Report

Proportioning Whole-Genome Single-Nucleotide–Polymorphism Diversity for the Identification of Geographic Population Structure and Genetic Ancestry

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The identification of geographic population structure and genetic ancestry on the basis of a minimal set of genetic markers is desirable for a wide range of applications in medical and forensic sciences. However, the absence of sharp discontinuities in the neutral genetic diversity among human populations implies that, in practice, a large number of neutral markers will be required to identify the genetic ancestry of one individual. We showed that it is possible to reduce the amount of markers required for detecting continental population structure to only 10 single-nucleotide polymorphisms (SNPs), by applying a newly developed ascertainment algorithm to Affymetrix GeneChip Mapping 10K SNP array data that we obtained from samples of globally dispersed human individuals (the Y Chromosome Consortium panel). Furthermore, this set of SNPs was able to recover the genetic ancestry of individuals from all four continents represented in the original data set when applied to an independent, much larger, worldwide population data set (Centre d'Etude du Polymorphisme Humain–Human Genome Diversity Project Cell Line Panel). Finally, we provide evidence that the unusual patterns of genetic variation we observed at the respective genomic regions surrounding the five most informative SNPs is in agreement with local positive selection being the explanation for the striking SNP allele-frequency differences we found between continental groups of human populations.

The identification of geographic population (sub)structure is an important prerequisite for finding genes of complex traits through association mapping (Ziv and Burchard 2003; Freedman et al. 2004; Marchini et al. 2004; McKeigue 2005), and the identification of genetic ancestry and recent genetic admixture is crucial for admixture mapping (Chakraborty and Weiss 1988; Parra et al. 1998; Montana and Pritchard 2004). Furthermore, DNA testing in forensics can potentially use genetic ancestry identification to predict the geographic origin of a person, which might help police direct investigations (Shriver and Kittles 2004; Ray et al. 2005). However, finding genetic markers that clearly differentiate populations has turned out to be difficult in practice, because the neutral genetic diversity in human populations tends to be distributed throughout the worldwide continents without sharp discontinuities (Cavalli-Sforza et al. 1994; Ramachandran et al. 2005) and with only small discontinuities due to geographic barriers (Rosenberg et al. 2005). Although small, the observed genetic differentiation between continents is statistically significant (Romualdi et al. 2002), suggesting that, if the number of loci analyzed is large enough, it may be possible to correctly infer the geographic origin of an individual (Edwards 2003). However, this weak genetic differentiation also implies that powerful clustering algorithms are required to detect existing genetic population (sub)structure by use of a large number of markers, but the final result is going to depend not only on the number of markers used but also on the evolutionary assumptions of the procedure applied (Corander et al. 2004).

Despite that it has been shown that nonrecombining markers from paternally inherited Y chromosomes and maternally transmitted mtDNA are highly suitable for detecting geographic population (sub)structure and genetic ancestry (Jobling and Tyler-Smith 2003; Jobling et al. 2004), their analysis can yield conflicting results for populations that have experienced sex-mediated genetic

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admixture throughout their history (Carvalho-Silva et al. 2001). Thus, it seems logical to use autosomal genetic markers in addition to sex-specific markers to correctly identify geographic population structure and genetic ancestry. Elsewhere, it has been shown that individuals from different geographic origins can be classified according to their continental region of sampling by use of the genetic information of several hundred autosomal microsatellites (Rosenberg et al. 2002, 2003) as well as autosomal *Alu*-insertion polymorphisms and microsatellites (Bamshad et al. 2003). Although the number of microsatellites can be reduced to ~40 when the statistical parameter I_n ("informativeness of assignment" index) is applied to marker ascertainment (Rosenberg et al. 2003), their relatively high mutation rates (Kayser et al. 2000; Holtkemper et al. 2001; Xu and Fu 2004) keeps the number of markers relatively high. In principle, the number of genetic markers could be reduced by using SNPs that mutate ~100,000 times more slowly than do microsatellites (Thomson et al. 2000). Recent studies suggest that there is a considerably large number of autosomal SNPs showing a geographically restricted allele-frequency distribution (Hinds et al. 2005). However, only a small number of populations from a small number of geographic regions have been analyzed so far (HapMap 2003; Hinds et al. 2005).

In this study, we used global whole-genome SNP variation and a newly developed ascertainment algorithm to identify a minimal set of markers with maximal ability to detect geographic population structure and genetic ancestry. In principle, genetic markers with the largest genetic distances between populations—determined, for example, by applying the genetic distance F_{ST} (Weir et al. 2005)—are the best candidates for population differentiation (Shriver et al. 2004). However, the redundancy of ancestry information between markers needs to be considered when aiming to minimize the number of genetic markers. Therefore, we developed a new method that is based on the informativeness of assignment index I_n (Rosenberg et al. 2003) to find a set of markers that tends to maximize the genetic differentiation between populations while minimizing the number of markers needed. This statistic computes the amount of (nonredundant) assignment information that a particular locus or set of loci contains, to differentiate a particular set of groups defined a priori. Since I_n computes the nonredundant amount of ancestry information, we thereby avoid the usually observed ascertainment bias toward markers that only differentiate between African and non-African groups, caused by the fact that genetic differences are usually largest between African and non-African populations (Hinds et al. 2005). This index ranges from 0 (when the frequency of all alleles of one locus are equally distributed between populations) to the natural logarithm of the number of considered populations (when the different alleles are able to unequivocally differentiate the populations). The informativeness of assignment index under the assumption of a population model without admixture (I_n) was computed because it has been shown that the I_n statistic produces similar estimates and has similar properties as the informativeness of assignment index under the assumption of a population model with admixture (I_a) (Rosenberg et al. 2003). The I_n statistic is preferred over the I_a statistic for defining informative markers, because I_a can produce denominators of 0 when two or more populations have the same allele frequencies, whereas I_n cannot (Rosenberg et al. 2003). We have overcome the problem of extremely large computational efforts needed to consider all possible allele combinations for a large number of loci by applying a genetic algorithm (Haupt and Haupt 2004).

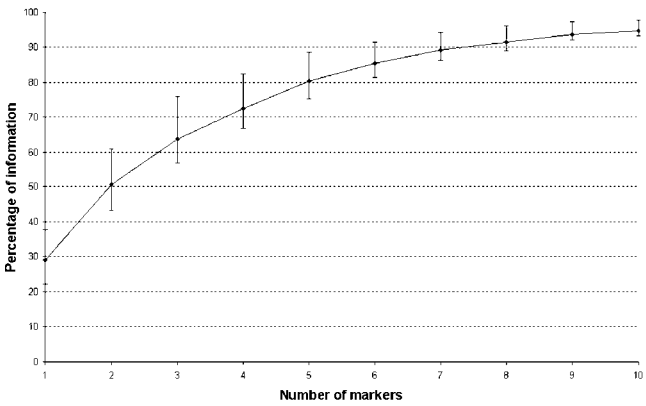

Figure 1 Percentage of information explained when the number of markers that are ascertained from 8,491 SNPs by use of the genetic algorithm based on the informativeness of assignment index (I_n) is increased from 1 to 10, given four continental groups and the YCC panel (see main text for details). The 95% CI of each SNP combination was computed by resampling the same number of chromosomes from the populations and computing I_n 1,000 times.

We analyzed >11,500 SNPs, using the Affymetrix 10K Array Xba 131, in 76 human individuals from 21 sampling localities representing six worldwide geographic areas: Africa, South Africa, America, Asia, North Asia, and Europe (Y Chromosome Consortium [YCC] panel). In short, 250 ng of DNA from each individual was digested, ligated, and amplified. PCR products were fragmented and biotin-labeled after pooling and purification. The biotin-labeled DNA fragments were hybridized to the probes on the Affymetrix GeneChip Mapping 10K array. Finally, the arrays were washed, stained, scanned, and analyzed. All procedures were done in accordance with the recommendations of Affymetrix (Sellick et al. 2003; Shriver et al. 2005). SNPs typed in <90% of the individuals or located on the X chromosome were re-

Table 1

The 10 Most Informative SNPs for Identification of Continental Population Structure and Genetic Ancestry

Marker	Chromosome	Gene Name	I_n for Four Groups and YCC Panel (%)	I_n for Seven Groups and CEPH-HGDP (%)
rs722869	14	*VRK1*	29.066	7.960
rs1858465	17	...	25.637	9.228
rs1876482	2	*LOC442008*	24.589	10.290
rs1344870	3	...	22.810	11.074
rs1363448	5	*PCDHGB1*	19.418	4.552
rs952718	2	*ABCA12*	18.739	9.472
rs2352476	7	...	18.317	5.603
rs714857	11	...	18.083	6.157
rs1823718	15	...	17.845	5.451
rs735612	15	*RYR3*	14.315	5.530

moved from the final data set, which resulted in usable genotypes for 8,491 SNPs per individual.

One might expect that the relatively small number of individuals we used per locality (on average, 3.5 individuals) would tend to decrease the observed genetic variance within populations and spuriously increase the amount of genetic variance explained between populations and continents. To test whether the sample size used here could spuriously increase the genetic differences between continents, we applied an analysis-of-molecular-variance approach (Excoffier et al. 1992), using the Arlequin 2.000 software (Schneider et al. 2000) and the 8,491 SNPs and grouping the populations into five continental regions: Europe, Africa, America, Asia, and Oceania. The amount of genetic variance explained within populations was 85.5% ($P < .0005$), which is within the range of the usually observed values of genetic variance within populations when neutral markers are used (Romualdi et al. 2002). This result seems to contradict expectations, but it can be explained by the fact that all SNPs used on the Affymetrix arrays were originally selected from The SNP Consortium repository (Matsuzaki et al. 2004), showing a similar degree of genetic variation based on a small set of population samples from different continents (Hao et al. 2004). In fact, this array has been successfully used for linkage mapping in different human populations (Kelsell et al. 2005). Thus, these SNPs do not represent the true underlying genetic variation of human populations, and it can be expected that the ascertainment bias would tend to increase the genetic variance within populations, compensating for the expected reduction of the genetic variance within populations when small sample sizes are used.

Each individual of the YCC panel could be genetically assigned—by use of all 8,491 SNPs and the STRUCTURE analysis—to one of the four groups considered. These four genetic groups correlate with four geographical regions: western Eurasia, East Asia, Africa, and America. Each group was then considered artificially as a population, and the I_n statistic was computed per marker. Loci with $I_n < 10\%$ of the maximum value ($\ln 4$) were excluded. We applied the genetic algorithm to the remaining set of 977 SNPs. Different runs were performed with an increasing number of SNPs, to quantify how the total amount of ancestry information changes with the number of markers included. The confidence intervals of I_n were determined by resampling the same number of chromosomes in each population for each locus and computing I_n. As seen in figure 1, the extra informativeness generated by the addition of new SNPs to the set of achieved markers increased rapidly, with the first eight SNPs already reaching 90% of the maximum informativity value. With only 10 SNPs, 94.6% of the maximum informativity value was obtained (table 1 and fig. 1). Further increasing the number of loci hardly increased the amount of extra information contributed by the additional SNPs. This indicates that almost all the information of genetic ancestry an additional SNP can contain in this data set was already described using the 10 markers considered previously. When the STRUCTURE algorithm (Pritchard et al. 2000) is applied to the YCC genotypes of these 10 SNPs, all individuals become clearly assigned to the correct continental region of sampling (fig. 2).

We then wished to know whether the high ability of the ascertained set of 10 SNPs to identify continental population structure and genetic ancestry persists when they are applied to an independent set of population samples. Therefore, we genotyped these 10 SNPs in the CEPH–Human Genome Diversity Project Cell Line Panel (CEPH-HGDP) samples, using TaqMan technology (for details, see appendix A [online only]). The CEPH-HGDP comprises 1,064 samples from 51 human populations of global distribution, including all continental regions: America, Central and East Asia, Europe, the Middle East, North Africa, sub-Saharan Africa, and

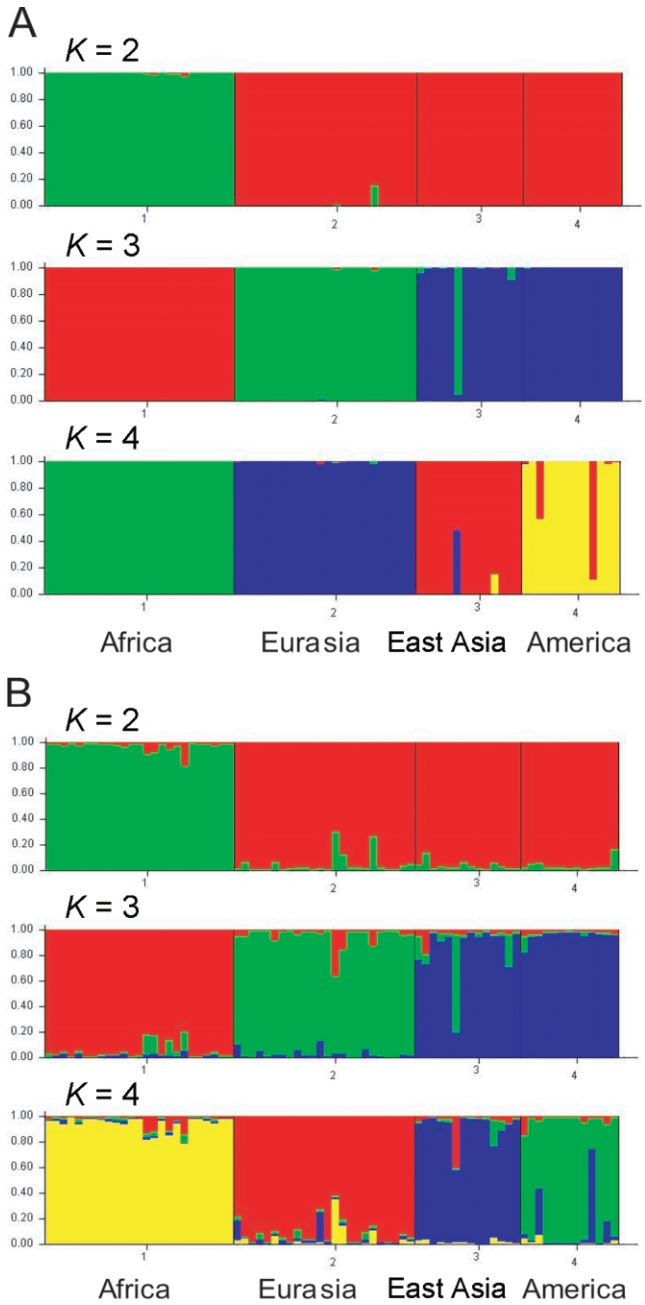

Figure 2 STRUCTURE analysis of the YCC samples, with $K = 2$, 3, or 4 groups, performed using genotypes of the 10 most informative SNPs ascertained using the genetic algorithm with the total YCC data. STRUCTURE analyses were computed using a model without admixture (*A*) and a model with admixture (*B*). Each analysis was repeated five times, after a Markov chain–Monte Carlo (MCMC) burning period of 50,000 and considering the next 200,000 MCMC iterations. In all five runs, good mixing was observed, and similar results were found in accordance with the model used. The natural logarithm of the estimated probability of the data ($\ln p$) is as follows. In panel A, for $K = 2$, $\ln p = -762.2$; for $K = 3$, $\ln p = -629.2$; and, for $K = 4$, $\ln p = -557.4$. In panel B, for $K = 2$, $\ln p = -764.9$; for $K = 3$, $\ln p = -631.2$; and, for $K = 4$, $\ln p = -559.5$.

Oceania (except Australia). However, we excluded 16 CEPH-HGDP individuals from the statistical analysis because of previously identified labeling errors (individuals 770 and 980 [Rosenberg et al. 2002, 2003]), sample duplication with another individual from the same population included in the panel (individuals 583, 650, 658, 657, 660, 472, 813, 762, 452, 457, 1149, and 1233 [Mountain and Ramakrishnan 2005]), and sample duplication between individuals with different population labels (individuals 111 and 220 [Mountain and Ramakrishnan 2005]); all respective sample pairs revealed identical genotypes in our SNP genotyping. In addition, we excluded two individuals with >10% missing genotypes (individuals 737 and 1007), on the basis of our SNP genotyping. As a result, we used 1,046 individuals from 51 populations. By use of the obtained HGDP data, I_n was computed for each SNP, with seven groups considered, and was compared with the I_n computed on the basis of YCC data, with four groups considered. A positive Spearman's correlation ($r = 0.564$; $P = .09$) was observed between the measures of informativeness of ancestry in both data sets; however, the slope of the lineal regression reached only 0.316, with a 95% CI (−0.026 to 0.658) that did not include 1. This indicates a substantial loss of information from the markers to differentiate groups of populations in the new HGDP population data set compared with the original YCC data set. As expected, when the same comparative analysis was performed but with HGDP samples assigned to the

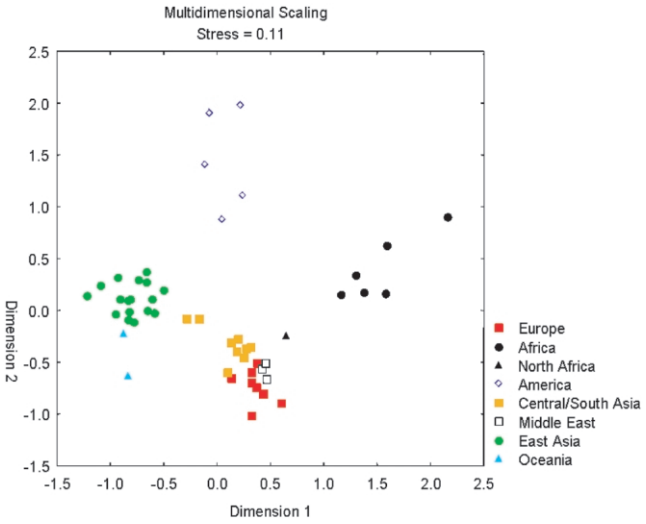

Figure 3 MDS plot based on the I_n matrix computed between pairs of populations by use of the genotypes of the 10 most informative SNPs in the 51 population samples from CEPH-HGDP. Four clusters of population can be identified: (i) sub-Saharan African populations, (ii) American populations, (iii) Eastern Asian and Oceanian populations, and (iv) European, Middle Eastern, North African, and Central/South Asian populations.

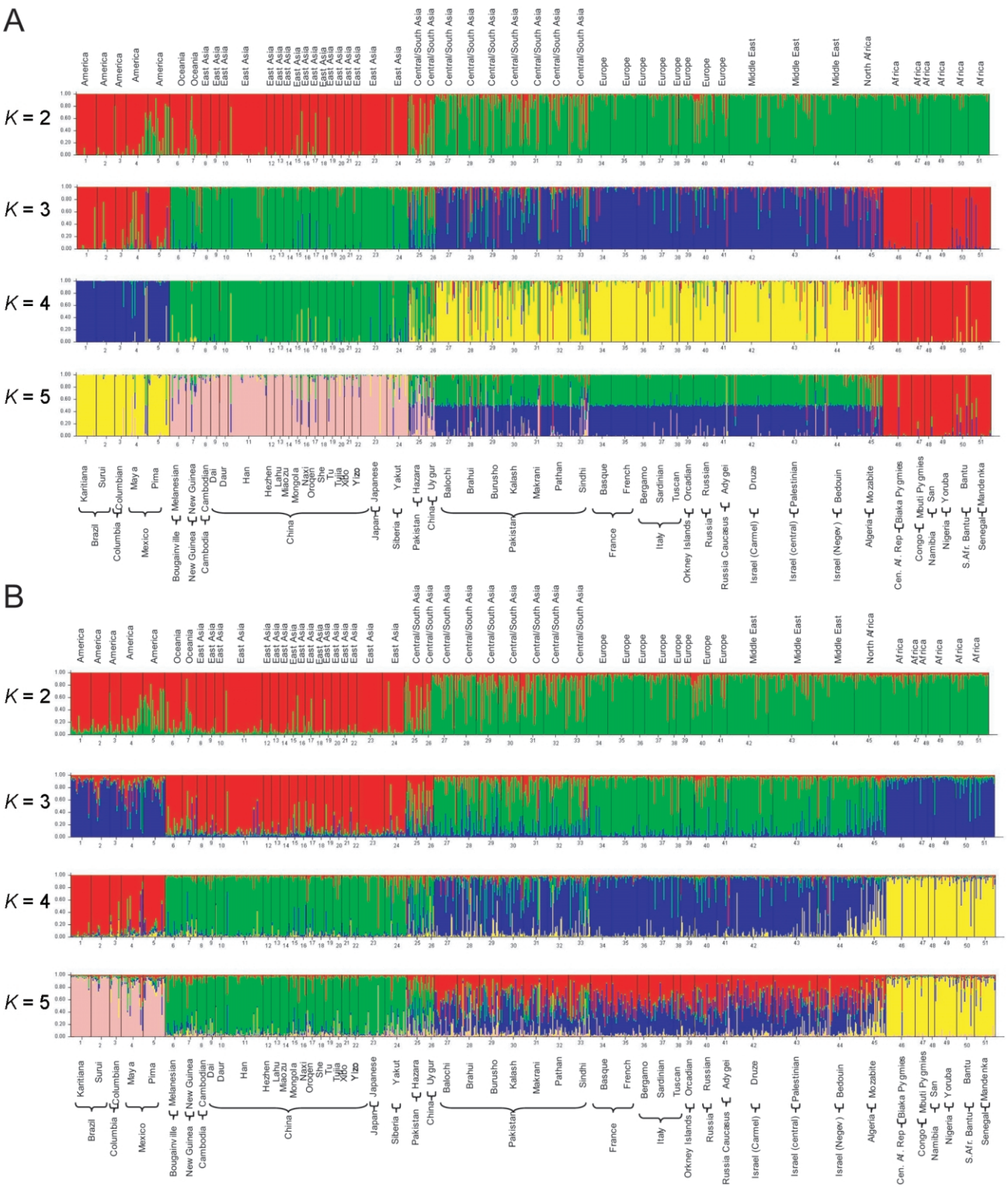

Figure 4 STRUCTURE analysis of the CEPH-HGDP samples, with $K = 2$, 3, 4, or 5 groups, performed using genotypes of the 10 most informative SNPs ascertained using the genetic algorithm with the total YCC data. Two different STRUCTURE analyses were computed: a population model without admixture (*A*) and a population model with admixture (*B*). Each analysis was repeated five times after an MCMC burning period of 100,000 and considering the next 10,000 MCMC iterations. In all five runs, good mixing was observed, and similar results were found in accordance with the model used. The $\ln p$, assuming K groups, is as follows. In panel A, for $K = 2$, $\ln p = -11{,}801.2$; for $K = 3$, $\ln p = -10{,}977.3$; for $K = 4$, $\ln p = -10{,}279.2$; and, for $K = 5$, $\ln p = -10{,}324.9$. In panel B, for $K = 2$, $\ln p = -11{,}886.2$; for $K = 3$, $\ln p = -11{,}070.6$; for $K = 4$, $\ln p = -10{,}345.5$; and, for $K = 5$, $\ln p = -10{,}456.9$. Cen. Af. Rep. = Central African Republic; S. Afr. = South Africa.

The figure is available in its entirety in the online edition of *The American Journal of Human Genetics*.

Figure 5 Clustering results using STRUCTURE software separately for each of the four groups detected by previous STRUCTURE analysis of the worldwide HGDP-CEPH samples by use of the 10 most ancestry-informative SNPs. The legend is available in its entirety in the online edition of *The American Journal of Human Genetics*.

four groups used for the YCC panel, a higher and statistically significant correlation ($r = 0.66$; $P = .04$) was obtained, with a higher slope of lineal regression of 0.655 and a 95% CI (0.046–1.264) that did include 1. However, since these analyses are based on the use of single markers, it is not justifiable to exclude the possibility of differentiating the seven groups when all 10 SNPs are considered at the same time. The I_n value considering seven groups and using all 10 markers simultaneously was 1.01 (95% CI 0.989–1.063), representing only 52% of the total amount of information that could be obtained with seven groups.

Given that different clustering algorithms can produce different results (Corander et al. 2004), we applied three different ways of detecting clusters of populations in the CEPH panel. In the first approach, the I_n statistic was computed for each pair of populations on the basis of the genotypes of the 10 ascertained SNPs, and the matrix was plotted by means of multidimensional scaling (MDS) (Kruskal and Wish 1978) with the STATISTICA 6.0 software (StatSoft 2001). Although the I_n statistic is an index of the informativeness of markers for ancestral inference, it correlates with classical measures of genetic distances (such as F_{ST}) when computed between pairs of populations; thus, it can be considered a measure of genetic distance in this special case (Rosenberg et al. 2003). We were able to detect four differentiated clusters of populations in the graphical representation of the MDS (fig. 3): (i) all sub-Saharan African populations, (ii) all American populations, (iii) all East Asian and Oceanian populations and two Central/South Asian populations (the Uygur from China and the Hazara from Pakistan), and (iv) all European and Middle Eastern populations and all other Central/South Asian populations not in cluster iii, with the North African population somewhat separated but in close proximity. A permutation test was performed to assess the statistical significance of the clustering suggested by the MDS analysis. First, each population was assigned at random to one of the four clusters; then the I_n value, given this new clustering, was computed; and the process was repeated 1,000 times. The resulting P value was highly statistically significant ($P < .0005$), thus supporting the observed clustering. We repeated the permutation test for clusters iii and iv, which appear somewhat close to each other in the MDS plot. The P value was also highly statistically significant ($P < .0005$), suggesting a clear differentiation between both clusters of populations, despite the presence of the two somewhat-intermediate populations of Uygur and Hazara.

The second approach was performed using the individual-based STRUCTURE algorithm, with an increasing number of groups, from $K = 2$ to $K = 5$. STRUCTURE analyses were repeated twice, once using a population model without admixture and once using a population model with admixture; results for each configuration can be seen in figure 4. When the number of groups was two, the algorithm clustered the African, North African, European, Middle Eastern, and Central/South Asian individuals separately from the East Asian, Oceanian, and American individuals, under both models (fig. 4). When three groups ($K = 3$) were specified, the STRUCTURE algorithm yielded the following clusters: (i) African and American individuals; (ii) Oceanian and East Asian individuals, together with the Hazara from Pakistan and the Uygur from China (although the latter two show somewhat more features of cluster iii, yet still belong to cluster ii); and (iii) European, Middle Eastern, and Central/South Asian individuals and North African individuals from Algeria (although the latter show some features of cluster i). When the number of selected groups was four, STRUCTURE clustered the individuals as follows: (i) sub-Saharan African individuals; (ii) American individuals; (iii) Oceanian and East Asian individuals, together with the Hazara and the Uygur (although the latter two show somewhat more features of cluster iv, yet still belong to cluster iii); and (iv) European, Middle Eastern, Central/South Asian, and North African individuals (although the latter show some features of cluster i). Increasing the number of structure groups beyond $K = 4$ did not increase the number of population groups identified. To test for putative presence of population substructure in each of the four genetically defined groups, STRUCTURE analyses were repeated, with one group being considered at a time. No population substructure was detected in the European, Middle Eastern, and Central/South Asian group, the Oceanian and East Asian group, or the African group, when two or more clusters were applied (fig. 5). Only

The figure is available in its entirety in the online edition of *The American Journal of Human Genetics*.

Figure 6 Clustering results using BAPS 3.2 software on the worldwide HGDP-CEPH samples by use of the 10 most ancestry-informative SNPs. The legend is available in its entirety in the online edition of *The American Journal of Human Genetics*.

The figure is available in its entirety in the online edition of *The American Journal of Human Genetics.*

Figure 7 Sliding-window and haplotype analyses for the genomic region surrounding SNP *rs952718* and including the *ABCA12* gene. The legend is available in its entirety in the online edition of *The American Journal of Human Genetics.*

in the case of America was a certain degree of genetic heterogeneity between populations observed (see fig. 5), but it disappeared when the number of clusters was increased from two to three (results not shown).

Finally, we applied a different Bayesian clustering algorithm, defined in the BAPS 3.2 program (Corander et al. 2004), and specified two, three, four, and five groups as previously specified in the STRUCTURE program. Almost identical results were observed for this method as for STRUCTURE when two, three, and four groups of populations were considered (see fig. 6), and the results for four groups were consistent with MDS and STRUCTURE results. However, when five groups were considered, BAPS 3.2 was able to differentiate the two Oceanian populations and create a fifth group (fig. 6). Overall, we obtained identical and statistically significant clustering results for the population samples when we applied three independent methods of clustering, which suggests consistency in the ability of the 10 ascertained SNPs to identify population (sub)structure and genetic ancestry in accordance with four continental groups.

All these results suggest that it is possible to substantially reduce the amount of markers needed to recover a particular population structure when carefully ascertained SNPs are used. Applying to the HGDP samples the 10 most informative SNPs that we ascertained by means of the genetic algorithm from a set of ~8,500 SNPs in the YCC panel, we were able to recover almost all the geographic population structure that was observed by others previously in HGDP samples with the use of 377 microsatellites (Rosenberg et al. 2002) or with a subset of the 40 most informative microsatellites (Rosenberg et al. 2003). Thus, the same results were obtained using four times fewer SNPs than microsatellites. Furthermore, our results demonstrate a reduction in the number of markers by >10 times the amount of SNPs used in previous studies to identify a similar geographic population structure (Turakulov and Easteal 2003; Yang et al. 2005). Our set of 10 SNPs infers incorrectly only the genetic ancestry of HGDP samples from those populations that were underrepresented or not considered in the original YCC data set, such as Oceania (although Oceania is detected using the BAPS 3.2 approach). It should be noted that, because the marker selection is based on maximizing the genetic differences between the populations, the capacity to correctly infer the ancestry of an individual from a new, previously unanalyzed population, will depend on the degree of genetic variation shared with the original set of populations. In the case of human populations, this is of particular relevance when geographical groups are being considered, because isolation by distance has played a major role in shaping human genetic diversity (Harpending and Rogers 2000; Barbujani and Goldstein 2004).

Since many of the environmental factors tend to be geographically restricted, genetic markers associated with a local positively selected genomic region are expected to show large differences between populations from different geographic regions because of different genetic adaptation processes in response to different environmental factors (Bamshad and Wooding 2003; Kayser et al. 2003; Bamshad et al. 2004). Consequently, these markers will be informative not only to understand human genetic adaptation toward environmental factors but also to infer the genetic ancestry of an individual and detect geographic population structure. We wished to know whether the strong continental differences in allele frequencies we observed at the ascertained SNPs can be explained by local positive selection in the different geographic areas or whether these patterns need to be explained by stochastic processes such as genetic drift. Therefore, we tested for signatures of positive selection the genomic regions of the five most ancestry-informative SNPs from our analysis: *rs1344870, rs1876482, rs952718, rs1858465,* and *rs722869*. Three of the SNPs (*rs1876482, rs952718,* and *rs722869*) fall within known or predicted genes (table 1). The presence of footprints of positive selection was tested by analyzing the genetic diversity of the surrounding region and by studying how the homozygosity of the haplotypes in such regions decays when the distance to the putatively selected region is increased (Sabeti et al. 2002). The SNP frequencies of the surrounding regions were taken from the Perlegen database (Hinds et al. 2005), which includes a large number of SNP genotypes distributed throughout the whole genome in populations from three of the four continents considered in our original YCC panel data set: Africa, Europe, and Asia (although, for the Perlegen data set, Africans, Europeans, and Asians who reside in

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Figure 8 Sliding-window and haplotype analyses for the genomic region surrounding SNP *rs722869* and including the *VRK1* gene. The legend is available in its entirety in the online edition of *The American Journal of Human Genetics.*

The figure is available in its entirety in the online edition of *The American Journal of Human Genetics*.

Figure 9 Sliding-window and haplotype analyses for the genomic region surrounding SNP *rs1858465*. The legend is available in its entirety in the online edition of *The American Journal of Human Genetics*.

North America were used). We computed I_n for each SNP of the surrounding region, considering these three continental groups, and performed a sliding-window approach, considering a window size of 2 kb and computing the mean I_n value of each window. To see whether the mean of the window was significantly different from other regions of the genome, we compared the obtained values with the mean values from windows of the same size and with the same number of markers in 10,145 genes from the entire genome in the Perlegen database (genes on the X chromosome were excluded because of the different effective population size for this chromosome (Schaffner 2004). The haplotype phases were inferred using the PHASE program (Stephens and Donnelly 2003). The extended haplotype analysis was performed using the SWEEP program (kindly provided by P. Sabeti), and the markers defining the core haplotype were ascertained from the window with the largest I_n mean.

In four of the five genomic regions analyzed, we detected unusual patterns of genetic variation that are compatible with the hypothesis of local positive selection—namely, (1) large regions of closely located markers showing high informativeness-of-ancestry scores, statistically significantly higher than the expected scores from other genes from the genome, and (2) large, extended haplotypes in at least one of the three populations analyzed in the haplotype bifurcation analysis as well as in the extended haplotype homozygosity (EHH) analysis (see figs. 7–10). Only in the case of *rs1344870* have we not found patterns that are suggestive of local positive selection on the basis of the three populations included in the Perlegen data set. For that region, only a few sliding windows are statistically significant ($P < .05$), there are similar frequencies in the core haplotypes between the three populations, and there is similar decay in the haplotype homozygosity of the different haplotypes in the three populations (see fig. 10). However, the hypothesis of positive selection cannot be rejected, because more continental regions were considered in the YCC samples used for SNP ascertainment than in the Perlegen samples used for analyzing the surrounding regions (i.e., Native Americans were not considered in the Perlegen sample set but were considered in YCC sample set). One of the regions (surrounding marker *rs1876482*) for which we found unusual patterns of genetic variation in agreement with evidence of local positive selection includes the predicted gene *LOC442008* (NCBI GeneID 442008), which shows a highly frequent (frequency 73%) and long extended core haplotype that is practically absent outside the Asian population (fig. 11). Thus, although the SNPs from the whole-genome analyses used to identify ancestry-informative markers were noncoding, our data indicate that the significant population differences of the markers with maximum informativeness of ancestry seem to be shaped by positive selection rather than by genetic drift.

In summary, we have shown that it is possible to substantially reduce the number of markers needed to identify geographic population structure on a continental level by applying carefully ascertained and validated SNPs. With 10 SNPs, we obtained a level of geographic population structure similar to that previously identified using 377 or 40 microsatellites in the same set of samples (Rosenberg et al. 2003), which suggests that carefully ascertained SNPs are more suitable than microsatellites for detecting geographic population structure and identifying genetic ancestry. Furthermore, we can show here that the frequency distributions of SNPs with maximal ability to detect continental population structure and genetic ancestry are most likely shaped by local positive selection rather than by genetic drift. However, our results also show that there is a considerable lack of power when applying ancestry-informative markers ascertained from the original data set to another set of population samples, and the portability of ancestry-informative SNPs depends on the relationship between the populations used. Further studies using more SNPs and/or more population samples from many geographic regions and localities in the world are needed to test whether an ultimate set of SNP markers can be found to identify geographic population structure and genetic ancestry on a more detailed level than the continental level that we achieved here.

Finally, we emphasize that the methodology we have developed here for minimizing the number of genetic markers that are necessary for maximizing the genetic differences between clusters of populations can be applied to any kind of population grouping. In this study, we were interested in finding markers that differentiate

The figure is available in its entirety in the online edition of *The American Journal of Human Genetics*.

Figure 10 Sliding-window and haplotype analyses for the genomic region surrounding SNP *rs1344870*. The legend is available in its entirety in the online edition of *The American Journal of Human Genetics*.

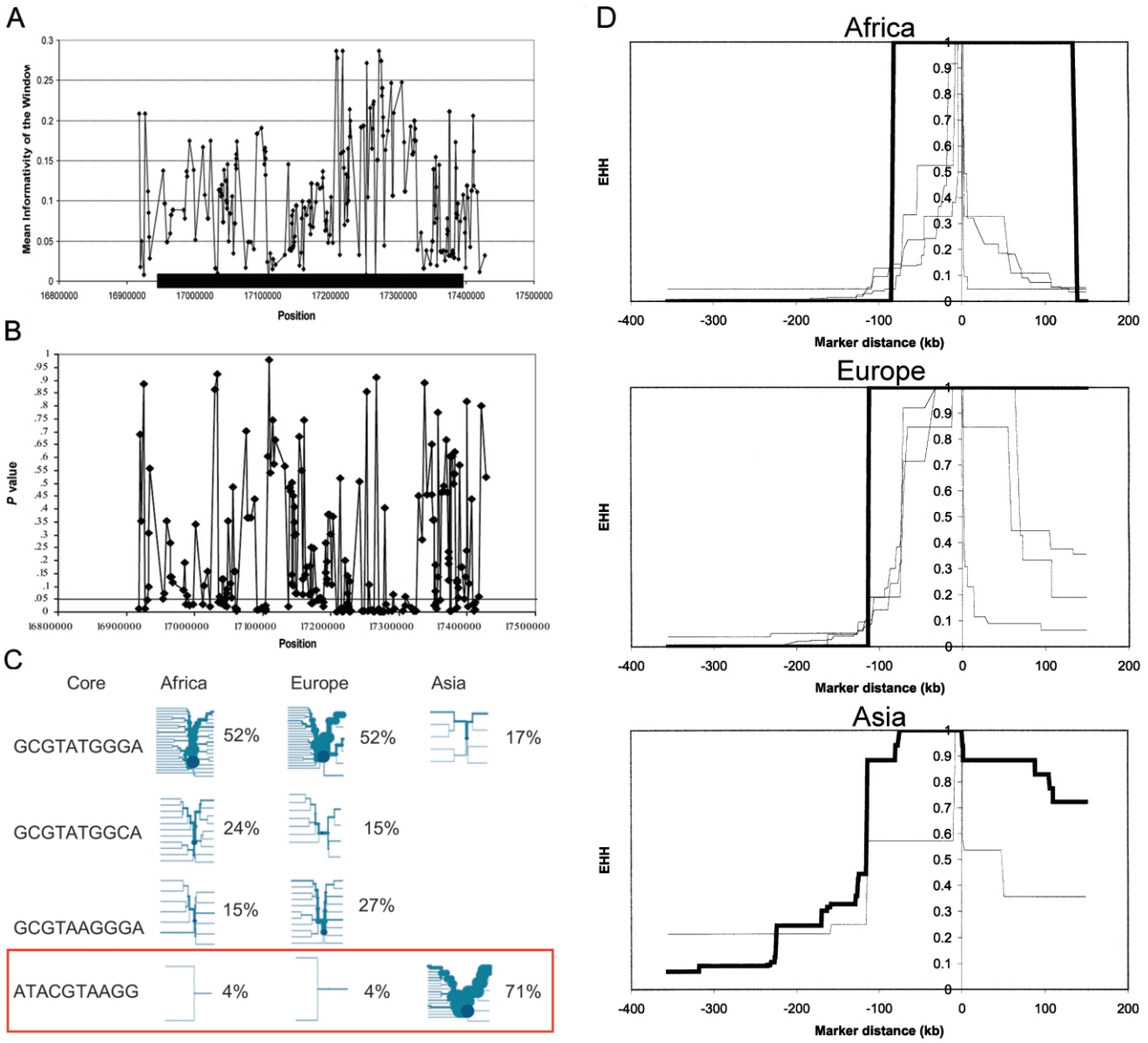

Figure 11 Sliding-window and haplotype analyses performed on the genomic region that includes SNP *rs1876482* (1 of the 10 most informative SNPs identified), which is located in the *LOC442008* gene, by use of Perlegene data. *A*, Sliding-window plot of the mean value observed for each window (the gene is represented by a black bar). *B*, Associated *P* value for comparison with an empirical distribution based on >10,000 genes (see main text). The $P = .05$ cutoff is represented by a black line. *C*, Bifurcation plots of the main core haplotypes in the three populations considered. *D*, Extended homozygosity versus genomic distance to the core haplotype. The region of the core haplotype was selected on the basis of the largest region that was statistically significant in the sliding-window analysis (from *rs12619554* to *rs4832712*; see main text for details). Note the high frequency of the third haplotype in the case of Asian populations and the slow decay of the EHH of that haplotype compared with the other haplotypes both within and between populations.

geographic groups of populations, and we succeeded on the continental level. Clearly, some events in human population history and some forces of local positive selection are expected to enhance the success of finding suitable markers for population differentiation on the continental level. In fact, we have shown here that the surrounding regions of the most ancestry-informative SNP markers show unusual patterns of genetic variation that are compatible with the hypothesis of local positive selection. However, local positive selection is not always restricted to the continental level (e.g., malaria resistance [Tishkoff et al. 2001]) nor equally distributed within a continent (e.g., lactose persistence into adulthood [Bersaglieri et al. 2004]); thus, it can be expected that as-

sociated markers that are able to differentiate respective (noncontinental) populations can be found when appropriate samples are used for marker ascertainment. In addition, when neutral markers are applied, the presence of statistically significant genetic differences between populations (Romualdi et al. 2002) allows other sets of informative markers that specify other population clusters to be found, especially if the number of markers is large enough, no matter what the biological sense of the clusters is. Therefore, making inferences from data sets of genetic markers obtained by the procedures used here should be done with care and needs to be conditioned to the biological meaning of the clustering obtained. However, the fact that we were able to identify four continental groups in the CEPH-HGDP samples by using three different clustering algorithms and applying SNP markers that were ascertained in a different set of global populations (YCC panel), for which the SNPs identified the same continental groups, in addition to the obtained evidence of local (continental) positive selection at the respective genomic regions, clearly emphasizes the value of the identified markers in recognizing continental population (sub)structure and continental genetic ancestry.

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Web Resource

The URL for data presented herein is as follows:

Affymetrix, http://www.affymetrix.com/index.affx

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