INTRODUCTION

Modern human populations exhibit large phenotypic differences in morphological and physiological traits such as pigmentation, hair shape, body shape and composition, and enzymatic activities. Such phenotypic divergences between populations are considered to result from genetic adaptation to local environments; people have experienced changes in their environment due to migration and establishment of civilizations over the past 100,000 years. In order to reveal the genetic bases of population-specific phenotypes, it is necessary to decode the signatures of gene histories engraved on the human genome. In one important “engraving” process known as the “hitchhiking effect”, positive selection of an advantageous mutation alters patterns of genetic variation in the adjacent sequence [1]. During fixation of an advantageous mutation, this process causes a “selective sweep,” i.e., elimination of variation in the neighboring region. To detect recent hitchhiking events, one practical approach is to search for genetic regions that have undergone few previous recombination events [2]. Another selection clue is high local genetic differentiation that can be measured with FST [3] when at least two populations are examined. To detect loci that have reached fixation, low nucleotide diversity should be considered along with the aforementioned points.

To date, numerous different approaches have been applied to genome-wide scans for positively selected loci in humans. Some of these rely on the McDonald-Kreitman test [4] or the dn/ds test [5], in which nucleotide sequences are compared with those of the chimpanzee [6–8]. Other techniques employ polymorphisms within species, and mainly utilize summary statistics such as FST, heterozygosity, or neutrality tests based on the site frequency spectrum (Tajima’s D [9], Fu and Li’s D [10], Fay and Wu’s H [11], and Kim and Stephan’s composite likelihood tests [12]), as well as linkage disequilibrium (LD)-based statistics [13–23]. In particular, LD-based methods using the concept of extended haplotype homozygosity (EHH) have been frequently employed because of their power for detecting a rapid increase in the frequency of an advantageous mutation under recent selection [24,25]. However, since these approaches require at least two alleles for comparison within a population, they are not applicable to already fixed loci, which are most likely to be under strong positive selection. In addition, when applied to a genome-wide scan for candidate loci, the current methods must be applied to every polymorphism or core region that may exhibit strong LD with another.

Since summary statistics are usually affected by demographic history, in previous genome-wide analyses, candidates of selected loci were identified at the extreme tails of empirical distributions. However, recent studies have suggested that such outlier approaches provide a number of false positive loci because of the large size of the human genome [26,27]. Given the trade-off that exists between Type I error rate and the power to detect true selected loci, the appearance of false positives is inevitable. Even so, improving current detection methods would certainly reduce the number of false positives. For example, one reason for the loss of detection power and the generation of false positives is the heterogeneity of recombination rates across the genome, which is a major roadblock to the accurate mapping of selected loci and may lead to the loss of detection power.

To overcome this limitation, a new approach called the “hitchhiking method” has been proposed, which relies on the detection of patterns of genetic variation within populations using SNP data. The hitchhiking method utilizes the concept of haplotype homozygosity (EHH) and searches for loci that have undergone recent positive selection. This method has been shown to be more robust against the loss of detection power and to generate fewer false positives compared to previous methods.

In summary, the hitchhiking method provides a practical approach for identifying positively selected loci in human populations. By utilizing the concept of haplotype homozygosity (EHH) and searching for loci that have undergone recent positive selection, this method enables the accurate mapping of selected loci and reduces the generation of false positives. The hitchhiking method thus represents an important advancement in the field of population genetics and offers a new way to study the role of positive selection in the evolution of human populations.
human genome [28]. In some previous methods, the genome is divided into windows of constant physical size in order to calculate summary statistics. Such methods can be complicated by the heterogeneity of recombination rates. Therefore, local recombination rates should be considered when window sizes are defined.

In order to reduce the limitations of the previous LD-based approaches, we developed a method for scanning genome-wide SNP genotype data for population-specific selective sweeps that have reached fixation, in which homozygosity for haplotypes in two populations are compared. In addition, to measure haplotype differentiation between populations, we compared two populations in homozygosity for the most frequent haplotype instead of using \( F_{ST} \). Although SNP genotype data do not always include selected polymorphisms, our method can efficiently detect the region where a selected polymorphism is located. Another advantage of our method is that the use of observed values of homozygosities allows us to omit haplotype phasing that needs to be either determined from family data or estimated from population data by means of a time-consuming computation.

**RESULTS**

The ratio of haplotype homozygosity between populations

To identify population-specific selective sweeps, we used data from multiple populations, where one population is selected as the test population and another is used as the reference population (Fig. 1). Statistics used in our method are haplotype homozygosity (HH), the probability that two haplotypes sampled at random from the population are same, and most frequent haplotype homozygosity (MHH), the probability that both the two haplotypes sampled are the most frequent haplotype in the population. Here, two sequences that have the same nucleotide for all the typed SNPs were regarded as the same haplotype. HH and MHH can be estimated as observed values \( \left( X_{ii} \right) \) or expected values \( \left( \Sigma x_i^2 \right) \), where \( X_i \) and \( x_i \) denote the frequency of individuals homozygous for the \( i \)th frequent haplotype and the frequency of the \( i \)th frequent haplotype, respectively. When we use the observed values, we do not have to determine or estimate haplotype phase.

The first process of our method is to define blocks for calculation of the statistics by the use of MHH (or HH) itself (Fig. 1). The block partition is made using the data of the test population in order to capture regions with low haplotype diversity. Because we are interested in population-specific fixations in this study, we set the definition of blocks as having at least two SNPs with MHH \( \geq 0.9 \), which corresponds to the frequency of the most frequent haplotype \( \geq \) approximately 0.95. Blocks were partitioned in the direction from small to large number of the chromosomal nucleotide position. Then, using the same block, we calculate homozygosity for the test population’s most frequent haplotype (HTMH) in the reference population, and HH in both the reference and test populations.

To detect population-specific change in haplotype frequency, the ratio of MHH (\( r_{MHH} \)) is calculated as

\[
r_{MHH} = \frac{HTMH in \text{the reference population}}{MHH in \text{the test population}}.
\]

If the test population’s most frequent haplotype is rare in the reference population, \( r_{MHH} \) shows an extremely small value, which may occur when the frequency of an advantageous mutation increased only in the test population. Therefore, this value is inversely correlated with the extent of local haplotype differentiation between populations. On the other hand, the ratio of HH (\( r_{HH} \)) is calculated as

\[
r_{HH} = \frac{HH in \text{the reference population}}{HH in \text{the test population}}.
\]

If a region showing MHH \( \geq 0.9 \) is unusually extended in the test population, \( r_{HH} \) exhibits a small value. Therefore, \( r_{HH} \) represents the extent of the population-specific decrease in haplotype diversity and thus can be an index for detecting a recent rapid increase in the frequency of an allele. Here, we can control the heterogeneity of recombination rates if the reference population can be regarded as neutral. However, \( r_{MHH} \) and \( r_{HH} \) would not show small values in the loci where the same allele is selected and fixed in both populations used in the comparison. In case different mutations in the same region were selected in the two populations, \( r_{MHH} \) would show a small value, but \( r_{HH} \) would not.

**Power and false positives in detection of selection with \( r_{MHH} \) and \( r_{HH} \)**

To estimate the detection power of \( r_{MHH} \) and \( r_{HH} \), we performed computer simulations (Fig. S1). In the simulations, we assumed two divergent populations: one is the reference
population under neutral conditions, and the other is the test population either under neutral conditions for null distribution or under genic selection on the derived allele. In the selection model, we assumed strong selective pressure so that the advantageous allele reaches fixation. The selected polymorphism was not used for calculation of the statistics. We first compared the observed and expected values of homozygosities. When we considered the case that a new advantageous mutation has fixed, rMHH and rHH exhibited low values regardless of the way of homozygosity estimation as shown in Fig. 2A and B, respectively. The observed and expected values showed the same distribution when the test population is neutral as well. Thus, we used the observed values of MHH and HH in the analyses described below. When we evaluated the effect of the density of typed SNPs, we found neither statistic was much affected (Fig 2C and D). In addition, under strong selective sweeps resulting in fixation, the distribution of rMHH was robust against changes in strength of selection and demographic parameters such as generations after divergence and population size (Fig 2C), whereas the distribution of rHH was susceptible to the difference in population size between the test and reference populations (Fig 2D). In the case of the test population under neutral condition, demographic parameters had a great influence on the distribution of both rMHH and rHH (Fig. 2E and F). This indicates that an increased number of generations after divergence and a decreased size of the test population raise the number of false positives due to genetic drift when we set certain threshold values of rMHH and rHH. In all the cases simulated, rMHH<0.05 and rHH<0.5 corresponded to approximately 90% detection power (81.5%–91.5% for rMHH and 86.8%–94.5% for rHH) (Fig 2G and H). Therefore, we used these values as thresholds in subsequent analyses. Type I error rates for rMHH<0.05 and rHH<0.3 ranged from 0.24%–1.43% and 0.76%–4.62%, respectively.

To examine the ability of rMHH to capture genetic differentiation without typing of the selected polymorphism, we compared rMHH with maxFST, i.e., the highest FST for all the typed SNPs in the block. In contrast to rMHH, maxFST was strongly affected by the density of typed SNPs when the test population was under selection models (Fig 3A). Fig. 3B indicates that rMHH is inversely correlated with maxFST, showing an ability to capture highly differentiated regions where the maxFST is low. When exactly 90% detection power was assured for both the statistics, maxFST yielded false positives twice more than rMHH for any condition tested (Fig. 3C).

We also considered the case in which natural selection acts on an old standing allele. Here we assumed that a previously neutral derived allele became advantageous after the split of two populations. The distribution of rMHH and rHH depended on the initial frequency of the advantageous allele at the time positive selection began to act. As shown in Fig. 2I and J, the power of rMHH<0.05 and rHH<0.3 gradually decreased as the initial frequency increased. For a 1% initial frequency, regions under selection could be detected with these thresholds, comparable to the case of a new advantageous mutation. For a 20% initial frequency, however, the power drastically decreased. When we considered the effect of the initial frequency of the advantageous allele on the length of blocks, we found their inverse correlation (Fig. S2), which indicates that variable block size as defined in this study is essential for detection of selection events under different situations.

**Analyses of the HapMap data and neutral genome simulation**

For analyses of real data, we used autosomal SNP genotype data of 180 unrelated individuals from Phase I (release 16c.1) of the International HapMap Project (60 individuals from Yorba in Ibadan, Nigeria, YRI; 60 individuals of northern and western European ancestry from Utah, CEU; 30 Han Chinese individuals from Beijing, CHB, and 30 Japanese individuals from Tokyo, JPT) [16]. Here, we refer to both of the East Asian groups (CHB and JPT) together as EAS. In addition, we performed a coalescent simulation, with non-uniform recombination rates, using the program and best-fitting demographic model of Schaffner et al. [29] (Fig S3A). From this simulation, a data set imitating genome-size chromosomes (2.7 Gbp) under neutrality was obtained. To empirically correct the ascertainment bias in the HapMap data, the probabilities that SNPs were “genotyped” were determined with the minor allele frequency spectra in the HapMap and the simulation (Fig S3B).

We subjected the three populations (YRI, CEU and EAS) to our method in order to detect selective sweeps that have reached fixation or near fixation. Scatter plots between rMHH and physical length of block (Fig. S4) show their inverse correlation but suggest that the elongated block length alone is not adequate for efficient detection of rapid fixations caused by selective sweeps. The number of candidate regions detected is shown in Table 1. Since the distribution of rMHH and rHH in the neutral genome simulation fitted well to that in the HapMap (Fig. 4), this means that the number of detected regions depends on demographic history rather than on natural selection. This result indicates that a relatively large proportion of the candidates are false positives. Many of these false positives probably can be attributed to the distant divergence time between African and non-African populations and to bottleneck events that occurred after the non-African population left Africa. When EAS and CEU were compared with each other as the test and reference populations, a small number of candidates (less than 1% of the defined blocks) were detected (Table 1). By using the combination of rMHH and rHH, we could narrow the candidates down further. Only 12 blocks in EAS, 4 blocks in CEU, and 1 block in YRI satisfied the criterion of rMHH<0.05 and rHH<0.3 for two reference populations (Table 1 and 2). It is worth noting that a few blocks exhibited low rMHH and high rHH in the HapMap CEU data (arrows in Fig. 4), but not in the neutral simulation. Some of these cases are likely to be due to errors in the HapMap project. If they are not due to errors, such blocks might be regions under recurrent fixations; the reference or common ancestor population may also have experienced at least one sweep.

**Candidates for genes fixed under positive selection**

Among regions with strong signatures of selection (Table 2 and Data S1), we observed overlaps with genes that previously had been reported to be targets of strong selection such as *DARC (F)I* [30] (YRI [test] vs. CEU [reference]) and *ABCC11* [31] (EAS vs. YRI/CEU) that are associated with malaria resistance and earwax type, respectively. Of four regions with the strongest signatures of European-specific selective sweeps (Table 1), two regions include pigmentation-related genes, *SLC24A5* (NCKX) [32] and *SLC45A2* (*MATP*) [33]. When we consulted EntrezGene and OMIM for gene functions, we also found a number of genes that may be involved in certain traits on which natural selection is likely to have acted [34–42] (Data S1). For example, *BMP2K* (CEU vs. YRI) is a kinase inducing bone morphogenic protein-2 that participates in skeletal development and patterning. *IGFBP2* (EAS vs. YRI) may have a potential role in growth through IGF-1 action. *EDA* (EAS vs. YRI/CEU) is related with hair and tooth morphogenesis and *ENAM* (EAS/CEU vs. YRI) is the largest protein in the enamel matrix of developing teeth. Fertility-related genes such as *PGR* (EAS vs. YRI/CEU), *MORC1* (EAS vs. YRI) and *SPAG5* (EAS/
Figure 2. Distributions of rMHH and rHH under simulations. (A and B) Comparison of the observed (obs) and expected (exp) values of homozygosities. Distributions of rMHH (A) and rHH (B) under the selection (sel) and neutral (neu) models were shown. The parameters used in the simulations are default settings (def): size of the test population, $N_t = 1000$; size of the reference population, $N_r = 1000$; generations after the population split, $G = 200$; selection coefficient, $s = 0.15$; SNP interval = 2 kb/SNP. (C and D) rMHH (C) and rHH (D) under different selection models. (E and F) rMHH (E) and rHH (F) under neutral different models. The parameters were changed accordingly in these simulation models. (G and H) Accumulative distributions of rMHH (G) and rHH (H). Denotation of each line is same as in C–F. (I and J) Accumulative distributions of rMHH (I) and rHH (J) for the case of selection on a standing allele. Selection models for several initial frequencies (IF) of the advantageous allele and a neutral model (neu) were analyzed.

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CEU vs. YRI) also exhibit strong signatures of selection. \textit{MYLK} (EAS/CEU vs. YRI) and \textit{MYLK2} (EAS vs. YRI) are key enzymes in contraction of smooth and skeletal muscles, respectively. \textit{PET112L} (YRI vs. EAS/CEU) plays an important role in mitochondrial gene expression, most likely in translation. We detected various adiposity-related genes that may be involved in fatty acid metabolism (\textit{PRKAG3}, EAS/CEU vs. YRI), glucose metabolism (\textit{DOK5}, EAS vs. YRI), and vitamin B metabolism (\textit{TPK1}, EAS/CEU vs. YRI). These genes could be candidates for “thrifty genes” that explain variation in the efficiency of energy expenditure among populations [43]. Like pigmentation-related genes, sunlight conditions, which depend on degrees of latitude, may be selective pressures on \textit{PPEF2} (EAS/CEU vs. YRI) involved in photoreception in the visual system and \textit{CSNK1D} (EAS vs. YRI) involved in circadian rhythms and sleep phase. Local epidemic diseases can drive population-specific selection acting on immunity-related genes such as \textit{CD226} (EAS/CEU vs. YRI) and \textit{IL4R} (EAS vs. YRI). It is interesting that two anthrax toxin receptors, \textit{ANTXR1} (CEU vs. YRI) and \textit{ANTXR2} (EAS vs. YRI), show signatures of selective sweeps, which may indicate cases in human history of fights against specific infectious diseases. A drug response-related gene, \textit{CYP3A4} (EAS/CEU vs. YRI), and olfactory receptor genes, \textit{OR3A2} and \textit{OR1G1} (EAS vs. YRI), might also be targets of local natural selection.

**DISCUSSION**

The previous LD-based methods using EHH are not applicable to already fixed alleles and also have reduced power for detecting alleles near fixation because relative EHH between different alleles is calculated as the statistic [22,24]. However, to understand the genetic factors that determine the phenotypic differences between populations, we think it is better to focus on completely differentiated loci. For this purpose, we provide an alternative method of genome-wide scanning for swept loci that have reached fixation in a population. Here, the test population is compared with the reference population in \textit{HH} and \textit{MHH} in order to control for the heterogeneity of recombination rate across the human genome. In the procedure of this method, we partitioned genomic data into blocks depending on \textit{MHH} in the test population. This enables us to detect those regions with low diversity and few past

| Test | Defined blocks | Ref1 | Ref1 \( r_{\text{MHH}<0.05} \) | Ref1 \( r_{\text{HH}<0.3} \) | A \( \cap B \) | Ref2 | Ref2 \( r_{\text{MHH}<0.05} \) | Ref2 \( r_{\text{HH}<0.3} \) | C \( \cap D \) | A \( \cap B \) \( r_{\text{MHH}} \) C \( \cap D \) | A \( \cap B \) \( r_{\text{MHH}} \) C \( \cap D \) |
|------|----------------|------|-------------------------------|----------------------|-------------|------|-------------------------------|----------------------|-------------|----------------------|------------------|
| YRI  | 30237          | EAS  | 843 (2.8%)                    | 210 (0.69%)          | 31 (0.10%)  | CEU  | 506 (1.7%)                    | 290 (0.96%)          | 39 (0.13%)  | 1 (0.0033%)          |                   |
| EAS  | 53717          | YRI  | 1376 (2.6%)                   | 3505 (6.5%)          | 874 (1.6%)  | CEU  | 139 (0.26%)                   | 350 (0.65%)          | 39 (0.073%) | 12 (0.022%)         |                   |
| CEU  | 42709          | YRI  | 657 (1.5%)                    | 1927 (4.5%)          | 384 (0.90%) | EAS  | 91 (0.21%)                    | 69 (0.16%)           | 12 (0.028%) | 4 (0.0094%)         |                   |

Blocks were defined as at least two SNPs with \textit{MHH} \( \geq 0.9 \). Candidate regions were detected as blocks with \textit{rMHH} \( < 0.05 \) and \textit{rHH} \( < 0.3 \).

Fig. 3. Comparison between \textit{rMHH} and \textit{maxFST}. (A) Accumulative distribution of \textit{maxFST}. (B) The distribution of \textit{rMHH} and \textit{maxFST} under a selection model (default settings as in Fig. 2). (C) Type I error rate of \textit{rMHH} and \textit{maxFST} at the threshold of 90% detection power under different models. The difference between \textit{rMHH} and \textit{maxFST} was significant \( (P<10^{-17} \) to \( 10^{-100} \)).

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Figure 4. Scatter plots between rMHH and rHH for the HapMap data and neutral genome simulation. Arrows indicate blocks with low rMHH and high HH values, which is rare in the simulation.

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Population-Specific Sweeps

In our method, rHH detects population-specific reduction in LD under the condition that the divergence time of the two populations is so short that the selection event began before the divergence. Therefore, we can set the threshold for both statistics that assures approximately 90% detection power regardless of demographic history and SNP density. Using this approach, we can reduce false positives overall while maintaining high detection power for true selected loci. Given that the potential for false positives in a population can increase depending on that population’s demographic history, the combination of the two statistics (and more) is necessary. Specifically, we used the threshold i.e. 1st percentile of the empirical distribution) [13,15,18,19].

Although recent advancement of statistical genetics and computer technology enables us to estimate haplotype phase in a large amount of genotype data [46], this procedure is still time consuming. In this study, however, the summary statistics, especially rMHH, are relatively robust against population demographic history when the test population has experienced fixation of a new advantageous mutation. Moreover, these statistics are relatively independent of the density of typed SNPs. Therefore, we can set the threshold for both statistics that assures approximately 90% detection power regardless of demographic history and SNP density. Using this approach, we can reduce false positives overall while maintaining high detection power for true selected loci. Given that the potential for false positives in a population can increase depending on that population’s demographic history, the combination of the two statistics (and more) is necessary. Specifically, we used the threshold i.e. 1st percentile of the empirical distribution) [13,15,18,19].

Table 2. Candidate regions with the strongest signs.

| Test | Chr | Position | First_rs..Last_rs | Length | SNP | Ref1 | rMHH | rHH | Ref2 | rMHH | rHH | Genes |
|------|-----|----------|------------------|--------|-----|------|-------|------|------|-------|------|-------|
| EAS  | Chr2:9715614..9721513 | rs6432017..rs13426930 | 5900 | 5 | YRI | 0.000 | 0.232 | CEU | 0.036 | 0.214 | ADAM17 |
| EAS  | Chr2:22298089..22450516 | rs396757..rs10196529 | 15248 | 51 | YRI | 0.000 | 0.000 | CEU | 0.036 | 0.125 |
| EAS  | Chr3:43672161..36814135 | rs6531489..rs6531493 | 21975 | 11 | YRI | 0.000 | 0.276 | CEU | 0.034 | 0.276 |
| EAS  | Chr3:117480167..117642506 | rs770537..rs1479211 | 162340 | 77 | YRI | 0.000 | 0.091 | CEU | 0.000 | 0.073 |
| EAS  | Chr6:100307973..100327281 | rs6916883..rs4640896 | 19309 | 7 | YRI | 0.037 | 0.148 | CEU | 0.019 | 0.185 |
| EAS  | Chr6:126422953..126649896 | rs11963634..rs9375427 | 46944 | 16 | YRI | 0.019 | 0.093 | CEU | 0.037 | 0.167 |
| EAS  | Chr7:882099819..82206515 | rs7833919..rs9283847 | 106697 | 44 | YRI | 0.000 | 0.073 | CEU | 0.037 | 0.145 | LOC392238 |
| EAS  | Chr13:9024425..139054833 | rs6986279..rs577869 | 30409 | 16 | YRI | 0.019 | 0.037 | CEU | 0.037 | 0.111 | LOC401478 |
| EAS  | Chr10:901695196..917342724 | rs10509597..rs2104483 | 48079 | 24 | YRI | 0.000 | 0.200 | CEU | 0.018 | 0.218 |
| EAS  | Chr16:47969205..48226046 | rs8058886..rs9938490 | 25684 | 80 | YRI | 0.000 | 0.074 | CEU | 0.019 | 0.204 | ABCC11, LONP, SIAH1 |
| EAS  | Chr20:574657..583089 | rs282152..rs282163 | 8433 | 6 | YRI | 0.000 | 0.224 | CEU | 0.000 | 0.138 | TCF15 |
| EAS  | Chr22:41774895..41787863 | rs138903..rs113515 | 12969 | 10 | YRI | 0.018 | 0.088 | CEU | 0.035 | 0.298 | BZRP |
| CEU  | Chr3:33991644..34012464 | rs35406..rs35412 | 21003 | 17 | YRI | 0.034 | 0.119 | EAS | 0.000 | 0.220 | MATP |
| CEU  | Chr12:49117161..49164730 | rs4424740..rs12581494 | 47570 | 14 | YRI | 0.036 | 0.236 | EAS | 0.036 | 0.200 | LOC113251 |
| CEU  | Chr15:46081373..46155554 | rs2470110..rs2433359 | 74182 | 42 | YRI | 0.000 | 0.019 | EAS | 0.000 | 0.130 | NCKX5, MYEF2 |
| CEU  | Chr15:46160804..46237972 | rs2459394..rs3784614 | 77169 | 32 | YRI | 0.000 | 0.017 | EAS | 0.000 | 0.186 | MYEF2, LOC400369, SLCL2A1 |
| YRI  | Chr4:153211294..153243151 | rs1355413..rs17360461 | 31858 | 11 | EAS | 0.019 | 0.222 | CEU | 0.000 | 0.278 | PET112L |

The blocks with rMHH < 0.05 and rHH < 0.3 to both the reference populations are shown. Their positions and overlapping genes were referred to NCBI Build 34. doi:10.1371/journal.pone.0000286.t002

The variable block size is adaptive also to detection of selection events under different situations, because the length of range showing LD decay depends on the strength of selection and on the frequency of the advantageous allele at the time that selective pressure began to act. By contrast, when a constant physical size of windows is used, summary statistics can greatly be confounded by the strength of selection, the initial frequency of the advantageous allele, and/or the heterogeneity of recombination rate [26,44]. Although blocks were defined as having at least two SNPs with MHH ≥ 0.9 in this study, the definition can be changed. For example, when blocks are defined as having at least two SNPs with HH ≥ 0.5, we would expect to capture population-specific LD decay if the frequency of the advantageous allele had risen up to approximately 70% or more. Under this definition, indeed, we could detect strong signatures of selection not only on fixed loci but also on loci without fixation such as LCT, a representative selected gene in Europeans [22,45] (data not shown).

In our method, rHH detects population-specific reduction in haplotype variation, which is a signature of a recent rapid increase in the frequency of an allele. Unlike relative EHH that examines each allele or core haplotype [24], rHH is applied to each block with a long range. Therefore, we can avoid independently testing adjacent loci in strong LD with each other. On the other hand, rMHH is an indicator of haplotype differentiation. Our simulation analyses suggested that rMHH has a higher ability to capture highly differentiated regions than maxFST does when the density of the typed SNPs is low and the selected polymorphism is not typed. However, maxFST would become effective if dense SNPs are available.

Summary statistics used for neutrality tests usually are confounded by population demographic history. Therefore, recent genome-wide scans for selection have resorted to outlier approaches based on empirical distribution, where a certain threshold is set (i.e., 1st percentile of the empirical distribution) [13,15,18,19]. Such approaches do not always guarantee high power to detect true selected loci [26]. In our method, the summary statistics, especially rMHH, are relatively robust against population demographic history when the test population has experienced fixation of a new advantageous mutation. Moreover, these statistics are relatively independent of the density of typed SNPs. Therefore, we can set the threshold for both statistics that assures approximately 90% detection power regardless of demographic history and SNP density. Using this approach, we can reduce false positives overall while maintaining high detection power for true selected loci. Given that the potential for false positives in a population can increase depending on that population’s demographic history, the combination of the two statistics (and more than one reference population, if possible) allows one to efficiently narrow the candidates down to those with the strongest selection signatures. In this study, we observed only a fairly small number of candidates in the comparison between EAS and CEU, which indicates that the divergence time of the two populations is so short that new mutations could not reach fixation without strong selection. However, a number of candidates still remained when the non-African populations were tested against YRI. At the time of “out of Africa”, strong positive selections must have acted since the African and non-African populations show distinct phenotypic differences. To distinguish true selected loci from false positives, the length of block would also be a clue (Data S1).

Although recent advancement of statistical genetics and computer technology enables us to estimate haplotype phase in a large amount of genotype data [46], this procedure is still time consuming. In this study, however, the approach using observed values of MHH and HH without phasing works fairly well. The conciseness and powerfulness, together with the applicability to data with relatively low SNP density, are convenient for analyses of genotype data produced by DNA microarray or other technolo-
gies. Thus, the present method can be a practical tool for future studies on other human populations and other species, such as domesticated animals and plants under artificial selection, in which evolutionary studies have only recently been performed [47,40].

It should be noted again that many of candidate regions identified in this study might be false positives generated by genetic drift. Strong signatures can be dependent on a neighboring selected region. Nevertheless, the set of candidates here must include true selected loci that determine interpopulation phenotypic differences, since approximately 90% detection power was assured in each statistic used. Indeed, we observed the strongest signatures on genes previously reported to show association with traits such as pigmentation and earwax type. In addition, our method detected several candidates for selected genes that have previously been identified by scans using other methods and other data sets [19,20]. Such genes are most likely to be true selected genes. To validate this, however, association studies between genotypes and phenotypes are required, using appropriate populations. It is also essential to carry out molecular analyses that identify location of selected polymorphisms, functions of genes in which selected polymorphisms are located, and effect of selected polymorphisms on gene functions. More importantly, this work may help reveal morphological and physiological characteristics of population specificity in detail. Such micro- and macro-level studies enable us to understand what factors have been selective pressures on modern humans and how we have adapted to them during the course of evolution.

MATERIALS AND METHODS

Estimation of the detection power of rMHH and rHH with computer simulations

To estimate the detection power of rMHH and rHH, computer simulations were divided into two phases, divergent population phase and ancestral population phase (Fig. S1A). The divergent population phase was simulated with a forward-time simulation program for neutral or genic selection models, where the frequency of the advantageous allele was deterministically increased. In the ancestral population phase, a coalescent simulation program [29] for neutral or genic selection models, where the selected SNP was not used for the analyses and only the block including or adjacent to the selected SNP was considered. In some cases, there was no such block, but those cases were also included in the power calculation as “undetected”. By contrast, every defined block in the simulated region was used in the neutral models.

HapMap data

The individual genotype data from Phase I (release 16c.1) of the International HapMap Project were downloaded from the website (www.hapmap.org) [18]. Of the 270 individuals examined in the project, we used 180 unrelated individuals (60 from YRI; 60 from CEU; 30 from CHB; 30 from JPT). Autosomal SNPs typed in all the populations (383,697 SNPs) were analyzed in this study. We referred gene positions to NCBI Build 34.

Neutral genome simulation

To obtain a set of genome-size data for 120 chromosomes each from the three human populations (YRI, CEU, and EAS) under neutral condition, a coalescent simulation was performed using the program and best-fitting demographic model of Schaffner et al. (Fig. S3A) [29]. In this simulation program, recombination rate can be varied within the region being simulated (500 kb). The simulation runs were replicated 5,400 times and all the regions simulated were connected to make chromosomes with the length of 2.7 Gbp.

To compare the HapMap data with the simulation data, correction of the ascertainment bias is essential. However, the accurate process of determining the ascertainment for the HapMap data is complicated. Consequently, since the SNP discovery rate depends on the minor allele frequency, we determined the probabilities that SNPs were “genotyped” according to the minor allele frequency spectra in the simulation and the HapMap (Fig. S3B). We obtained a set of “genotyped” SNP data from the simulation and constructed 180 individual diploid data from the haploid data.

SUPPORTING INFORMATION

Figure S1 Schema of simulation procedure for power estimation. (A) The simulation procedure was divided into two phases: divergent and ancestral population phases were performed with forward-time and coalescent simulation, respectively. (B) From the results of the coalescent simulation, a SNP was chosen as the selected SNP (orange circle) according to its derived allele frequency and the SNP with the highest minor allele frequency in each window was chosen as “genotyped SNPs” and relocated to be at constant intervals of 2 kb (Fig. S1B). The simulation runs were replicated 100 times. The result from each run in the ancestral population phase was regarded as an initial allelic state in the divergent population phase.

Finally, we obtained 120 chromosomes with allelic state and constructed 60 diploid individuals for each population. Blocks (containing at least two SNPs with MHH ≥0.9) were defined as described in the Results section. In the selection models, the selected SNP was not used for the analyses and only the block including or adjacent to the selected SNP was considered. In some cases, there was no such block, but those cases were also included in the power calculation as “undetected”. By contrast, every defined block in the simulated region was used in the neutral models.
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