response involved in BD pathogenesis.

BD is a systemic vasculitis characterised by the deregulation of both innate and adaptive immune responses with enhanced pro-inflammatory activity, strong genetic association and distinctive geographical distribution. Several inflammatory related variants, such as TNF-α *-1031C* (OR 1.3; 95% CI 5 1.1 to 1.7) (5), IL10 rs1518111 A/G (OR 1.4; 95% CI 1.3 to 1.6) (6) and IL23R-IL12RB2 rs924080 A/G (OR 1.3 95% CI 1.2 to 1.4) (6) have been reported as associated with a small risk to develop BD, whereas HLA-B*51:01 is still considered the major genetic susceptibility factor to BD (OR 5.8; 95% CI 5.0 to 6.7) (7). Distinctively, BD geoepidemiology, of which the highest prevalence is seen from the Mediterranean basin to the Far East between latitudes 30°N and 45°N, mirrors the geographical distribution of HLA-B*51:01 across the globe (Fig. 1B) (8). HLA-B*51:01 is speculated to have been spread, together with closely linked genes, by the migration of early *Homo Sapiens* from Africa to the Americas (8), and then fixed along the historical Silk Road (9). However, the reasons why HLA-B*51 and BD are rare or virtually absent in some areas such as Sub-Saharan Africa, and which forces have driven the fixation of genetic susceptibility factors to BD, are still unknown.

We recently pointed out that the evolutionary selection of HLA-B*51:01 and susceptibility genes in linkage disequilibrium (LD) with it might have taken place over millennia (10). The speculative evolutionary adaptation model described here is based on two assumptions. Firstly, on the negative selective pressure exerted in an endemic setting by *Plasmodium Falciparum* Malaria on

the ancestral *Homo Sapien* populations and their human descendants. Secondly, on a subsequent counterbalancing and a more recent positive selection by the epidemic *Yersinia Pestis* Plague. Both driving forces have contributed to the non-homogeneous distribution of HLA-B*51:01 and linked genes (8) among world populations and to their fixation in a defined area of the world, thus influencing the geoepidemiology of BD (Fig. 1A-B). Finally, this model provides an insight into how such a dual pathogen-driven genetic selection might have favoured the co-selection of variants (*e.g.* TNF-α, ICAM1, IL-10) implicated in the polygenic pro-inflammatory trait involved in BD pathogenesis.

Mutual exclusion of Behçet's disease and malaria endemic areas?

*Plasmodium Falciparum as a negative selective agent for HLA-B*51 and linked genes*

Malaria from *Plasmodium Falciparum* is an infectious disease burdened by high mortality, mostly caused by cerebritis, especially in children younger than 5 years-old, whose pressure has determined the selection of several polymorphisms and haplotypes both within and outside the HLA region (2). The other strains of *Plasmodium* (*e.g. Vivax*) are not selective agents. The co-evolution of Humans and *Plasmodium Falciparum* started approximately 40–80,000 years ago in sub-Saharan Africa (Fig. 1A), before the out-of-Africa migration (11). It is recognised that *Plasmodium Falciparum* had infected humans before the Great Human Expansion, the rapid spread of population across the Eurasian continent which started approximately 45–60,000 years ago (11, 12). The parasite accompanied the ancestors of modern humans in the colonisation of the planet that led to the spread of *Plasmodium Falciparum* across the tropics and laid the foundations for its current geographical distribution (11-13). The worldwide parasite population remained relatively small for a considerable period of time followed by rapid expansion about 10,000 years ago, concurrent with the emergence of agricultural societies in humans, which coincides with an increase in the spread of Malaria and a boost in the selective effect of *Plasmodium Falciparum* on modern humans (14).

In Africa, the malaria endemic from *Plasmodium Falciparum* and HLA-B*51:01 have an inverse correlation suggesting a negative, pathogen-driven, evolutionary selection (Fig. 1B). It is noteworthy that HLA-B*51:01 allelic frequency is significantly lower in West African and Sub-Saharan ethnic groups (0.00 to 0.02), which are still plagued by *Plasmodium Falciparum* infection, than in closely related populations inhabiting Southern Europe (0.15) and the Middle East (0.20). More epidemiologic evidence comes from Sardinia, a Mediterranean island with an isolated and genetically homogeneous population which was plagued by endemic *Plasmodium Falciparum* infection for millennia, where Contu *et al*. (15) have demonstrated that HLA-B*51 frequency had an inverse correlation with malaria prevalence in differently plagued island subareas. Further proof has emerged from molecular investigations. HLA-B*53:01 and HLA-B*35:01 are protective against severe malaria from *Plasmodium Falciparum* in West Africans and in Sardinians respectively (15, 16). Their amino acid sequences are identical to each other and to that of HLA-B*51:01 in the B pocket but differ from it in the F pocket in position 116, where the HLA-B*51:01 harbours a tyrosine instead of a serine (17). This change modifies the structure of the peptide binding pocket and may be responsible for the lack of the protective effect afforded by HLA-B*51:01 against malaria. Noteworthy, the HLA-B*27 allele, which has a latitude-related gradient inverse to that of malaria endemic (18) and whose sub-allele B*27:02 resulted associated with BD in Turkish patients (19), shows similarity with HLA-B*51 in the presentation of intracellular epitopes (20).

Recently, a genome wide association study identified a strong LD in the HLA region of BD patients, mainly due to the fact that HLA-B*51 was found almost exclusively on a single extended haplotype (6). Successively, two distinct extended haplotypes harbouring HLA-B*51:01 were identified in Sardinia, but only one of them was associated with BD (21). Therefore, it is conceivable that distinct HLA-B*51:01 haplotypes predispose to different degrees of inflammatory response. Several studies have reported the association of enhanced TNF-α activity and TNF-α promoter polymorphisms, which are in strong LD with the locus B, with cerebral malaria as a fatal complication of *Plasmodium Falciparum* infection. Noteworthy, some of these functional polymorphisms have been reported as associated with BD, either independently or synergistically with HLA-B*51:01. As an example, TNF-α *-1031C* is associated with stronger inflammatory response, mortality from cerebral malaria and BD susceptibility (22, 23). Based on this data it is reasonable to assume that HLA-B*51:01 haplotypic distribution may, in part, be the result of the ancestral selective pressure exerted by *Plasmodium Falciparum* which negatively selected those complex traits conferring an enhanced counterproductive and lethal pro-inflammatory phenotype.

*The origin and distribution of HLA-B*51, and linked genes, in modern humans*

Verity *et al*. have suggested that the HLA-B*51:01 was spread by the migration of early *Homo Sapiens* from Africa to the Americas (8), which by itself does not clarify why this allele is so rare in Africa. The highly conserved structure of HLA-B*51:01 in Caucasian, Japanese and Afro-Americans supports its evolvement prior to the divergence of the major ethnic groups (24). However, whether or not the HLA-B*51 was already present in ancestral *Homo Sapiens* before the out-of-Africa migration is still debated (25). Despite a number of controversial viewpoints concerning the genetic differentiation of modern *Homo Sapiens* (26), it has been suggested that the presence of HLA-B*51 in Eurasians, together with B*07, C*07:02, C*16:02, might be the result of genetic contribution from the Neanderthals, which occurred after the early out-of-Africa migration until 40–30,000 years ago. Such an admixture with the Neanderthals has been indicat-

ed as the sole source in modern humans of these alleles, whose current presence in Africa was due to a back-migration (25, 26). Accordingly, the virtual absence of HLA-B*51:01 and other Neanderthal alleles in some African areas may be explained by a small back flow of migrants rather than a negative selection by *Plasmodium Falciparum* (Fig. 1). However, the higher frequency of the B*07 allele, which is not associated with Malaria susceptibility, in modern populations of Western and sub-Saharan Africa compared to those of the Middle East and Central Asia clashes with the fact that this is the only acceptable explanation. If Neanderthals were confirmed as the sole source of these alleles in modern humans, it could be assumed that early HLA-B*51:01 positive humans returning to Africa, likely before the transition from hunter-gatherer society to agricultural society, would have been negatively selected as more susceptible to dying from severe malaria. On the other hand, as *Plasmodium Falciparum* had already infected early Homo Sapiens at the time of their admixture with Neanderthals, only a few HLA-B*51:01 positive individuals may have managed to return to Africa as a result of the malarial selection that took place along the path of their back-migration.

In the event of a negative selection by *Plasmodium falciparum*, the burden of HLA-B*51:01 allele in the early settlers of the Eurasian continent would have been reduced as would its frequency around the world be today, unless a founder effect or a subsequent decisive and rapid positive selective pressure or both occurred in those areas where it now appears to have greater frequency. It is thought that during the Great Expansion across the Eurasian continent there was a continuous decrease of genetic diversity, understood as heterozygosity, with geographic distance, a process called serial founder effect (12). A major founder effect is deemed to have occurred in North America as the result of the crossing of the Bering Strait by a small number of Homo Sapiens 15–10,000 years ago (12). Such a founder effect, which occurred in isolate versus neighbouring populations, might have favoured the fixing of those haplotypes harbouring the HLA-B*51, which previously escaped the selection by *Plasmodium Falciparum* because not associated with a lethal pro-inflammatory response, preserving this genetic background in a neutral environment due to the absence of selective pressure (*e.g.* the Americas were probably colonised by *Plasmodium Falciparum* far more recently and primarily in the lowlands and swamps and not in the mountains or mesas). This possible explanation corroborates a major prediction of Verity's hypothesis (8) in which the virtual absence of BD in those Native American populations carrying a high incidence of HLA-B*51 might be due to the absence of susceptible HLA-B*51 sub-alleles or closely linked genes, as TNF-α promoter polymorphisms, or both (8). The finding that Amerindian populations carry the highest frequencies reported worldwide of apparently non BD susceptible HLA-B*51:02 sub-allele (0.13 to 0.15) (10) and TNF-α *-857T* (0.30 to 0.45) (27), as well as the lowest frequency of the BD associated TNF-α *-1031C* (0.07 to 0.09) (27), further support Verity's hypothesis.

Behçet's disease along the path of the Black Death?

It has been claimed that the HLA-B*51:01 subtype has been preserved in European and Asian populations by a unifying selection (28). To confirm the validity of our model, such selection is expected to have positively occurred after the overland migration across the Beringia Strait and to have been more intense in those areas where today's populations have a higher incidence of BD. Moreover, the responsible infectious agent should have favoured the fixation of pro-inflammatory phenotypes and complex traits containing and associated to the HLA-B*51:01 because of their protective role in the host response against the infection.

Phylogenetic analysis suggested that *Yersinia Pestis* evolved in China 20–15,000 years ago, therefore after the human migration across the Bering Strait, and spread worldwide, through multiple radiations along the ancient trading routes, resulting in historical epidemics (29, 30). Noteworthy, the regions where humanity was hit by lethal epidemics from *Yersinia Pestis,* and particularly the Black Death (1330-1350 AD), correspond to the same areas of greatest HLA-B*51:01 and BD incidence, and roughly trace the Silk Road (Fig. 1). Although it is common knowledge that the Black Death and its successive outbreaks until 17th century reached and struck Northern European countries, it has been clarified that the plague hit central Asia, the Middle East, North Africa and Southern Europe hardest, spreading both across land and by ship (31, 32). Causing the loss of one-third to half the world's population at that time, the enormous impact of plague pandemics on human mortality led to the suggestion that *Yersinia Pestis* may have acted as a devastating selective agent (31).

Several reports have suggested that strong innate and adaptive host responses are needed to overcome virulence factors of *Yersinia Pestis*. *In vitro* and animal models show that the activity of TNF-α, IFN-γ and Nitric Oxide Synthase 2 are directly correlated with protection against lethal plague during the early intracellular stages of *Yersinia Pestis* infection (33, 34). Such an observation fits with the evidence that neutrophils may kill *Yersinia Pestis* through the enhanced production of superoxide and oxidative killing induced by high serum TNF-α levels, leading to protection against lethal plague (35). Moreover, experimental observations suggest that HLA class I restricted cytotoxic T-lymphocyte play a pivotal role in the protective immunity against plague (33, 36). Therefore, it is reasonable to assume that a genetically determined trait conferring a pro-inflammatory immune response results protective against *Yersinia Pestis*.

Although the exact pathogenesis of BD remains unknown, it is established knowledge that BD patients have neutrophil, T-lymphocyte and natural killer (NK) hyperactivity with high serum TNF-α levels and enhanced pro-inflammatory cytokine production. Peripheral blood mononuclear cells from BD patients exhibit a hypersensitivity response, to both *non-self* (*e.g.* strep-

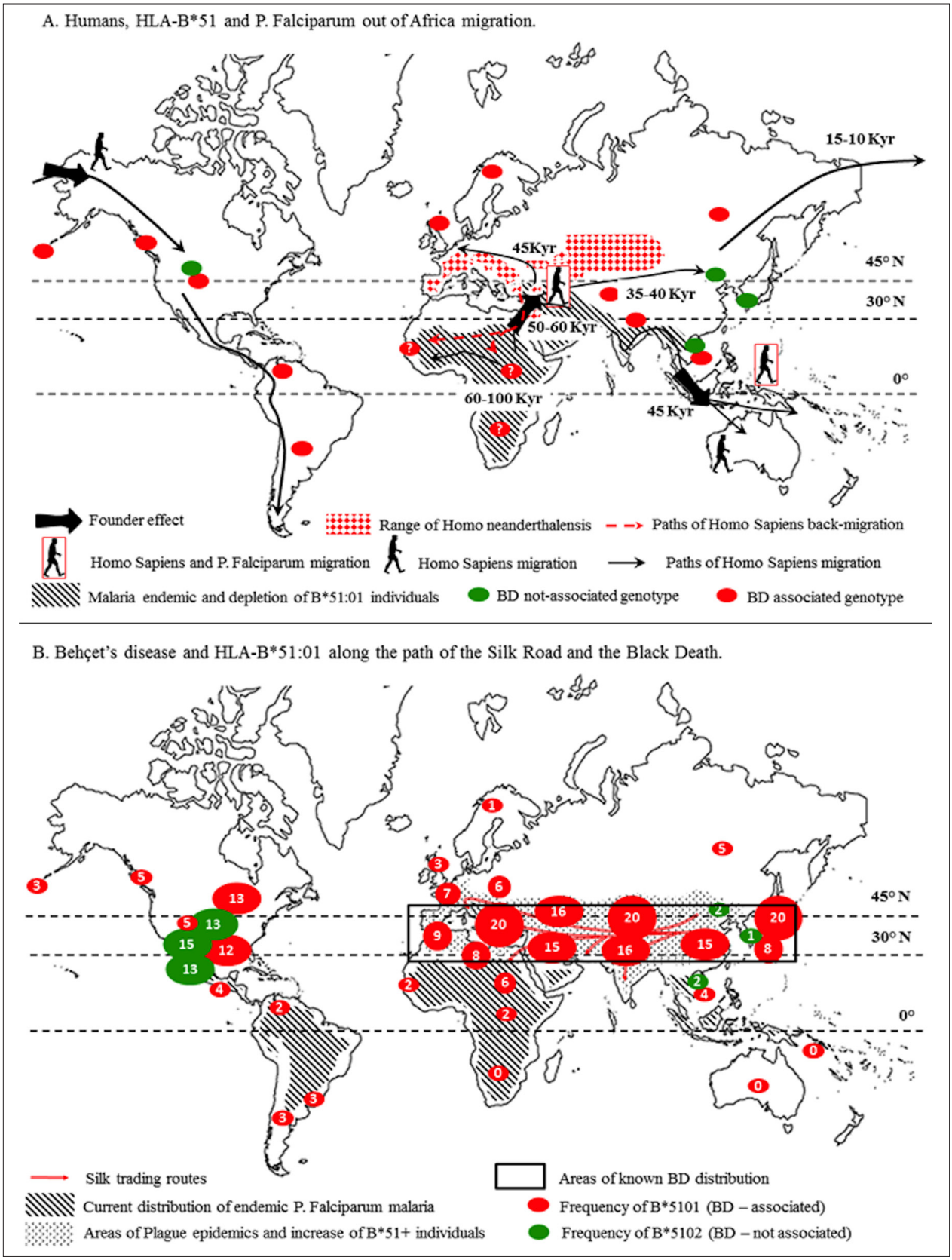

Fig. 1. Image representative of the possible ways of distribution and fixation of HLA-B*51 and its sub-alleles around the world under the selective effect of Plasmodium Falciparum endemic (**A**) and Yersinia Pestis epidemics (**B**) and according to ancient dispersal patterns of humans during the past 100,000 years (100 Kyr) (13). The ancestral origin of HLA-B*51:01 or its later origin from inbreeding with Neanderthals is called into question. It is likely the high B*51 frequency seen in areas in North America is due to a founder effect that occurred in a neutral environment. Only the most representative values of HLA-B*51 sub-alleles frequency (http://www.allelefrequencies.net/) in indigenous pre-Columbian population are reported.

tococcal antigens, super-antigens) and *self* ("molecular mimicry model") antigens, able to up-regulate the expression of activated T-lymphocytes and neutrophils in patients but not in controls (37). It remains unclear whether a virus or bacterium initiates and/or prolongs the characteristic mucosal and endothelial immune/inflammatory hyper-reactivity observed at the gastrointestinal barrier and vascular surface, respectively. Such an increased pro-inflammatory response is strongly believed to be part of a complex genetically determined trait (37).

We speculate that this trait has been fixed as a by-product of the positive genetic selection exerted by *Yersinia Pestis* because of its protective role in the host response against infection. Hence, our model is genuine under the assumption that the bottleneck effect secondary to the high mortality rate of plague epidemics might have caused the expansion of this advantageous complex trait, counterbalancing the primitive negative selection of HLA-B*51:01 individuals by *Plasmodium Falciparum* malaria endemic (Fig. 1). In light of these considerations, *Yersinia Pestis* might represent the driving force, hypothesised by Ohno *et al*. (9), that favoured the fixing of the complex trait closely linked to HLA-B*51:01 and associated with susceptibility to BD along the Silk Road thus contributing, together with the selective effect of *Plasmodium Falciparum* but independently from it, to the distinctive geoepidemiology of BD in world populations.

Unfortunately, no data are available on which polymorphisms, both within and outside the HLA region, are associated with susceptibility to or protection against plague in humans. However, since the functional polymorphisms of TNF-α cause higher serum level of TNF-α, they might have been fixed in individuals exposed to *Yersinia Pestis* infection due to their protective role and might have played a part in fixing the BD-associated HLA-B*51:01 haplotypes. A major role of HLA-B*51:01 against *Yersinia Pestis* cannot be excluded, nevertheless, laboratory evidence is absent and this hypothesis remains open to further investigation.

Finding the complex trait associated with Behçet's disease

Although environmental factors are thought to have putative responsibility, ethnic background is considered more important in determining BD susceptibility (38).

Patients with BD are characterised by a pro-inflammatory phenotype that varies in degree and can be attributed to a complex trait consisting of several genes co-operating with distinct contribution in determining the complex pathogenetic mechanisms of the disease (39). Considering that the highest contribution of HLA-B*51:01 to the overall BD genetic susceptibility was estimated to be only 19% (40), it is conceivable that the complex trait conferring BD susceptibility may lead to disease development even in the absence of HLA-B*51:01, as proved by the fact that only 50–60% of patients are HLA-B*51:01-positive in endemic areas. A recent study has questioned the role of the HLA-B*51:01 as a genetic determinant of BD by saying that its robust association with the disease is explained by a variant (rs116799036: OR 3.9) located between the HLA-B and MICA genes (41). Multiple genes other than HLA-B*51:01, both within and outside the HLA region, somewhat involved in the innate and adaptive immune responses are also likely to contribute with smaller effect (41-44) and it is reasonable to assume that an effective combination of them may confer disease susceptibility. Accordingly, evidence of linkage to several HLA and non-HLA susceptibility loci in BD patients has been provided (41-46).

Why the allelic effect of variants associated with BD is relatively small and disease hereditability is low (sibling recurrence rate 4.2%; 95% CI 1.2 to 7.2) (47), rather than absent, could be satisfactorily explained by Darwin's theory of evolution, through a pathogen-driven genetic selection, operating in a hostile environment to either decrease or increase the frequency of mutations that have an effect on the individual's reproductive ability (48). Many examples of genetic variations conferring risk to BD development are polymorphisms of loci thought to be involved in susceptibility to cerebral malaria or protection against plague (Fig. 2). The stratified selection possibly operated by the two lethal infections identified in our model might have played a role in the fixation of the whole complex trait associated with BD development, and not only the HLA-B*51:01, taking part in the co-selection of genes in LD within the HLA region (*e.g*. HLA-MICA, HLA-TNF-α), and/or functionally linked genes (*e.g*. HLA-KIR, cytokine-cytokine, cytokine-thrombophilic factors, HLA-B*51:01-ERAP1), which may explain the low prevalence of BD outside those areas with high frequency of HLA*B51:01. As an example, an increase in some KIR-HLA combinations, that were more effective in controlling infections and promoting survival in different regions of Europe, has been envisaged (49). Another example comes from IL10. Low levels of IL10 are associated with enhanced TNF-α production and, in experimental models, with susceptibility to lethal malaria by *Plasmodium Falciparum* and protection against *Yersinia Pestis* infection (50, 51); therefore, IL10 locus would be an attractive candidate for a strong selective pressure by these infectious agents. It is noteworthy that IL10 levels in BD patients are low and the BD-associated IL10 variants are associated with decreased expression of this anti-inflammatory cytokine (6).

A major caveat in our model is why the HLA-B*51 link is scarce in BD patients from non-endemic areas. Outside of those regions of the world where HLA-B*51:01 has a higher frequency, there is a lower or very low prevalence of BD suggesting that also the other susceptibility alleles, with smaller effects, have been negatively selected in these areas. Interestingly, however, an increasing number of BD cases unrelated to HLA-B*51 among individuals of African ancestry is reported in literature and this might be due to a different genetic distribution (52). In non-isolated populations of sub-Saharan Africa, the long-term selection driven by Malaria might have negatively selected the extended haplotypes harbouring HLA-B*51:01, as the most disadvantageous in the *Plasmodium Falciparum* endemic en-

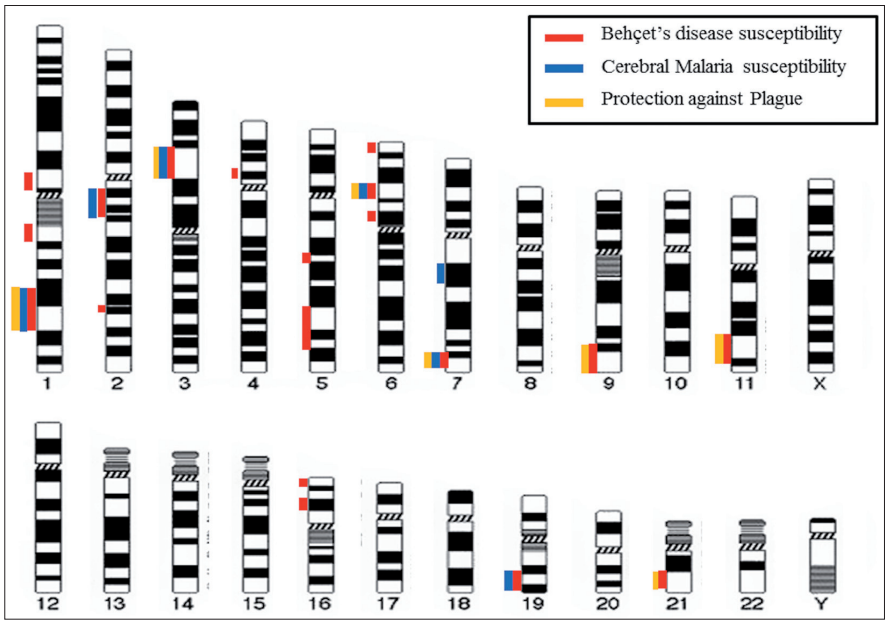

Fig. 2. Representation of the 23 human chromosomes. The figure depicts those loci which have been associated with Behçet's disease (see text for references) and cerebral malaria susceptibility (as reported in OMIM). Moreover, those loci whose products have been reported as associated with protection against *Yersinia Pestis* infection are highlighted (see text for references).

vironment, but may have less strongly selected other advantageous pro-inflammatory genes. They may represent those genes less relevant to BD pathogenesis, that however, in some combinations, might reach the threshold necessary for the clinical appearance of the disease, which would explain the lower frequency of BD reported in the indigenous South African, West African and Afro-Caribbean populations. As an example, HLA alleles different from HLA-B*51 have been described as associated with BD in some populations but not in others, in particular those B alleles sharing the Bw4 motif, a specific sequence of amino acids at residues 77-83 in the alpha-1 helix, which may be causally related to BD due to its involvement in NK cell recognition and recruitment (10). Such a possible explanation may lie in changes in genetic distribution and selection of the human genome due to biogeography, where the host heterogeneity met the microbial polymorphisms and where additional selective pressure was exerted by other infectious diseases (*e.g.* Tuberculosis, Smallpox), and might also be responsible for the reported variability of genetic susceptibility and disease expressions across and within world regions (53).

Conclusion

Our digression can be a working hypothesis for researchers dedicated to documenting the action of lethal infectious agents on the genetics of human populations. We hope that this may open completely new areas of research and attract the attention of a new community of scientists from various disciplines. Future studies, addressed to prove the hypothesis as described here, might also shed some light on the major role of HLA-B*51:01 and its ligand(s) in BD pathogenesis, for which definitive experimental proof is still missing. Although it would be difficult, based only on historical data and indirect experimental evidence, to explain how the selective pressures acted on the complex trait associated with BD, it is possible to further test this hypothesis using experimental methods. To establish whether differences in frequency of genes involved in susceptibility to BD and protection against lethal infections have increased or arisen after the great epidemics of *Yersinia Pestis*, the genotype from victims of the Black Death could be analysed when less expensive and more effective techniques become available. A recognised feature of HLA-B*51 concerns its role in conferring susceptibility to some infectious diseases (54) and protection against others (55-57). *In vitro* experimental model might address the activity of neutrophils, NK and T-lymphocytes from HLA-B*51:01 positive BD patients and normal controls when challenged by *Plasmodium Falciparum* and *Yersinia Pestis* antigens to identify a different response against these infectious agents.

Given how little has actually been explained of the genetic influences on most common pro-inflammatory diseases, a better understanding of the underlying evolutionary mechanisms will help to elucidate the functional relevance of genetic variants associated not only with BD, but even with other inflammatory immune-mediated diseases, possibly allowing the design of novel therapeutic strategies.

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