The Case for Selection at CCR5-Δ32

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<td>doi:10.1371/journal.pbio.0030378</td>
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The Case for Selection at CCR5-Δ32

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The C-C chemokine receptor 5, 32 base-pair deletion (CCR5-Δ32) allele confers strong resistance to infection by the AIDS virus HIV. Previous studies have suggested that CCR5-Δ32 arose within the past 1,000 y and rose to its present high frequency (5%–14%) in Europe as a result of strong positive selection, perhaps by such selective agents as the bubonic plague or smallpox during the Middle Ages. This hypothesis was based on several lines of evidence, including the absence of the allele outside of Europe and long-range linkage disequilibrium at the locus. We reevaluated this evidence with the benefit of much denser genetic maps and extensive control data. We find that the pattern of genetic variation at CCR5-Δ32 does not stand out as exceptional relative to other loci across the genome. Moreover using newer genetic maps, we estimated that the CCR5-Δ32 allele is likely to have arisen more than 5,000 y ago. While such results can not rule out the possibility that some selection may have occurred at C-C chemokine receptor 5 (CCR5), they imply that the pattern of genetic variation seen at CCR5-Δ32 is consistent with neutral evolution. More broadly, the results have general implications for the design of future studies to detect the signs of positive selection in the human genome.


Introduction

The impact of evolutionary selection on the human population is of central interest and, with increasing information about genetic variation, has become a subject of intense examination [1–6]. Knowledge of selective events and selected loci provide insight into the genetic etiology of human disease, past and present, and into the events that have shaped our species. As infectious diseases pose a major selective force, selected variants may give insight into immunological defense mechanisms—highlighting important pathways in pathogen resistance.

Evolutionary pressure generates a number of potentially detectable signals at a locus under selection as compared to the neutrally evolving genome. Because different populations are subject to distinct selective environments, selection may produce population-specific alleles and greater population differentiation at an affected gene, which can be measured with the FST statistic [7]. Positive selection may also cause a rapid rise in an allele’s frequency, creating a disparity in the age of an allele estimated from its high frequency in the population (characteristic of an old allele) and its long-range linkage disequilibrium (LD, characteristic of a young allele). LD-based methods such as the Long-Range Haplotype test have been developed to detect this signal [3,8–10].

C-C chemokine receptor 5 (CCR5) is one of the most prominent reported cases of recent natural selection in the human genome. First identified as encoding a principal entry receptor for HIV-1 infection of CD4+ T lymphocytes, CCR5 has been the subject of intense focus by geneticists [8,11–14]. A well-established association exists between a 32 base-pair deletion variant in CCR5 (CCR5-Δ32) and protection from HIV infection, demonstrating that CCR5 plays an important biological role in HIV entry into cells.

The first suggestion that CCR5 may have been subject to positive selection was a high proportion of nonsynonymous mutations at CCR5, suggesting selective pressure for amino acid divergence [12]. More compelling evidence for selection on CCR5-Δ32 came from work by Stephens et al. [8]. This study found that Δ32 occurs at high frequency in European Caucasians (5%–14%, with north-south and east-west clines) but is absent among African, Native American, and East Asian populations, suggesting that the Δ32 mutation occurred after the separation of the ancestral founders of these populations. Moreover, Stephens et al. [8] reported strong LD between CCR5-Δ32 and two microsatellite markers, suggesting an estimated age for the allele of only ~700 y (range 275–1,875 y). The apparent rapid rise in frequency implied strong positive selection, and the specific age raised intriguing possibilities for the selective agent, such as the bubonic plague in Medieval Europe.

With the recent availability of comprehensive information...
about patterns of allelic diversity in the human genome, we
can now reexamine the case for selection at CCR5 by
comparison with extensive empirical data and more sophis-
ticated predicted distributions. We carried out high-density
single-nucleotide polymorphism (SNP) genotyping around
CCR5 in multiple populations, and analyzed the data with the
benefit of large genomic comparison datasets and revised
physical and genetic maps. Our results show that CCR5-Δ32
does not clearly stand out in terms of genetic diversity or
long-range haplotypes relative to other variants at the locus
or throughout the human genome.

Results/Discussion

We genotyped CCR5-Δ32, two microsatellites, and 70 SNPs
837 kbp centromere-distal and 430 kbp centromere-
proximal to the CCR5 locus (Table S1). We studied 340
chromosomes from three populations: European-Americans,
Chinese, and Yoruba from Nigeria. Eight of the European-
American chromosomes bore the Δ32 mutation. In addition,
genotyped a subset of the SNPs in 12 Δ32/Δ32 individuals
from the original study. This provided a total of 32
chromosomes bearing the Δ32 allele. We carried out all
analyses on both datasets (Table S2).

We first examined the allele frequencies at SNPs around
CCR5 in the European-American, Yoruba, and Chinese
population samples for evidence of selection. As a genome-
wide empirical comparison, we used two datasets. The first is
2,359 SNPs genotyped in the same 340 samples in the three
populations. These SNPs are distributed in 168 immunologic
genes from 64 loci across the genome; they were chosen
according to the same methodology and have a similar
physical distribution as for CCR5 (15) (see Materials and
Methods). The second is data for 63,149 SNPs on Chromo-
some 3 from the International Haplotype Map Project
(HapMap, data release 16) genotyped in the same three
populations.

CCR5 is not a significant outlier relative to the 168 genes or
HapMap Chromosome 3 with respect to heterozygosity and
FST (Table 1; Figure S1). The heterozygosity statistic assesses
the genetic diversity in a population; a selective sweep can
reduce genetic diversity and balancing selection can increase
genetic diversity. The FST statistic (7) compares the frequency
of an allele between populations; a population-specific
selective pressure may produce greater population differ-
entiation at an affected gene. We also looked at the derived
allele frequency (DAF) distribution, which can detect the
genetic hitchhiking of variation linked to an allele under
positive selection, and found no evidence for selection (16)
(Table 1; Figure S2). All of these tests have limited power,
with genotyping data ascertained to favor common shared SNPs
and using the chimpanzee sequence for comparison. There-
fore, while the results provide no evidence for selection, it
can not be ruled out; this could be further explored with
sequencing of a large number of chromosomes.

We also assessed the significance of the observation that
Δ32 is at moderately high frequency (8%) in the European-
Americans but absent in the Chinese and Yoruba populations
sampled. The observation is not exceptional in our available
polymorphic data: of SNPs present at similar frequency (7%–
9%) in European-Americans, ~7% are not found in the
Chinese and Yoruba populations for the 168 genes, and 6%
are not found for the same populations for the HapMap data.
These estimates are likely to be conservative considering that
the ascertainment of these studies favors shared polymor-
phisms. As more data become available, this analysis should
be extended by larger sample sizes, more populations, and
more closely matched data (including insertion/deletion
polymorphisms and functional polymorphisms).

We next tested for signatures of selection by examining the
extent of LD around CCR5-Δ32. For this purpose, we used the
Long-Range Haplotype test for selection (3) (see Materials
and Methods). Specifically, we calculated the relative ex-
tended haplotype homozygosity (REHH), which is sensitive
to recent directional positive selection, and extended haplotype
homozygosity (EHH), which is more sensitive to multiple
selective sweeps at a locus. To estimate the recombination
rate, we used two measures: the genetic distance from a
family-based linkage study (17) and the number of observed
historical recombination events (3) (Material and Methods).

We initially examined the centromere-distal side of CCR5
using the approach of Stephens et al. (8) (Figure 1A).
Specifically, we sorted the chromosomes into two groups:
Δ32-bearing and non-Δ32-bearing chromosomes. Consistent
with the previous study (8), we found that the Δ32-bearing
chromosomes have much longer LD than non-Δ32-bearing
chromosomes: the EHH is 5.96 times greater than the average
EHH of other variants at this locus \( \text{REHH} = 5.96 \) at a distance
of 500 kbp or 0.25 centimorgans (cM) (Figure 1B).

We reasoned, however, that the apparent long-range LD
might be a result of sorting the chromosome into only two

---

Table 1. Genetic Diversity at CCR5 in Comparison with Genetic Diversity for Regions from Two Large Empirical Datasets

<table>
<thead>
<tr>
<th>Measure</th>
<th>Population</th>
<th>CCR5</th>
<th>Comparison Regions (64)</th>
<th>HapMap Chromosome 3</th>
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<tr>
<td>Average heterozygosity</td>
<td>European-American</td>
<td>0.34</td>
<td>0.27 (0.16–0.39)</td>
<td>0.29 (0.14–0.44)</td>
</tr>
<tr>
<td></td>
<td>Chinese</td>
<td>0.26</td>
<td>0.24 (0.11–0.37)</td>
<td>0.26 (0.09–0.43)</td>
</tr>
<tr>
<td></td>
<td>Yoruba</td>
<td>0.22</td>
<td>0.27 (0.17–0.37)</td>
<td>0.31 (0.19–0.41)</td>
</tr>
<tr>
<td>Average F_{ST}</td>
<td>European-American versus Chinese</td>
<td>0.11</td>
<td>0.14 (0.02–0.27)</td>
<td>0.09 (0.03–0.15)</td>
</tr>
<tr>
<td></td>
<td>European-American versus Yoruba</td>
<td>0.12</td>
<td>0.16 (0.01–0.30)</td>
<td>0.14 (0.07–0.21)</td>
</tr>
<tr>
<td></td>
<td>Chinese versus Yoruba</td>
<td>0.19</td>
<td>0.17 (0.02–0.31)</td>
<td>0.16 (0.06–0.23)</td>
</tr>
<tr>
<td>Average DAF distribution</td>
<td>European-American</td>
<td>0.34</td>
<td>0.35 (0.21–0.48)</td>
<td>0.41 (0.24–0.58)</td>
</tr>
<tr>
<td></td>
<td>Chinese</td>
<td>0.26</td>
<td>0.35 (0.21–0.49)</td>
<td>0.4 (0.23–0.58)</td>
</tr>
<tr>
<td></td>
<td>Yoruba</td>
<td>0.22</td>
<td>0.29 (0.19–0.40)</td>
<td>0.34 (0.2–0.49)</td>
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DOI: 10.1371/journal.pbio.0030378.001
classes based on their genotype at CCR5-Δ32, rather than dividing them according to the full variation seen at CCR5. Figure 2 shows how an apparent signal of long-range LD can readily arise in this fashion. Briefly, one class (for example, the non-Δ32) may contain multiple distinct haplotypes whose individual signals of long-range LD may be obscured when grouped together, with the result that the other class (for example, the Δ32) appears to have much longer relative LD.

In fact, this is precisely the case for CCR5. We fully delineated the variation at CCR5 by genotyping seven additional SNPs within the gene and defined haplotypes as previously described [18] (Figure S3). There are five distinct haplotypes, including the Δ32-bearing haplotype with frequency 8% (Table S3). The relative LD of the Δ32-bearing haplotype is significantly lower than for two other haplotypes (REHH = 1.92 versus 6.77 and 3.29 at distance 500 kbp or 0.25 cM; see Figure 1C), indicating that there is no significant evidence of long-range LD on the centromere-distal side of CCR5.

We next analyzed LD on the centromere-proximal side of CCR5. We first employed the approach used in the original study and again found the Δ32-bearing chromosomes had much longer LD than non-Δ32-bearing chromosomes (REHH = 20.22 at a distance of 250 kbp or 0.25 cM; see Figure 1B). We then reanalyzed the data by disaggregating the chromosomes into the five haplotypes described above. The relative long-range LD for Δ32-bearing chromosomes is much lower (REHH = 7.26), although it is still the highest among the five haplotypes.

We sought to assess whether the extent of LD in the centromere-proximal direction on Δ32-bearing chromosomes is unusual relative to that seen across the human genome. We first compared the results to the genome-wide distribution of REHH scores for the HapMap (Release 16, www.hapmap.org), and found that Δ32-bearing chromosomes do not clearly stand out from other haplotypes of similar frequency (6%–10%) (Figures 3A and S4). Because the 120 European-American chromosomes genotyped in the HapMap project have limited power for studying low-frequency haplotypes (P. V., B. F., E. S. L., and P. C. S., unpublished data), we augmented the analysis by comparing all 32 Δ32-bearing chromosomes to simulations with larger sample size [19] (see Materials and Methods). We simulated 1,000 1-mbp regions in 400 European-American chromosomes under a neutral model, generating 5,915 haplotypes matched with a frequency similar to the Δ32-bearing haplotype (6%–10%). The level of EHH for the Δ32-bearing haplotype was not unusually high on either the centromere-distal (p = 0.49) or centromere-proximal (p = 0.15) side of CCR5 when compared to the level seen at an equivalent recombination distance for the simulated regions. The REHH (we used the EHH of the two common haplotypes for a relative value) was also not unusually high (Figure 3B).

We further examined the extent of the Δ32-bearing haplotype in comparison to other haplotypes of similar frequency. For this purpose, we defined the extended haplotype length (EHL) on each side of a haplotype to be the genetic distance at which the EHH score falls to 0.5. The EHL for the Δ32-bearing haplotype is 0.212 cM on the centromere-distal side and 0.258 cM on the centromere-proximal side, corresponding to a total of 0.470 cM (Figures 3 and S5). We then determined the EHL for haplotypes of comparable frequency (6%–10%) for both the HapMap data (average EHL is 0.354; CCR5-Δ32 is the 88th percentile) and for the simulated data (average EHL is 0.453; CCR5-Δ32 is the 64th percentile). The distribution is presented in Figure 3. Long-range LD around rare alleles is a prevalent feature in the genome, and the EHL for CCR5-Δ32 therefore does not stand out in comparison to either the HapMap or simulation.
dataset (Table S4). The EHL for CCR5-Δ32 would only be significant if the recombination rate in this region were several-fold higher than that measured by the current recombinational maps or by counting of historical recombination events (Protocol S1).

Given that long-range LD is a common feature of rare alleles in European-Americans, we wanted to test if our method would have the power to detect selection of an 8% allele over the time scale previously proposed [8]. We simulated 500 regions of 1 mbp length in 400 and 120 European-American chromosomes that had undergone a partial selective sweep beginning either 700 or 2,000 y ago for both groups of chromosomes, carrying the selected allele to a frequency of 8%. We were able to detect recent selection in the 400 chromosomes; 69% of selected alleles originating 700 y ago and 39% of selected alleles originating 2,000 y ago have EHL values above the 95th percentile when compared to the neutral distribution. There is far less power in the 120 chromosomes (30% and 10% of selected alleles originating 700 y ago and 39% of selected alleles originating 2,000 y ago have EHL values above the 95th percentile when compared to the neutral distribution). With improvements in the genetic map over the past 7 y [17], the microsatellites were shown to be on opposite sides of CCR5 and at a much shorter genetic distance (0.18 cM, Figure S6). Using the methodology and data employed by Stephens et al. [20] (Table S5), but with the revised genetic map, the estimated age rises from 688 y (275–1,875 y, 95% confidence interval) to 7,000 y (2,900–15,750 y, 95% confidence interval.). When we expanded the analysis to include 32 genetic markers that have been genotyped in the Δ32-bearing chromosomes, the estimated age also rises, to a similar value of 5,075 y (3,150–7,800 y, 95% confidence interval). The SNP-based estimate of the age differs and has tighter error bars because the denser map holds more information about historical recombination events than the two microsatellites, whose genetic diversity is roughly equivalent to two SNPs (Figure S7). The older age estimate is consistent with unpublished work on DNA extracted from 3,000-y-old burial sites in central Germany showing that the CCR5-Δ32 was present at an appreciable frequency several millennia ago, at least in central Germany [21].

Finally, we revisited the estimated date of origin for the CCR5-Δ32 mutation. The original estimate [8] was based on the analysis of two microsatellites that were in strong LD despite apparently being at a considerable genetic distance away (0.91-cM interval and both centromere-distal, according to the genetic maps that were current at the time). With improvements in the genetic map over the past 7 y [17], the microsatellites were shown to be on opposite sides of CCR5 and at a much shorter genetic distance (0.18 cM, Figure S6). Using the methodology and data employed by Stephens et al. [20] (Table S5), but with the revised genetic map, the estimated age rises from 688 y (275–1,875 y, 95% confidence interval) to 7,000 y (2,900–15,750 y, 95% confidence interval.). When we expanded the analysis to include 32 genetic markers that have been genotyped in the Δ32-bearing chromosomes, the estimated age also rises, to a similar value of 5,075 y (3,150–7,800 y, 95% confidence interval). The SNP-based estimate of the age differs and has tighter error bars because the denser map holds more information about historical recombination events than the two microsatellites, whose genetic diversity is roughly equivalent to two SNPs (Figure S7). The older age estimate is consistent with unpublished work on DNA extracted from 3,000-y-old burial sites in central Germany showing that the CCR5-Δ32 was present at an appreciable frequency several millennia ago, at least in central Germany [21].

The revised age estimate suggests the high frequency of the CCR5-Δ32 allele cannot be attributed solely to a strong...
selective event within the past millennium. If selection did play a role in the high frequency of the allele, the initial selection pressure must have occurred before the period calculated in the previous estimate [8]. It should be noted that the data do not rule out some additional selection occurring within the past millennia, but none that would be detected by the methodology used in Stephens et al. or in the current paper.

Our reanalysis of CCR5 shows that CCR5-D32 does not clearly stand out from the rest of the genome in terms of allele frequency distribution, population differentiation, or long-range LD (Figure S8). The high population differentiation and long-range LD found for CCR5-D32 are, in fact, far more common in the genome than previously believed, but none that would be detected by the methodology used in Stephens et al. or in the current paper.

Beyond the specific results for CCR5, our results have important implications for studies of selection in the human genome. First, accurate assessment of LD benefits from fully delineating the core haplotypes at a locus; it may not be sufficient to compare a haplotype of interest to the set of all other haplotypes. Second, long-range LD around specific alleles is a prevalent feature in the genome; the significance of LD results should therefore be assessed relative to empirical distributions observed in genome-wide studies with larger numbers of samples. Third, accurate estimates of an allele’s age require accurate genetic maps.

With the growing availability of genome-wide datasets, it should soon be possible to search the genome for signs of strong selective events [3] by studying the pattern of variation at every gene relative to a comprehensive genome-wide distribution. The results should shed light on important factors that have shaped our species and may provide valuable information about natural mechanisms of disease resistance.

Materials and Methods

Samples. DNA samples for 93 individuals from 12 multigenerational pedigrees of European-American ancestry were obtained from Coriell Repositories (http://locus.umdnj.edu/cr). DNA samples from 93 healthy individuals (31 mother–father–child clusters) from the Yoruba in Nigeria were obtained as part of the International Collaborative Study of Hypertension in Blacks. DNA samples from 30 Han Chinese trios from Guanchi were included. DNA samples
from a chimpanzee, gorilla, and orangutan were obtained from Corell Repositories.

**Genotype data.** We genotyped 71 SNPs in and around the CCR5-Δ32 using the mass spectrometry-based MassArray platform provided by Sequenom (San Diego, California, United States), implemented as previously described [18]. The names, locations, alleles, and allele frequencies for all SNPs used are given in Table S1. Microsatellite genotyping was conducted at the McGill University and Genome Quebec Innovation Center (Quebec, Canada), by use of MultiProbe and MiniTrak Liquid Handling Systems (Perkin-Elmer, Wellesley, California, United States) and 3730 DNA sequencers (Advanced Biosystems, Foster City, California, United States). PCR was performed with fluorescently labeled markers in standard conditions (annealing temperature of 54 °C).

We also used genotypes of 2,359 SNPs, distributed in 168 immunologic genes from 64 loci throughout the genome in the same three primate species. These SNPs were selected from public databases in multiple batches over a 1.5-y period from July 2002 to December 2003. Preference was given to “double hit” SNPs which have been shown to be more likely to be validated [24]. These criteria may bias our ascertainment of haplotype structure and may reduce the representation of rare and population-specific variation; we comment in the paper where this bias might affect our observations.

We used publicly available data from the International Haplotype Map Project as a comparative distribution of variance in the genome with which to compare our results (http://www.hapmap.org).

**Phase.** We prepared these files using Genehunter (http://www.broad.mit.edu/ftp/distribution/software/genehunter/) to uncover unambiguous phasing using family data [25]. The child chromosomes were then discarded, and we kept only the independent parent chromosomes. We then used PHASE (http://www.stat.washington.edu/stephan/code/software.html [26]) to obtain complete phased data.

**Simulations.** We used a computer program that simulates gene history with recombination based on a neutral model of evolution described elsewhere [19,28]. The program was modified to generate data comparable with that collected from the three populations—Chinese, European-American, and Yoruba. The simulations were calibrated to provide data consistent with the HapMap with respect to various genetic measures (including \( F_{ST} \), heterozygosity, and minor-allele frequency distribution) and used model parameters (including demography and recombination rate) consistent with current estimates [19]. We simulated a long region (1 mbp in length) of DNA and then mimicked the SNP selection strategy used by the SNP Consortium [29], which was the source of most of the SNPs in our study. We modified the program to generate simulations of a partial selective sweep in 400 European-American chromosomes, where 32 chromosomes had a common ancestor 2,000 y ago. We also tested where the 32 chromosomes had a common ancestor 700 y ago per Stephens et al. [8]. We also tested where the 32 chromosomes had a common ancestor 2,000 y ago.

\( F_{ST} \). Mean pairwise distance fixation index, \( F_{ST} \), was used to calculate genetic differentiation between the three populations [30,31]. \( F_{ST} \) partitions the total variance into within- and between-population components, quantifying the inbreeding effect of population substructure.

**Heterozygosity.** Net’s measure of heterozygosity [32], the probability that any two randomly chosen samples from a population are the same, was used to calculate SNP diversity:

\[
\pi = \frac{n}{n-1} \left(1 - \sum_{i=1}^{p} p_i^2\right)
\]

where \( n \) is the number of chromosomes in the sample, \( k \) is the number of alleles at a locus, and \( p_i \) is the frequency of the \( i \)th allele.

**DAF distribution.** We calculated the DAF distribution for all SNPs where it was likely that the ancestral allele could be determined by genotyping a representative chimpanzee, gorilla, and orangutan. If there was consensus primate allele across all successfully genotyped primates, it was identified as the ancestral allele. Otherwise, no ancestral allele was defined. \n
**EHH, EHH.** EHH assesses the age of each haplotype at a gene by measuring the decay of the extended ancestral haplotype (i.e., SNPs far away from the gene), which occurs over time with recombination. For a population of individuals sharing core haplotype \( X \), EHH is the probability that any two randomly chosen samples of core haplotype \( X \) have the same extended haplotype [3]. It is a measure of the decay of LD across a region of the genome that has two advantages: first, it can be used with multi-allelic markers so a core haplotype model can be studied if desired, and second, it measures LD across a region with many loci and not just between a pair of loci. The EHH is calculated as:

\[
EHH = \frac{\sum_{i=1}^{c} (F_{EHH})_{i}}{\left(\frac{c}{2}\right)}
\]

where \( i \) is the core haplotype tested, \( c \) is the number of samples of a particular core haplotype, \( e \) is the number of samples for a particular extended haplotype, and \( s \) is the number of unique extended haplotypes.

To correct for local variation in recombination rates, we can compare the EHH of a tested core haplotype to that of other core haplotypes present at the locus, using the relative EHH measure (i.e., REHH). REHH is the factor by which EHH decays on the tested core haplotype compared to the decay of EHH on all other core haplotypes combined. One must first calculate the \( \frac{EHH}{REHH} \), the decay of EHH on all other core haplotypes combined. For this, we use the following equation where \( n \) is the number of different core haplotypes:

\[
\frac{EHH}{REHH} = \frac{\sum_{i=1}^{c} (F_{EHH})_{i}}{\left(\frac{c}{2}\right)}
\]

The relative EHH (i.e., REHH) is simply \( EHH/\frac{EHH}{REHH} \). EHH and REHH were calculated for all haplotypes in all haplotype blocks for CCR5, HapMap Release 16 Chromosome 3, and the 1,000 simulated regions (120-chromosome and 560-chromosome sample sets). Haplotypes were placed into 20 bins based on their frequency. \( p \)-Values were obtained by log-transforming the EHH and REHH in the bins to achieve normality, and calculating the mean and standard deviation. All analysis was carried out using the Sweep software program (P. V., B. F., E. S. L., and P. C. S., unpublished data).

**Observed historical recombination (marker breakdown, all EHH).** When comparing EHH/REHH values across regions, it is important to make sure that the value is being calculated at a similar genetic distance. This will soon be replaced with better cM values, but, where they are not known, this can be matched by the “marker breakdown,” that is the degree to which each added marker at a further distance causes the extended haplotypes to decay for all core haplotypes [3]. This gives an evaluation of how much historical recombination (observed recombinants) has occurred over a distance from the core, and therefore what genetic distance is being looked at. This can be calculated as “all EHH.”

\[
allEHH = \frac{\sum_{i=1}^{c} (F_{EHH})_{i}}{\left(\frac{c}{2}\right)}
\]

where \( n \) is the number of different core haplotypes, \( e \) is the number of samples of a particular core haplotype, \( s \) is the number of samples for a particular extended haplotype, and \( s \) is the number of unique extended haplotypes.

**Bifurcation diagram.** To visualize the breakdown of LD on core haplotypes, we used bifurcation diagrams [3]. The root of each diagram is a core haplotype, identified by a dark-blue circle. The diagram is bidirectional, portraying both proximal and distal LD. Moving in one direction, each marker is an opportunity for a node; the diagram either divides or not, depending on whether both or only one allele is present. Thus, the breakdown of LD on the core haplotype background is portrayed at progressively longer distances.

The thickness of the lines corresponds to the number of samples with the indicated long-distance haplotype.

**Supporting Information**

**Figure S1.** \( F_{ST} \) and Heterozygosity for SNPs within 100 kbp of CCR5 Genotyped to 100-kbp Sliding Windows for HapMap Release 16 for European-Americans

Found at DOI: 10.1371/journal.pbio.0030378.sg001 (54 KB DOC).

**Figure S2.** The DAF Distribution of CCR5 Compared to 100-kbp Sliding Windows for HapMap Release 16 for European-Americans

Found at DOI: 10.1371/journal.pbio.0030378.sg002 (62 KB DOC).
Table S3. Extended haplotype length for haplotypes of different frequency on Hmap chromosome 3 in European-Americans found at DOI: 10.1371/journal.pbio.0030378.s004 (23 KB DOC).

Table S5. Details of CCR5-A32 date estimates found at DOI: 10.1371/journal.pbio.0030378.s005 (26 KB DOC).

Accession number

The locusLink (http://www.ncbi.nlm.nih.gov/LocusLink) accession number for the C-C chemokine receptor 5 is 1234.

Acknowledgments

PCS is funded by the Damon Runyon Cancer Research Foundation and by a L'Oreal award for women in Science. EW was funded by the Cancer Research Institute. We thank Andrei Verner and his colleagues at McGill University and Genome Quebec Innovation Center for their work on microsatellite genotyping. We thank Mary Carrington, Dan Richter, Parisa Sabeti, and three anonymous reviewers for their suggestions and reviews of our manuscript.

Competing interests. The authors have declared that no competing interests exist.

Author contributions. PCS, EW, MC, DA, SO, and ESL conceived and designed the experiments. PCS, EW, DA, and ESL performed the experiments. PCS, EW, DA, SO, and ESL contributed reagents/materials/analysis tools. PCS, EW, DA, and ESL wrote the paper.


