Parasites represent a major selective force for interleukin genes and shape the genetic predisposition to autoimmune conditions

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Many human genes have adapted to the constant threat of exposure to infectious agents; according to the “hygiene hypothesis,” lack of exposure to parasites in modern settings results in immune imbalances, augmenting susceptibility to the development of autoimmune and allergic conditions. Here, by estimating the number of pathogen species/genera in a specific geographic location (pathogen richness) for 52 human populations and analyzing 91 interleukin (IL)/IL receptor genes (IL genes), we show that helmints have been a major selective force on a subset of these genes. A population genetics analysis revealed that five IL genes, including IL7R and IL18RAP, have been a target of balancing selection, a selection process that maintains genetic variability within a population. Previous identification of polymorphisms in some of these loci, and their association with autoimmune conditions, prompted us to investigate the relationship between adaptation and disease. By searching for variants in IL genes identified in genome-wide association studies, we verified that six risk alleles for inflammatory bowel (IBD) or celiac disease are significantly correlated with micropathogen richness. These data support the hygiene hypothesis for IBD and provide a large set of putative targets for susceptibility to helmint infections.

It is commonly believed that infectious diseases have represented one of the major threats to human populations and have therefore acted as a powerful selective force. Even today, despite the advances in treatment and prevention, infectious diseases account for ~48% of worldwide deaths among people >45 yr of age (1). These figures do not include the heavy burden imposed by helmint infections, which have recently been designated as the “great neglected tropical diseases” (2). With an estimated 2 billion individuals infected worldwide (3), helmints represent the prevalent chronic infectious diseases of humans. Although parasitic worms determine severe clinical symptoms in a minority of heavily infected individuals, even apparently subclinical parasite burdens can result in impaired nutritional status and growth retardation (4, 5). It is therefore conceivable that several human genes have evolved in response to both microbial/viral infectious agents and parasitic worms; indeed, it has been suggested (6, 7) that human populations may have adapted to parasites to such a degree that the lower exposure to infectious agents in modern developed societies results in immune imbalances, with autoimmune and allergic conditions being the outcome.

Genes involved in immunity and inflammation are known to be frequent targets of natural selection; balancing selection, which is thought to be a relatively rare phenomenon in humans, has particularly shaped the evolutionary fate of

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genes involved in immune responses (8, 9). Balancing selection is the situation whereby genetic variability is maintained in a population via selection. The best known example in the human genome affects the MHC genes, which are characterized by extreme polymorphism levels. Recently, Prugnolle et al. (10) demonstrated that populations from areas with high pathogen diversity display increased MHC genetic variability, indicating the action of pathogen-driven balancing selection.

Quite obviously, the presence of a functional variant is a prerequisite for selection to act, and the identification of non-neutrally evolving genes has been regarded as a strategy complementary to classical clinical and epidemiological studies to provide insight into the mechanisms of host defense (11). Similarly, analysis of the evolutionary history of genes involved in immune defense might provide novel insights into the delicate balance between efficient response to pathogens and autoimmune/allergic manifestations.

In this study, we focused our attention on a large gene family that includes ILs and their receptors (hereafter referred to as IL genes). ILs are small secreted molecules that regulate most aspects of immune and inflammatory responses and exert their effects through binding to specific receptors expressed on target cells. Various IL genes have been associated with differential susceptibility to specific infections (12, 13), and with an augmented likelihood to develop autoimmune or allergic/atopic diseases (for review see reference [14]). Finally, whereas previous reports have demonstrated nonneutral evolution at single IL genes (15–17), no comprehensive analysis has been performed and no attempt to take into account pathogen richness across different human populations has ever been described.

RESULTS
Pathogen-driven selection acts on IL genes
Pathogen-driven selection is a situation whereby the genetic diversity at a specific locus is influenced by pathogens; this is expected to occur because one or more alleles are associated with the modulation of susceptibility to infectious agents. One way to identify loci or variants subjected to pathogen-driven selection is to search for correlations between genetic variability and pathogen richness (10, 18). The latter is a measure of pathogen diversity, and is basically calculated as the number of different pathogen species/genera in a specific geographic location (see Materials and methods for further details). The choice to use pathogen richness rather than more conventional epidemiological parameters such as prevalence/burden stems from several considerations, as follows: (a) comprehensive data on prevalence are impossible to retrieve for many infections; (b) even when prevalence data are available, they may vary considerably within the same country depending on the surveyed regions, the survey period (e.g., before or after eradication campaigns), the population surveyed (e.g., city dwellers rather than farmers/bushmen/nomads or children rather than adults); (c) the prevalence of specific infections might have changed greatly over recent years as a result of eradication campaigns, and historical prevalence data are rarely available; (d) we were not interested in a single species/genus. As a consequence, the prevalence of all (or at least the most common) pathogen species would be required. Still, prevalence data are difficult to combine; e.g., in endemic regions, individuals can be infected with multiple parasite species (2), and these subjects tend to harbor the most intense infections, possibly because of an additive and/or multiplicative impact on nutrition and organ pathology (19).

To verify whether pathogen-driven selection has been acting on IL genes, we exploited the fact that a set of >650,000 single-nucleotide polymorphisms (SNPs) has been genotyped in 52 populations (Human Genome Diversity Project-Centre d’Etude du Polymorphisme Humain [HGDP-CEPH] panel: http://www.cephb.fr/en/hgd/) distributed worldwide (Fig. S1) (20). Pathogen richness was evaluated by gathering information on the number of different pathogen species/genera present in different geographical areas of the world from the Gideon database. Specifically, pathogens were divided into two major groups: micro- and macropathogens. Macropathogens include viruses, bacteria, fungi, and protozoa. Macropathogens include insects, arthropods, and helminths; in this group parasitic worms were by far the most abundant class (90% of species/genera), so when we refer to macropathogens we basically mean helminths. Analyses were also separately performed for viruses, bacteria, protozoa, and fungi; data are available as supplemental material (Table S1). After data organization in Gideon, we calculated both micro- and macropathogen richness on a country by country basis (i.e., they represent the number of different micro- and macropathogen species/genera per country; Fig. S1). As expected, we observed that micro- and macropathogen richness strongly correlated across geographic locations (Kendall’s rank correlation coefficient = 0.67; P < 2^{-10}); this is likely caused by the major impact of climatic factors on the spatial distribution of both pathogen classes (21).

IL genes were retrieved from the HGNC Gene Families/Grouping Nomenclature website (http://www.genenames.org/genefamily.html). From the resulting 99 genes, IL3RA and IL9R were removed because they are located on the pseudoautosomal regions of sexual chromosomes; IL15RB and IL11RB were not analyzed because their sequence and chromosomal locations are not present in public databases. Finally, four IL9R pseudogenes were discarded. The remaining 91 genes (Table S2) included most known ILs, their receptors, and receptor-accessory proteins.

A total of 1,052 SNPs in IL genes had been typed in the HGDP-CEPH panel, allowing analysis of all genes except for IL2, IL6R1, IL6STP, IL8RBP, IL17C, IL23A, IL28A, IL28B, IL31, and IL34.

For all 1,052 IL gene SNPs in the dataset, we calculated Kendall’s rank correlation coefficient (r) between allele frequencies in HGDP-CEPH populations and micro- or macropathogen richness; a normal approximation with continuity correction to account for ties was used for p-value calculations. After Bonferroni correction for multiple tests, we observed that 48 and 94 SNPs significantly correlate with micro- and macropathogen richness, respectively, with 32 SNPs correlating with
| SNP       | Gene   | Micropathogens | Macropathogens |
|-----------|--------|----------------|----------------|
| rs17561   | IL1A   | 0.38           | 0.47           |
| rs1143634 | IL1B   | 0.55           | 0.58           |
| rs6761276 | IL1F10 | -0.31          | -0.51          |
| rs10496447| IL1F8  | 0.54           | 0.57           |
| rs3917304 | IL1R1  | -0.53          | -0.51          |
| rs6444444 | IL1RAP | -0.55          | -0.50          |
| rs6800609 | IL1RAP | 0.39           | 0.47           |
| rs2885373 | IL1RAP | 0.55           | 0.50           |
| rs17196143| IL2RAP | -0.49          | -0.51          |
| rs6444435 | IL1RAP | -0.47          | -0.54          |
| rs6630730 | IL1RAPL1| -0.41          | -0.53          |
| rs7052954 | IL1RAPL1| -0.48          | -0.52          |
| rs6526833 | IL1RAPL1| 0.43           | 0.52           |
| rs7890572 | IL1RAPL1| 0.44           | 0.50           |
| rs10521948| IL1RAPL1| 0.49           | 0.54           |
| rs7056388 | IL1RAPL1| 0.41           | 0.51           |
| rs196990 | IL1RAPL1| 0.41           | 0.56           |
| rs7881819 | IL1RAPL1| 0.38           | 0.51           |
| rs10521946| IL1RAPL1| -0.49          | -0.62          |
| rs1318832 | IL1RAPL1| -0.51          | -0.55          |
| rs5943559 | IL1RAPL1| -0.45          | -0.56          |
| rs12387961| IL1RAPL2| 0.37           | 0.46           |
| rs721953  | IL1RAPL2| 0.46           | 0.44           |
| rs1384360 | IL1RAPL2| -0.45          | -0.44          |
| rs6621992 | IL1RAPL2| -0.45          | -0.46          |
| rs7583215 | IL1R1L2| -0.55          | -0.54          |
| rs2287041 | IL1R1L2| 0.51           | 0.47           |
| rs3771188 | IL2RA   | -0.54          | -0.52          |
| rs315931  | IL2RN   | -0.41          | -0.46          |
| rs2637988 | IL2RN   | -0.44          | -0.47          |
| rs2009582 | IL2RN   | -0.30          | -0.46          |
| rs3087266 | IL2RN   | -0.34          | -0.46          |
| rs2386841 | IL2RA   | 0.46           | 0.47           |
| rs11256497| IL2RA   | -0.49          | -0.49          |
| rs2284034 | IL2RB   | 0.37           | 0.48           |
| rs1003694 | IL2RB   | 0.32           | 0.46           |
| rs2289966 | IL2RB   | -0.32          | -0.48          |
| rs2284033 | IL2RB   | 0.37           | 0.56           |
| rs228973  | IL2RB   | 0.44           | 0.52           |
| rs228975  | IL2RB   | -0.49          | -0.59          |
| rs2235330 | IL2RB   | 0.41           | 0.54           |
| rs2243268 | IL4     | -0.51          | -0.58          |
| rs2243288 | IL4     | -0.43          | -0.47          |
| rs2243290 | IL4     | 0.49           | 0.54           |
| rs2070874 | IL4     | -0.50          | -0.55          |
| rs3024672 | IL4R    | -0.40          | -0.53          |
| rs3024607 | IL4R    | -0.48          | -0.53          |
| rs17026370| IL5RA   | 0.47           | 0.55           |
| rs2066992 | IL6     | -0.41          | -0.46          |
Table 1. Correlations with micro- and macropathogen richness (Continued)

| SNP      | Gene  | Micropathogens | Macropathogens |
|----------|-------|----------------|----------------|
| rs2069835 | IL6   | -0.46          | 0.949          | -0.53          | 0.002 | 0.982 |
| rs17505589 | IL7   | 0.50           | 0.986          | 0.43           | 0.115 | 0.851 |
| rs11567697 | IL7R  | 0.43           | 0.904          | 0.51           | 0.005 | 0.972 |
| rs1554286 | IL10  | -0.45          | 0.956          | -0.56          | <0.0001 | 0.994 |
| rs3024490 | IL10  | -0.39          | 0.880          | -0.52          | <0.0001 | 0.988 |
| rs2512144 | IL10RA| -0.38          | 0.832          | -0.47          | 0.009 | 0.938 |
| rs999261  | IL10RB| 0.39           | 0.854          | 0.49           | 0.002 | 0.958 |
| rs2243115 | IL12A | -0.46          | 0.952          | -0.43          | 0.045 | 0.843 |
| rs17129789 | IL12RB2| -0.43         | 0.910          | -0.51          | 0.001 | 0.971 |
| rs10521698 | IL13RA2| -0.40        | 0.860          | -0.50          | 0.003 | 0.956 |
| rs1589241 | IL15  | 0.48           | 0.970          | 0.48           | 0.006 | 0.950 |
| rs2322262 | IL15  | 0.47           | 0.962          | 0.49           | 0.005 | 0.953 |
| rs13106911 | IL15 | -0.36          | 0.811          | -0.46          | 0.007 | 0.925 |
| rs8177636 | IL15RA| 0.39           | 0.858          | 0.47           | 0.006 | 0.942 |
| rs8177685 | IL15RA| -0.41          | 0.881          | -0.47          | 0.004 | 0.931 |
| rs3136614 | IL15RA| -0.47          | 0.957          | -0.40          | 0.125 | 0.798 |
| rs2296139 | IL15RA| 0.44           | 0.933          | 0.53           | <0.0001 | 0.985 |
| rs12437819 | IL16 | -0.50          | 0.981          | -0.41          | 0.071 | 0.846 |
| rs12438640 | IL16  | 0.50           | 0.982          | 0.41           | 0.060 | 0.854 |
| rs10484879 | IL17A| -0.49          | 0.970          | -0.39          | 0.425 | 0.770 |
| rs6518661 | IL17RA| -0.45          | 0.944          | -0.43          | 0.026 | 0.896 |
| rs879576  | IL17RA| 0.45           | 0.931          | 0.50           | 0.004 | 0.953 |
| rs999514  | IL17RB| 0.47           | 0.964          | 0.45           | 0.015 | 0.916 |
| rs708567  | IL17RC| -0.40          | 0.890          | -0.51          | 0.001 | 0.976 |
| rs6445854 | IL17RD| -0.43          | 0.912          | -0.52          | <0.0001 | 0.982 |
| rs4535195 | IL17RD| -0.44          | 0.942          | -0.48          | 0.003 | 0.957 |
| rs12487790 | IL17RD | 0.43        | 0.935          | 0.52           | <0.0001 | 0.983 |
| rs17216900 | IL17RD | 0.45        | 0.946          | 0.36           | 0.368 | 0.779 |
| rs12496746 | IL17RD | -0.46        | 0.951          | -0.42          | 0.048 | 0.866 |
| rs455583  | IL17RE| -0.46          | 0.957          | -0.57          | <0.0001 | 0.996 |
| rs279581  | IL17RE| 0.39           | 0.878          | 0.50           | 0.001 | 0.974 |
| rs279572  | IL17RE| 0.37           | 0.854          | 0.48           | 0.002 | 0.960 |
| rs172155  | IL17RE| -0.37          | 0.847          | -0.48          | 0.003 | 0.953 |
| rs2272128 | IL18RAP| -0.47        | 0.965          | -0.39          | 0.125 | 0.829 |
| rs2243193 | IL19  | 0.42           | 0.919          | 0.50           | 0.001 | 0.973 |
| rs12044804 | IL19 | -0.48          | 0.373          | -0.62          | <0.0001 | 1.000 |
| rs4845143 | IL19  | 0.41           | 0.910          | 0.49           | 0.001 | 0.969 |
| rs12409415 | IL19| -0.45          | 0.928          | -0.49          | 0.002 | 0.945 |
| rs12046559 | IL19 | 0.43           | 0.923          | 0.54           | <0.0001 | 0.989 |
| rs12145973 | IL19 | 0.59          | <0.0001        | 1.000          | 0.42  | 0.106 | 0.820 |
| rs2138992 | IL19  | 0.46           | 0.959          | 0.52           | <0.0001 | 0.984 |
| rs2232360 | IL20  | -0.38          | 0.858          | -0.46          | 0.007 | 0.934 |
| rs1322393 | IL20RA| 0.39           | 0.876          | 0.49           | 0.002 | 0.963 |
| rs1322394 | IL20RA| -0.38          | 0.844          | -0.48          | 0.003 | 0.948 |
| rs75977   | IL20RB| -0.43          | 0.934          | -0.47          | 0.005 | 0.940 |
| rs835634  | IL20RB| 0.43           | 0.932          | 0.46           | 0.008 | 0.929 |
| rs748794  | IL20RB| 0.43           | 0.929          | 0.46           | 0.008 | 0.930 |
| rs12934152 | IL21R| -0.57          | 0.999          | -0.50          | 0.005 | 0.952 |
| rs10903022 | IL22RA1| -0.42        | 0.920          | -0.49          | 0.002 | 0.971 |
| rs10751768 | IL22RA1| 0.42        | 0.926          | 0.50           | 0.001 | 0.974 |
| rs3795302 | IL22RA1| 0.39        | 0.898          | 0.49           | 0.001 | 0.971 |
both. These variants map to a total of 44 IL genes (Table I). We next verified whether the correlations between IL SNP frequencies and pathogens could be secondary to associations with other environmental variables (e.g., climatic factors). Hence, for each geographic location corresponding to HGDP-CEPH populations, the following parameters were obtained: average annual mean and maximum temperature, precipitation rate, and short-wave radiation flux. None of the SNPs reported in Table I significantly correlated with any of these variables (Table S3).

Allele frequency spectra in human populations are affected by selective and nonselective events; whereas selection acts on specific genes, nonselective forces (e.g., demography or distance from Africa [22]) are expected to affect all loci equally. We thus compared the strength of IL gene correlations to sets of control SNPs in the dataset. In particular, for each IL SNP in Table I, we extracted from the full HGDP-CEPH dataset all SNPs having an overall minor allele frequency (averaged over all populations) differing by <0.01 from its frequency; for all SNPs in the frequency-matched groups, we calculated Kendall’s rank correlation coefficient (τ) between micro- and macropathogen richness and allele frequencies. We next calculated the percentile rank of IL gene SNPs in the distribution of Kendall’s τ obtained for the control sets. In most cases (46 for micro- and 66 for macropathogens), percentile ranks higher than the 95th were obtained for IL SNPs. These data indicate that SNPs in IL genes are clearly more strongly influenced by pathogen richness compared with control SNPs, suggesting that selective forces (i.e., pathogen-driven selection), and not nonselective forces, are responsible for the observed associations. All data are gathered in Table I, which shows the compilation of all SNPs that significantly correlate with either micro- or macropathogen richness; the value of τ for both correlations, as well as Bonferroni-corrected P values and percentile ranks, are reported. It is worth noting that data in Table I have been organized to keep together all SNPs in the same gene and to group ligands and receptors; therefore, the order does not reflect greater association with micro- or macropathogens, which can instead be inferred from correlation values.

Another issue that deserves attention relates to the genomic organization of IL genes, because many of them are located in clusters. This raises the possibility that many of the observed allele associations are spurious and derive from linkage to a single selected allele. Yet analysis of linkage disequilibrium (LD; Fig. S2) indicates that linkage is not extensive across gene clusters, with the exception of IL17RE and IL17RC. Therefore, with the exception of these two genes, the remaining loci are independent targets of pathogen-driven selection.

### Balancing selection acts on IL1F5, IL1F7, IL1F10, IL7R, and IL18RAP

Pathogen-driven variations in allele frequencies can occur under different selection scenarios, such as directional or balancing selection. Given the aforementioned results and the role of IL genes in regulating immune responses, we next verified whether selection signatures could be identified at IL genes by using classical population genetic analyses. To this aim, we exploited the observation that 68 out of 91 IL loci have been included in genetic variation projects (i.e., the SeattleSNPs program and the Innate Immunity in Heart, Lung and Blood Disease Programs for Genomic Applications) so that resequencing data (although with some gaps) are available in at least two populations: one with European ancestry (EU) and one with African ancestry (either Yorubans [YRI] or African Americans [AA]). Common population genetics tests include Tajima’s D (D$_T$) (23) and Fu and Li’s D* and F* (24). D$_T$ tests the departure from neutrality by comparing two nucleotide diversity indexes: θ$_w$ (25), which is an estimate of the expected per site heterozygosity, and π (26), which is the average number of pairwise sequence nucleotide differences. Positive values of D$_T$ indicate an excess of intermediate frequency variants and are a hallmark of balancing selection; negative D$_T$ values indicate either purifying selection or a high representation of rare variants as a result of a selective sweep. Fu and Li’s F* and D* (24) are also based on SNP frequency spectra and differ from D$_T$ in that they also take into account whether mutations occur in external or internal branches of a genealogy. Population history, in addition to selective processes, affects frequency spectra and all related statistics; for this reason, statistical significance was evaluated by performing coalescent simulations using a population genetics model that

### Table I. Correlations with micro- and macropathogen richness (Continued)

| SNP        | Gene      | Microorganisms | Macropathogens |
|------------|-----------|----------------|----------------|
| rs4486393  | IL22RA1   | 0.39           | 0.146          | 0.863 | 0.50 | 0.002 | 0.968 |
| rs4292900  | IL22RA1   | 0.40           | 0.109          | 0.882 | 0.47 | 0.005 | 0.947 |
| rs16829209 | IL22RA1   | 0.39           | 0.156          | 0.856 | 0.50 | 0.002 | 0.959 |
| rs11570915 | IL26      | 0.40           | 0.132          | 0.852 | 0.53 | 0.001 | 0.980 |
| rs3814240  | IL26      | 0.42           | 0.026          | 0.929 | 0.49 | 0.002 | 0.964 |
| rs3814241  | IL26      | 0.50           | 0.001          | 0.982 | 0.47 | 0.004 | 0.946 |
| rs10878789 | IL26      | 0.47           | 0.004          | 0.962 | 0.41 | 0.052 | 0.868 |
| rs9632389  | IL31RA    | −0.48          | 0.005          | 0.960 | −0.43 | 0.058 | 0.837 |
| rs10055201 | IL31RA    | 0.39           | 0.120          | 0.869 | 0.45 | 0.009 | 0.927 |
| rs1554999  | IL32      | −0.48          | 0.002          | 0.965 | −0.55 | <0.0001 | 0.988 |

*Bonferroni-corrected p-value.
*Percentile rank relative to the distribution of SNP control sets matched for allele frequency.
incorporates demographic scenarios (27). Simulations were performed using the cosi package (27) and were used to derive a p-value that indicates whether or not the value obtained for a given IL locus is expected under a given demographic scenario; a significant P value indicates that the obtained value is unlikely under the specified conditions and, therefore, that neutrality can be rejected.

Another method of figuring out the effects of selection and population history again lies in the assumption that selection acts on a single locus, whereas demography affects the whole genome; therefore, we calculated test statistics for 238 genes resequenced by the National Institute of Environmental Health Science (NIEHS) program (Table S4). These empirical distributions were used to calculate the percentile rank of D_f, D*, and F* values for analyzed IL genes; this procedure provides a direct comparison of the locus under analysis with a sample of human genes and allows an estimation of how unusual the value obtained is (e.g., a percentile rank of 0.99 suggests that neutral evolution is unlikely).

5 of the 68 IL genes available for population genetic analysis gave significant results in at least one population; three of them (IL1F10, IL7R, and IL18RAP) also display at least one SNP correlating with pathogen richness (Table I). Data concerning θ_W and π, as well as D_f, D*, and F*, are reported in Table II. For IL1F5, IL1F7, and IL1F10, θ_W and π were close to or higher than the 95th percentile in the distribution of NIEHS genes in both populations, indicating an excess of polymorphisms at these loci; conversely, no exceptional θ_W and π were observed for IL18RAP in AA and for IL7R in either population. Calculation of D_f, D*, and F* for the five genes indicated that one or more statistics rejected neutrality in both Africans and Europeans for IL1F5, IL1F10, and IL18RAP; conversely, unusually high values were obtained only for YRI and EU in the case of IL1F7 and IL7R, respectively. Overall, these data suggest the action of balancing selection. It should be noted that IL7R is encompassed by a copy number variant (CNV; http://projects.tcag.ca/variation/); yet the CNV only occurs in 1 out of 110 chromosomes, and thus should not affect our results.

Another commonly used test to verify departure from selective neutrality is the Hudson, Kreitman, and Aguade (HKA) test (28); it is based on the assumption that under neutral evolution, the amount of within-species diversity correlates with levels of between-species divergence because both depend on the neutral mutation rate. An excess of intraspecific diversity compared with divergence (k > 1) is considered a signature of balancing selection. Here, we performed a maximum likelihood HKA test (MLHKA) (29) by comparing each IL gene in Table II to 16 neutrally evolving genes resequenced in the same individuals (see Materials and methods for details). The MLHKA test rejected the neutral evolution model for IL1F5 and IL7R (in both populations), as well as IL1F10 (in YRI), but not for IL1F7 and IL18RAP. Indeed, in the latter case, interspecific divergence higher than the genome average paralleled the high levels of intraspecific diversity (unpublished data).

Population genetic diversity, measured as F_ST, can also provide information on selective processes. Under selective neutrality, F_ST is determined by genetic drift, which is mainly accounted for by demographic history and similarly affects all genom loci. Conversely, natural selection being a locus-specific force, it can affect F_ST values for specific genes. Balancing selection may lead to a decrease in F_ST compared with neutrally evolving loci (8); specifically, low F_ST values among continental populations strongly suggests the action of balancing selection worldwide (i.e., irrespective of local environmental

### Table II. Summary statistics for five IL genes

| Gene   | L* | P* | N* | S* | θ* | π* | D_f | D* | F* |
|--------|----|----|----|----|----|----|-----|----|----|
|        |    |    |    |    |    |    | Value | Value | Value |
| IL1F5  | 6.4| YRI| 48 | 17.61| 21.73| 0.81| 0.033| 0.97 | 0.43 | 0.11 | 0.85 | 0.68 | 0.045| 0.93 |
|        |    | EU | 46 | 39 | 13.87 | 25.33 | 2.84 | 0.0004 | >0.99 | 1.68 | 0.0018 | >0.99 | 2.49 | 0.0002 | >0.99 |
| IL1F7  | 6.1| YRI| 48 | 16.57 | 22.07 | 1.14 | 0.016 | >0.99 | 0.82 | 0.037 | 0.95 | 1.11 | 0.012 | 0.98 |
|        |    | EU | 46 | 35 | 13.02 | 8.69 | −1.13 | 0.10 | 0.15 | 1.18 | 0.046 | 0.96 | 0.43 | 0.28 | 0.69 |
| IL1F10 | 4  | YRI| 48 | 19.36 | 18.98 | −0.066 | 0.21 | 0.76 | 1.15 | 0.012 | 0.98 | 0.85 | 0.027 | 0.95 |
|        |    | EU | 46 | 16 | 13.92 | 12.68 | 1.19 | 0.070 | 0.89 | 1.59 | 0.0041 | >0.99 | 1.72 | 0.0090 | 0.99 |
| IL18RAP| 17.8| AA | 48 | 97 | 12.30 | 14.24 | 0.56 | 0.039 | 0.95 | 0.69 | 0.013 | 0.94 | 0.77 | 0.011 | 0.96 |
|        |    | EU | 46 | 88 | 11.27 | 15.56 | 1.36 | 0.057 | 0.91 | 1.26 | 0.0099 | 0.97 | 1.54 | 0.013 | 0.98 |
| IL7R   | 22 | AA | 48 | 119 | 12.80 | 9.94 | −0.80 | 0.42 | 0.39 | 0.26 | 0.10 | 0.82 | −0.16 | 0.21 | 0.71 |
|        |    | EU | 46 | 70 | 7.60 | 10.33 | 1.28 | 0.084 | 0.90 | 1.53 | 0.0029 | >0.99 | 1.71 | 0.011 | 0.99 |

- **L*:** Length of analyzed resequenced region (in kilobasepairs).
- **P*:** Population.
- **N*:** Sample size (chromosomes).
- **S*:** Number of segregating sites.
- **θ_W:** Estimation per site (×10^-3).
- **π:** Estimation per site (×10^-4).
- **P-values obtained by applying a calibrated population genetics model, as described in the text.
- **Percentile rank relative to the distribution of values obtained for 238 NIEHS genes (i.e., comparison with an empirical distribution).
pressures), whereas reduced population differentiation within continents is consistent with a locally exerted selective pressure resulting in balancing selection. We calculated $F_{ST}$ for the 5 IL genes and compared it to the distribution of this parameter among 238 NIEHS genes. Unusual $F_{ST}$ values were only observed for IL18RAP; in this case, a negative $F_{ST}$ was obtained, indicating that the real value is close to 0, therefore corresponding to a percentile rank lower than the 2.5th.

One other effect of balancing selection is the maintenance of divergent haplotype clades separated by deep coalescence times (30). We therefore studied haplotype genealogies by constructing median-joining networks. In particular, regions displaying low recombination rates were selected (see Materials and methods and Fig. S3); in the case of IL18RAP and IL1F10, network analysis was not performed because of the low LD throughout the gene region.

Haplotype genealogies for IL1F5, IL1F7, and IL7R revealed two major clades separated by long branches (Fig. 1), each containing common haplotypes. To estimate the time to the most recent common ancestor (TMRCA) of the haplotype clades, we applied a phylogeny-based method (31) based on the measure $\rho$, which is the average pairwise difference between haplotypes and a root. TMRCA estimates of 2.76 million years (MY; SD = 660 KY), 2.10 MY (SD = 404 KY), and 1.68 MY (SD = 408 KY) were obtained for IL1F5, IL1F7, and IL7R, respectively. In all cases, we verified these results using GENETREE, which is based on a maximum-likelihood coalescent analysis (32). Consistently, the resulting gene trees, rooted using the chimpanzee sequences, are partitioned into two deep branches (Fig. 2). Using this method, a second estimate of the TMRCA for the three genes was obtained (Fig. 2 and Table S5). Estimates of coalescence times for neutrally evolving autosomal human loci range between 0.8 and 1.5 MY (33); the TMRCA values we estimated for IL1F5, IL1F7, and IL7R, although not exceptional, are deeper than for most neutrally evolving loci. Again, this finding suggests the action of balancing selection (30).

Heterozygote advantage (also known as overdominance) is one of the possible causes of balancing selection. To verify whether this is the case for the five selected IL genes, for each population we calculated the ratio of observed heterozygosity to expected gene diversity. This same ratio calculation has recently been applied to human HapMap SNPs, and threshold values for inference of overdominance have been set to 1.160 and 1.165 for YRI and EU, respectively (34). To obtain an additional estimate of this parameter distribution in the human genome, ratios were also calculated for NIEHS genes. Whereas all other genes showed nonexceptional values, the observed heterozygosity to expected gene diversity ratio for IL1F5 amounted to 1.07 and 1.20 for YRI and EU, respectively. This value is higher than the previously set threshold for EU and falls above the 99th percentile value obtained from NIEHS genes in this same population.

Overall, these data strongly suggest the action of balancing selection on these 5 IL genes reported in Tables II and III; it is worth mentioning that the MLHKA test failed to reject neutrality for IL1F7 and IL18RAP, suggesting that relaxed constraint rather than balancing selection might be responsible for the observed high $D_T$, $D^*$, and $F^*$ values. Still, we noticed that the 6 polymorphisms within IL1F7 exons in YRI are all

Figure 1. Haplotype genealogy for IL1F5, IL1F7, and IL7R gene regions. The analyzed regions correspond to the largest LD block for each gene (Fig. S2). Each node represents a different haplotype, with the size of the circle proportional to the haplotype frequency. Nucleotide differences between haplotypes are indicated on the branches of the network. Circles are color-coded according to population (gray, AA or YRI; white, EU). The chimpanzee sequence is also shown (black). Fig. S2 is available at http://www.jem.org/cgi/content/full/jem.20082779/DC1.
accounted for by nonsynonymous substitutions; application of the MK tests (35) using orangutan for divergence yielded a p-value of 0.058. Although not fully significant, this result indicates an unusual frequency of replacement polymorphic variants and, together with the deeper-than-average TMRCA, is in line with balancing selection rather than with functional relaxation.

With respect to IL18RAP, the extremely low $F_{ST}$ value we observed (Table III) is not consistent with relaxed functional constraints, but instead supports the idea that a balanced polymorphism is maintained in AA and EU, suggesting response to a widespread selective pressure (i.e., not local adaptation). Finally, it should be noted that IL1F5 and IL1F10 are located nearby, raising the possibility that the signatures we observe at both gene regions are caused by hitchhiking with a single functional variant; yet, LD is low across the region because of the presence of a recombination hotspot within the IL1F10 gene region. Moreover, although hitchhiking has the potential to affect large genomic regions, the signatures of balancing selection are predicted to extend over relatively short distances (36, 37). We therefore consider that the two genes might be independent selection targets.

**Risk alleles for inflammatory bowel and celiac disease correlate with pathogen-richness**

Previous analyses showed that a polymorphism (rs917997) located ~1,500 bp downstream of IL18RAP is significantly associated with both celiac disease (CeD) and inflammatory bowel disease (IBD; with the SNP also influencing the level of gene expression (38, 39). This variant was not included in our analysis of pathogen correlations because of its location outside the gene region (as defined in Materials and methods). Still, we observed that rs917997 is in strong LD ($D^' = 1$ and 0.87 in EU and YRI, respectively) with rs2272128, which we found to correlate with macro- and micropathogen richness. We therefore checked the presence of rs917997 and verified that the risk allele for CeD and IBD also correlates significantly with pathogen richness (Table IV). Stimulated by this finding, we next verified whether the frequency distribution of other

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**Table III.** MLHKA test and $F_{ST}$ for five IL genes

| Gene | Population | $P$-value | MLHKA | $F_{ST}$ (rank) |
|------|------------|-----------|-------|----------------|
| IL1F5 | YRI        | 2.92      | 0.0043| 0.13 (0.53)    |
|       | EU         | 2.85      | 0.0032|                |
| IL1F7 | YRI        | 1.82      | 0.29  | 0.28 (0.89)    |
|       | EU         | 1.91      | 0.29  |                |
| IL1F10| YRI        | 2.38      | 0.017 | 0.16 (0.62)    |
|       | EU         | 1.46      | 0.56  |                |
| IL18RAP| AA         | 1.18      | 0.22  | 0 (<=0.025)    |
|       | EU         | 1.62      | 0.38  |                |
| IL7R  | AA         | 2.53      | 0.0042| 0.019 (0.084)  |
|       | EU         | 2.14      | 0.028 |                |

*Population.*

*Percentile rank relative to the distribution of $F_{ST}$ values calculated for 238 NIEHS genes.

*Selection parameter.*
sustainability alleles in IL genes has been influenced by pathogen richness. To analyze only variants that have been identified in an unbiased manner (i.e., without a priori hypothesis on the genes involved), we searched among published genomewide association studies for SNPs in IL genes that have been associated with any trait. For available variants in the CEPH-HGDP panel, we next calculated correlations with micro- and macropathogen richness. Results reported in Table IV indicate that six out of nine risk variants for CeD or IBD/Crohn’s disease (CD) were associated with micro- and macropathogen richness; in particular, two of them located within IL23R are in tight LD, and thus do not represent independent observations. Noticeably, in all six cases, the risk allele correlates with pathogen richness and correlations with micropathogens were stronger compared with macropathogens (with the exception of rs11465804), a situation different from the general observation that macropathogens have represented a more powerful selective pressure on IL genes.

**DISCUSSION**

We analyzed the recent evolutionary history of IL genes in humans by integrating information on environmental variables with classical population genetics and association studies. Results herein suggest that microbes and parasitic worms played a relevant role as selective agents, but the pressure imposed by helminths on IL genes has been stronger than the one caused by viral and microbial agents. Helminths were present among our ancestors before the emergence of humans as a species (for review see reference [40]). These parasites evolve at lower rates than viruses and bacteria and, in contrast to most viral/microbial agents, are able to maintain themselves in small human communities (41). Notably, by establishing chronic infections, parasitic worms affect the susceptibility of their host to viruses, bacteria, and protozoa (for review see reference [2]). Therefore, helminths might have represented a stable threat to human populations and their distribution, which is not associated with sudden epidemics and, as in the case of micropathogens, might have left stronger genetic signatures.

A limitation of our study is that we implicitly assumed that the number of different pathogen species/genera per country has been maintained proportionally unchanged along human evolutionary history. Although clearly an oversimplification, this might reflect reality to some degree, given that climatic variables (e.g., precipitation rates and temperature) have a primary importance in driving the spatial distribution of human micro- and macropathogens (21). Therefore, while the fitness cost imposed by specific species/genera might have evolved rapidly, the relative number of pathogen species per country might have changed proportionally less.

Another possible caveat of our results concerns the definition of “pathogen,” in that we included any organism that can cause a disease irrespective of its virulence or pathogenicity. The reason for this choice is that the fitness of a pathogen is a direct measure of the ability of such pathogen to replicate within a given environment. Fitness is dependent on both the features of the pathogen and of the host. The features of the

**Table IV.** Correlations with micro- and macropathogen richness for SNPs associated with different traits

| SNP       | Gene/location | Allele | Micropathogen | Macropathogen | Trait/disease               |
|-----------|---------------|--------|---------------|---------------|-----------------------------|
| rs6897932 | IL7R          | C      | 0.22          | n.s.           | Multiple sclerosis/1 diabetes |
| rs917997  | IL18RAP       | A      | 0.42          | <0.001        | CeD/IBD                     |
| rs10045431| IL12B         | C      | 0.43          | <0.001        | CD                          |
| rs7517847 | IL23R         | C      | 0.23          | n.s.           | IBD                         |
| rs11209026| IL23R         | G      | 0.47          | <0.001        | IBD                         |
| rs11465804| IL23R         | T      | 0.39          | 0.004         | CD                          |
| rs6822844 | IL2/IL21 (intergenic) | G | 0.40          | 0.004         | CeD                         |
| rs3024505 | IL10          | T      | -0.28         | n.s.           | Ulcerative colitis           |
| rs17810546| IL12A         | G      | 0.03          | n.s.           | CeD                         |
| rs13015714| IL18R1       | C      | 0.47          | <0.001        | CeD                         |
| rs2250417 | IL18          | A      | 0.23          | n.s.           | Protein quantitative trait loci |
| rs7626795 | IL1RAP        | G      | 0.20          | n.s.           | Lung cancer                  |
| rs4129267 | IL6R          | C      | -0.17         | n.s.           | Protein quantitative trait loci/pulmonary function |
| rs6761276 | IL1F10        | n.s.   | -0.31         | 0.030         | Protein quantitative trait loci |
| rs12251307| IL2RA         | T      | -0.16         | n.s.           | Type 1 diabetes              |

*Ancestral state based on chimpanzee sequence.*

*The correlation coefficient is calculated between pathogen richness and the risk allele frequency;
*Bonferroni-corrected p-value (15 tests).
*Nonsignificant (P > 0.05).*

*This SNP is located within IL1F10, but affects IL1RN protein levels.*

*Not reported.*
Pathogen include its abilities to (a) replicate within the host, (b) select a proper cell target (tropism), (c) avoid the immune response, and (d) escape the obstacles posed by drugs. The features of the host include the immune response, the genetic background, and the availability of target cells. The reciprocal balance between these factors determines the virulence of a pathogen, a feature that, therefore, cannot be considered as a constant, but rather evolves dynamically over time.

Despite these limitations, we were able to identify several variants that are likely candidates for pathogen-driven selection, and the suitability of this approach is confirmed by the previous demonstration that variability at HLA genes correlates with a similar measure of pathogen richness (10). Our data point to the SNPs in Table I as good candidates for experimental analyses aimed at inferring their role in IL gene function, given that a signature of natural selection necessarily implies the presence of a functional variant (either the correlated variant itself or a linked one). Also, genes subjected to a selective pressure from infectious diseases should be regarded as candidates for genetic epidemiology studies (e.g., case-control studies).

It is worth noting that most members of the IL-1 signaling pathway were observed to correlate with pathogen richness. IL-1A and IL-1B are pleiotropic cytokines with central roles in immune and inflammatory responses (43) required for the development of Th2-mediated immunity and protection against chronic infection in mice (44). The observation that SNPs strongly correlated with micro- and macro-pathogen richness map to IL1RAPL1 is more puzzling, as this gene is not known to be associated with infection and immune response, but is involved in brain development and function (45); nonetheless, this gene is expressed in tissues that are different from brain (46), suggesting that it plays other roles apart from neurodevelopment.

Strong correlations with macropathogen richness were obtained for IL4, IL4R, and IL10. These molecules are pivotal to the elicitation of Th2 response, which is the immune response central to helminth resistance (40, 47). The strongest correlation with macropathogens was observed for rs12044804 in IL19 (rs12044804). This gene is located within an IL cluster, which also comprises IL10 and IL20, and is encompassed by a low-frequency CNV. The low levels of LD across the IL cluster (Fig. S2) suggest that IL19, IL20, and IL10 SNPs are independently associated with pathogen richness. IL19, IL20, IL22, IL24, and IL26 all belong to the IL-20 subfamily, are all produced by different leukocyte populations, and all bind to partially shared receptors that are mainly expressed by epithelial cells and known to promote keratinocyte growth and to induce skin inflammatory responses (48, 49). The correlation of SNPs in most of these genes with macropathogen and helminth richness suggests that modulation of skin immunological properties might represent an adaptive response to parasite species that infect humans through the skin.

SNPs in IL2RB, IL15, and IL15RA also correlated with macropathogen richness; these genes converge on the same pathway as IL-2RB and IL-15RA are part of a trimeric complex that binds IL-15 (50). IL-15 has a central role in intestinal inflammatory processes and in the pathogenesis of CD and CeD (51), possibly participating in the immune protection of gut tissues. An interesting observation is that IL-2RB, via IL-15 binding, regulates the intestinal epithelial barrier by transducing signals that result in tight junction formation (52). Variation of mucosal permeability after nematode infection is a host defense response and is important for efficient parasite expulsion (53); the correlation we observed between SNPs in these genes and macropathogen richness might indicate selection for improved intestinal clearance of nematodes. Again, mouse models will prove central in addressing the role of these loci in parasite expulsion; e.g., in the case of IL4R, which also correlates with helminth richness (Table I), treatment with antibodies or use of il4r-deficient mice prevented expulsion of Heligmosomoides polygyrus, Trichuris muris, and Nippostrongylus brasiliensis (54).

A major locus for susceptibility to Schistosoma mansoni infection (SM1) has previously been mapped to 5q31-q33 (55). This region covers IL4, IL5, IL3, IL13, IL9, and IL12B, as well as other candidate loci (IRF1 and CSF2). Although our data do not allow inference on which gene is responsible for increased susceptibility to schistosomiasis, it is worth noting that four SNPs in IL4 display very strong correlation with helminth richness. IL4 might therefore be regarded as a candidate locus for susceptibility to S. mansoni infection, with dedicated association studies being required to verify this prediction. Conversely, the SM2 region (6q22-q23), where a locus controlling hepatic fibrosis in S. mansoni infection has been mapped (56), harbors no IL genes.

Pathogen-driven variations in allele frequencies can occur under different selection scenarios, such as directional or balancing selection; the latter itself is the result of an initial stage of positive selection that favors the spread in a population of a new allele until selection opposes its fixation and a balanced situation is established. Common causes of balancing selection include heterozygote advantage, changing environmental conditions, and frequency-dependent selection, all of these possibly applying to host–pathogen interactions. Also, given the pleiotropic roles of many IL genes, selective pressures different from pathogen richness might affect the evolutionary fate of these loci. Classical population genetics analyses indicated that five IL genes are likely targets of balancing selection. IL1F5, IL1F7, and IL1F10 are recently discovered IL-1 family members (57) that are located within the IL1 gene cluster; based on comparative analyses, IL-1F5 and IL-1F10 are predicted to act as antagonists (58). IL1F5 is a regulator of skin and brain inflammation (59, 60) and it is expressed in many different human tissues (57). Interestingly, an excess of heterozygotes was observed for IL1F5, suggesting that overdominance might underly the maintenance of a balanced polymorphism in the gene. Overdominance is rare in humans (36, 61) and is hypothesized to enhance immune response flexibility by modulating allele-specific gene expression in different cell types and in response to diverse stimuli/cytokines (62). Whether this is the case for IL1F5 remains to be verified.
**IL1F10** is a still relatively unknown protein mainly expressed in skin, proliferating B cells, and tonsils (63). One of the intermediate frequency SNPs in the gene is accounted for by a missense substitution that replaces an aspartic acid residue with an alanine (Asp51Ala); the presence of a negatively charged residue at this position is conserved among mammals (Fig. S4), possibly suggesting functional significance and awaiting experimental testing.

In analogy to **IL1F5**, the other IL1 family member we identified as rejecting neutral evolution, i.e., **IL1F7**, also acts as an antinflammatory molecule (64).

**IL1F5**, **IL1F7**, and **IL1F10** are not known to be involved in human diseases; in contrast, **IL18RAP** and **IL7R** play a role in triggering immune responses. The IL-7/IL-7R ligand-receptor pair is central to the proliferation and survival of B and T leukocytes. We identified one SNP in the gene as highly correlated with macropathogen richness (Table I). This is not surprising given the role of Th2 responses in helminth infection and the involvement of IL-7R in the TSLP signaling pathway (65), which in turn regulates Th2-mediated inflammatory responses (66).

Similar to **IL7R**, **IL18RAP** plays a known role in human pathology. The gene encodes a component of the protein complex involved in transducing IL-18 signal, resulting in the activation of NF-κB (67). The IL-18 receptor complex is expressed in the intestine (38), and one SNP immediately downstream **IL18RAP** (rs917997) has been associated with both CeD and IBD (38, 39). We found the predisposing allele of rs917997 and a linked variant (rs2272128) to correlate with pathogen richness. The location of rs2272128 in the 5′ gene region and its strong correlation with pathogens might suggest that it (rather than rs917997) represents or is in close LD with the functional allele. Moreover, the correlation of a risk allele for autoimmune diseases with pathogen-richness suggests an interesting link between adaptation and disease. Indeed, we observed that five more risk alleles for either IBD or CeD significantly correlate with micro- and macropathogen richness. Albeit preliminary, these data suggest that infectious agents have shaped the genetic variation at IL loci involved in intestinal inflammatory processes and, as a consequence, the genetic predisposition to both CeD and CD/IBD.

A north–south gradient for IBD prevalence has been described in both the US and Europe (68). This observation, together with the increase of IBD prevalence in the last 40 yr (68) and the hypothesis that helminths elicit Th2-mediated responses, led to the proposal that lower exposure to parasitic worms in the setting of industrialized countries results in unbalanced immune response, and eventually predisposes to IBD (68, 69). The so-called hygiene hypothesis, which clearly implies evolutionary considerations concerning human–pathogen interactions, has been supported by recent studies in both humans and mice (40, 68–70). Data herein seem to indicate that a portion of CeD- and IBD-predisposing alleles have been selected by micropathogen richness, pointing to an adaptive role for these variants. Although not directly supportive of the “IBD hygiene hypothesis,” these results indicate a higher disease predisposition in subjects carrying IL SNP variants that confer stronger protection against viruses/bacteria and therefore likely elicit more vigorous Th1 responses. Living conditions in industrialized countries have resulted in a reduction of both helminth and bacterial/viral infection. The effect of this environmental change on the homeostasis of immune responses might be difficult to reconcile with simple theories (71). In this complex scenario, we consider that evolutionary studies and population genetics approaches, such as the one proposed here, provide some insight into the genetic basis of predisposition to infectious and autoimmune diseases.

**MATERIALS AND METHODS**

**Data retrieval and haplotype construction.** Data concerning the HGDP-CEPH panel derive from a previous work (20). A SNP was ascribed to a specific gene if it was located within the transcribed region or no further than 500 bp upstream the transcription start site.

Genotype data for resequenced IL genes were retrieved from the Seattle-SNP (http://pga.mbb.washington.edu) and Innate Immunity PGA (http://innateimmunity.net/) web sites. A total of 68 genes were available for analysis. For each gene, genotypes deriving from 24 subjects of African ancestry and 23 of Caucasian ancestry were retrieved.

Genotype data for 236 resequenced human genes were derived from the NIEHS SNPs Program web site (http://eggr.gr.washington.edu). We specifically selected genes that had been resequenced in populations of defined ethnicity (NIEHS panel 2).

Haplotypes were inferred using PHASE version 2.1 (72), a program for reconstructing haplotypes from unrelated genotype data through a Bayesian statistical method.

Recombination rates were derived from the University of California at Santa Cruz genome browser web site (http://genome.ucsc.edu). Information concerning CNVs was derived from the database of genomic variants (http://projects.tcag.ca/variation/).

Variants and risk alleles identified in genome-wide association studies were retrieved from the National Human Genome Research Institute web site (http://www.genome.gov/) updated on December 1, 2008.

**Statistical analysis.** **D**<sub>1</sub> (23), Fu and Li’s D* and F* (24) statistics, and diversity parameters **θ**<sub>W</sub> (25) and **π** (26) were calculated using hudson’s software (73). Coalescent simulations were performed using the cobalt package (27) and its best-fit parameters for YRI, AA, and EU populations with 10<sup>5</sup> iterations. cosi is a simulation package based on a population genetics model calibrated on empirical data; it therefore allows incorporation of demographic scenarios in simulations.

The **F**<sub>ST</sub> statistic (74) estimates genetic differentiation among populations and was calculated as previously proposed (75).

The maximum likelihood ratio **HKA** test was performed using the ML-HKA software (29) as previously described (18). In brief, we used multilocus data of 16 selected genes and Pan troglodytes (NCBI panTro2) as an outgroup (except for **IL1F7**, where **Pongo pygmaeus abelii**, NCBI ponAb2, was used as the outgroup). The 16 reference genes were randomly selected among NIEHS loci <20 kb that have been resequenced across panel 2; the only criterion was that no reference gene rejected the neutral model (i.e., that no gene yielded significant **D**<sub>1</sub>). The reference locus used was as follows: **VNN3**, **PLA2G2D**, **MB**, **MAD2L2**, **HRAS**, **CYP17A1**, **ATOX1**, **BNIP3**, **CDC20**, **NGB**, **TUBA1**, **MT3**, **NUDT1**, **PRDX5**, **RET**, and **JUND**.

LD analyses were performed using haploview (76), and haplotype blocks were identified through an implemented method.

Median-joining networks to infer haplotype genealogy were constructed using NETWORK 4.5 (31). Estimate of the TMRCa was obtained using a phylogenoy-based approach implemented in NETWORK using a mutation rate based on the number of fixed differences between human and chimpanzee or orangutan and assuming a separation time from humans of 6 MY and 13 MY ago, respectively. A second TMRCa estimate was derived from application of a maximum-likelihood coalescent method implemented
in GENETREE (32). Again, the mutation rate $\mu$ was obtained on the basis of the divergence between human and a primate, assuming a generation time of 25 yr. Using this $\mu$ and the maximum likelihood $\theta (\theta_{ML})$, we estimated the effective population size parameter ($N_e$). With these assumptions, the coalescence time, scaled in $2N_e$ units, was converted into years. For the coalescence process, 10$^6$ simulations were performed. All calculations were performed in the R environment (www.r-project.org).

Environmental variables. Pathogen absence/presence matrices for the 21 countries where HGDP-CEPH populations are located were derived from the Gideon database (http://www.gideononline.com) following previous methods (10, 18). Information in Gideon is updated weekly and derives from WHO reports, National Health Ministries, PubMed searches, and epidemiology meetings. The Gideon Epidemiology module follows the status of known infectious diseases globally, as well as in individual countries, with specific notes indicating the disease’s history, incidence, and distribution per country. We manually curated pathogen absence/presence matrices by extracting information from single Gideon entries. These may refer to either species or genera (in case data are not available for different species of a same genus). Following previous suggestions (10, 18), we recorded only species/ genera that are transmitted in the 21 countries, meaning that cases of transmission caused by tourism and immigration were not taken into account; also, species that have recently been eradicated as a result, for example, of vaccination campaigns, were recorded as present in the matrix. A total of 283 pathogen species were retrieved (Table S6). Other environmental variables such as average annual mean and maximum temperature, precipitation rate, and short-wave radiation flux were derived for the geographic coordinates corresponding to HGDP-CEPH populations from the NCEP/NCAR database (http://www.cdc.noaa.gov/PublicData/).

Online supplemental material. Table S1 shows correlations between the richness of viruses, bacteria, protozoa, and fungi. Table S2 is a list of IL genes resequenced by the NIEHS program. Table S5 shows GENETREE block for IL-1F10. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20082779/DC1.

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