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Current relaxation of selection on the human genome: Tolerance of deleterious mutations on olfactory receptors

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ABSTRACT

Knowledge and understanding about the selective pressures that have shaped present human genetic diversity have dramatically increased in the last few years in parallel with the availability of large genomic datasets. The release of large datasets composed of millions of SNPs across hundreds of genomes by HAPMAP, the Human Genome Diversity Panel, and other projects has led to considerable effort to detect selection signals across the nuclear genome (Coop et al., 2009; Lopez Herraez et al., 2009; Sabeti et al., 2006, 2007; Voight et al., 2006). Most of the research has focused on positive selection forces although other selective forces, such as negative selection, may have played a substantive role on the shape of our genome. Here we studied the selective strengths acting presently on the genome by making computational predictions of the pathogenicity of nonsynonymous protein mutations and interpreting the distribution of scores in terms of selection. We could show that the genetic diversity for all the major pathways is still constrained by negative selection in all 11 human populations studied. In a single exception, we observed a relaxation of negative selection acting on olfactory receptors. Since a decreased number of functioning olfactory receptors in human compared with other primates had already been shown, this suggests that the role of olfactory receptors for survival and reproductive success has decreased during human evolution. By showing that negative selection is still relaxed, the present results imply that no plateau of minimal function has yet been reached in modern humans and therefore that olfactory capability might still be decreasing. This is a first clue to present human evolution.

1. Introduction

One of the constant efforts of molecular anthropology's fathers such as Morris Goodman was to identify and describe the forces that have shaped human genome evolution (Conrad et al., 1983; Wildman et al., 2003). Based on nucleic acid and protein sequence comparisons between species, they described the evolutionary driving forces that promoted the fixation of new variants on the human lineage (positive selection), and also the forces that have limited the fixation of any new variant (negative selection). Whereas positive selection findings taught us about adaptive evolution, negative selection results taught us about physico-chemical and physiological constraints acting on proteins. Because adaption of an organism to a new environment leads to new constraints, positive selection often causes subsequent negative selection (Goodman, 1982); conversely, a new environment or new way of life can release previous negative selection.

Although positive selection, the most common focus of research on adaptive genetic variants, defines the forces leading to the frequency increase of an adaptive genetic variant, such as for allowing adults to digest milk in farmer populations (Gerbault et al., 2009), negative selection defines the forces leading to the frequency decrease of a genetic variant. For example, some variants can favor the appearance of lethal and fetal diseases, with the result that the fitness of the variant carriers will be lower than the non-carriers and consequently the frequency of this variant will decrease. Thus, knowledge of the negative selection fingerprint on our genome is crucial for understanding the constraints acting on our genome and ultimately for understanding the origin of genetic diseases.

Results involving negative selection on human populations are often based on the d*N*/d*S* ratio (reviewed in Harris, 2010). However, due to limitations of this method, other studies have proposed using algorithms predicting the potential deleterious effect, mainly based on the observed diversity in other organisms. By showing that deleterious SNPs are on average younger and/or less frequent than non-deleterious SNPs, these studies suggest that negative selection is still active on human populations, or at least was active until recently (Barreiro et al., 2008; Lohmueller et al., 2008; Pereira et al., 2011; Pierron et al., 2011). However, these studies are limited in terms of populations and groups of genes studied.

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Here we have tested whether negative selection has played a significant role on current global genetic diversity observed by the HAPMAP project (Altshuler et al., 2010) by comparing the frequency of SNPs predicted as deleterious versus not deleterious. Our results confirm that the presently observed human genetic diversity is still shaped by negative selection; indeed, we could observe that the genetic diversities of all major pathways are shaped by negative selection. However, the results show an accumulation of deleterious SNPs on olfactory receptor genes in positions highly conserved relative to other primates. Because this result was observed in the 11 different human HAPMAP populations, we propose a release of negative selection on olfactory transduction on the current human population that is still going on.

2. Materials and methods

2.1. SNP allele and genotype frequencies

Allele and genotype frequencies of 1.5 million SNPs were downloaded from the HapMap3 Public Release #28 dataset (Altshuler et al., 2010). Including the original 270 samples used in Phases I and II, the HapMap3 sample collection comprises 1,301 samples collected using two platforms: the Illumina Human1M (by the Wellcome Trust Sanger Institute) and the Affymetrix SNP 6.0 (by the Broad Institute). The samples came from 11 populations: ASW, African ancestry in Southwest USA (90 individuals); CEU, Utah residents with Northern and Western European ancestry from the CEPH collection (180 individuals); CHB, Han Chinese in Beijing, China (90 individuals); CHD, Chinese in Metropolitan Denver, Colorado (100 individuals); GIH, Gujarati Indians in Houston, Texas (100 individuals); JPT, Japanese in Tokyo, Japan (91 individuals); LWK, Luhya in Webuye, Kenya (100 individuals); MEX, Mexican ancestry in Los Angeles, California (90 individuals); MKK, Maasai in Kinyawa, Kenya (180 individuals); TSI, Toscans in Italy (100 individuals); YRI, Yoruba in Ibadan, Nigeria (180 individuals).

2.2. Pathogenicity evaluation

Pathogenicity of amino acid variation encoded by HAPMAP nonsynonymous SNPs (nsSNPs) has been evaluated using two well-known algorithms, SIFT and POLYPHEN. SIFT predictions for the HAPMAP nsSNPs were extracted from the list of pre-computed predictions based on the NCBI's dbSNP (build 132) database available on the SIFT website (Kumar et al., 2009). Similarly, we have also extracted the POLYPHEN prediction for HAPMAP nsSNPs from a precomputed list of all human SNPs from dbSNP build 126 available on the POLYPHEN website (Ramensky et al., 2002).

2.3. Ancestral and derived allele

For each nsSNP studied, we have distinguished the "ancestral" and "derived" allele. The ancestral nucleotide comes from the ENSEMBL database, which has inferred ancestral sequences from primate EPO multiple alignments using Ortheus (Flicek et al., 2011). SNPs for which the ancestral status are unknown were removed from the analysis.

2.4. Non-unique SNPs

It has been shown that probes used in HAPMAP methodology can align to several positions in the genome, leading to false positives and to the existence of artificial polymorphisms. In order to avoid this problem, we removed from our analysis any SNP listed by Doron and Shweiki as putatively nonunique (Doron and Shweiki, 2011).

2.5. Maximum frequencies

Our hypothesis is that deleterious nsSNPs are subject to negative selection and thus can rarely reach high frequencies. Conversely, non-deleterious nsSNPs are not negatively selected; therefore, it is possible that these nsSNPs can randomly reach high frequencies in some populations. In order to study the existence of negative selection on the current human population, we have tested the influence of deleterious status of nsSNP-derived alleles on their maximal observed frequency in any studied human population. For each nsSNP we have collected the frequency of the derived allele in each different HAPMAP population and called the highest value collected as the nsSNP's maximal observed frequency. In order to reduce the influence of genotyping error on the results, throughout this study we retained only the nsSNPs with a maximal observed frequency higher than 1%. Then we compared by Mann–Whitney U-test the distribution of the maximal frequency between deleterious and not deleterious SNPs. This analysis was done twice: (i) based on SIFT predictions, and (ii) based on POLYPHEN predictions.

2.6. Population frequencies

We next studied whether the effect of negative selection is strong enough to be seen at a population level (supposedly more affected by a random component). Analysis was carried out as in Section 2.5.

2.7. Comparison between pathways

We have also studied the effect of negative selection on the different gene categories. Based on the genes in which they are located, the nsSNPs were categorized (when possible) into the major KEGG pathways (Kanehisa, 2002). The KEGG pathway map is a molecular interaction/reaction network diagram, and was manually created from published materials. Analysis for each pathway was carried out as described in Section 2.5.

In order to compare the effect of negative selection on the different pathways, we studied whether some pathways could support a high proportion of deleterious common polymorphisms. In this test we used the Fay et al. definition of common polymorphism, a SNP with a derived allele presenting an allele frequency higher than 15% (Fay et al., 2001). Using a chi-square test, we compared the ratio of deleterious/tolerated (using SIFT) common polymorphisms between two pathways. We performed the same test based on POLYPHEN by grouping the *possibly damaging* and *probably damaging* in one category, *damaging*, and then tested the ratio benign/damaging nsSNPs between two pathways. We performed this test at a global level using the maximal observed frequency and also at a population level using the observed frequency in each HAPMAP population.

2.8. Olfactory receptor nsSNPs

In order to study if the observed common nsSNPs classified by SIFT as *deleterious* and by POLYPHEN as *damaging* are due to positive selection on the carrier gene, we listed the empirical *p*-value for selection on each gene on HAPLOTTER database based on HAPMAP phase II (Voight et al., 2006). This *p*-value is based on the number of SNPs with an extreme iHS (Integrated Haplotype Score), which has high statistical power to study positive selection on non-fixed polymorphisms. We have used the significance threshold of 0.01 as proposed by the authors.

In order to study whether the observed results are due to a technical issue biasing the genotyping system caused by the sequence homology of numerous olfactory receptor genes, we have

compared the HAPMAP frequency and the frequency obtained by the project "1000 genomes" using SPSMART (Altshuler et al., 2010; Amigo et al., 2008).

3. Results

In order to study the influence of negative selection on current human genetic diversity, we have compared the frequency in 11 human populations between deleterious and non-deleterious SNPs, as described in Materials and Methods. As a result, the frequencies were available for 14,952 nsSNPs with a SIFT prediction (2550 *deleterious* nsSNPs, 12,402 *tolerated* nsSNPs) and for 15,571 nsSNPs with a POLYPHEN prediction (10,744 *benign* nsSNPs, 2513 *possibly damaging* nsSNPs, 1477 *probably damaging* nsSNPs). Taken together, 13,434 SNPs have a prediction from both algorithms. In addition, since SIFT predicts fewer deleterious SNPs than POLYPHEN, there are some conflicting predictions (3180 SNPs) (Supplementary Table 1), although this does not affect any conclusion.

When we studied the relation between the maximal frequencies observed in any population and the algorithm prediction (Supplementary Tables 2 and 3; Fig. S1), we observed that the nsSNPs predicted as *deleterious* by the SIFT algorithm presented a significantly lower maximal frequency than the nsSNPs predicted as *tolerated* (Fig. 1b, one-tailed Mann–Whitney U-test p-value < 10^{-15}). Conversely, the two categories of SNPs predicted as *probably damaging* and *possibly damaging* by POLYPHEN presents a significantly lower maximal frequency than nsSNPs predicted as *benign* (Fig. 1a, one-tailed Mann–Whitney U-test p-value < 10^{-15}). This result suggests that, as expected, negative selection is still limiting present human genetic diversity.

We examined the population dependence of nsSNP type frequency to test whether negative selection is strong enough to shape nsSNP diversity only in specific populations more sensitive to random events. We observed that in any population the nsSNPs predicted as *deleterious* by the SIFT algorithm represent a significantly lower frequency than nsSNPs predicted as *tolerated* (Fig. 1d, one-tailed Mann–Whitney U-test p-value < 10^{-15}). Conversely, the two categories of SNPs predicted as *probably damaging* and *potentially damaging* by POLYPHEN represent a significantly lower frequency than nsSNPs predicted as *benign* (Fig. 1c, one-tailed Mann–Whitney U-test p-value < 10^{-15}). These results demonstrate that negative selection is strong enough to shape the local SNP diversity independent of the population studied.

To examine whether negative selection favors any of the various gene categories, we clustered the nsSNPs following their categorization from the KEGG pathway map (available for 4782 nsSNPs predicted by SIFT (Supplementary Table 4) and 4557 nsSNPs predicted by POLYPHEN (Supplementary Table 5)). We found that, for any pathway under study, nsSNPs predicted as *deleterious* by the SIFT algorithm are present at a significantly lower frequency than nsSNPs predicted as *tolerated* (Fig. 2b, one-tailed Mann–Whitney U-test p-value < 5×10^{-4}). The range of p-values go from 10^{-4} to 10^{-16} depending on the pathway studied and varies mainly because of the number of nsSNPs studied in each pathway (Supplementary Tables 4 and 5). Conversely, nsSNPs predicted as *probably damaging* and *possibly damaging* by POLYPHEN are present at a significantly lower frequency than nsSNPs predicted as *benign* (Fig. 2a, one-tailed Mann–Whitney U-test p-value < 5×10^{-3}). Again, p-values range from 10^{-4} to 10^{-16} depending on the pathway studied and is mainly a function of the number of nsSNPs studied in each pathway (Supplementary Tables 4 and 5). This result demonstrates that negative selection is affecting all the major pathways.

Interestingly, the group of *deleterious* (SIFT) and *damaging* (POLYPHEN) nsSNPs affecting the pathway called "organismal system" presents a higher average compared to these groups for other pathways (Fig. 2a and b). The "organismal system" pathways also have the lowest percentage of reduction between *deleterious* and *tolerated* SIFT groups, 20% compared to 35–55% for the other pathways (Supplementary Table 4). Conversely, the "organismal system" pathways have the lowest percentage of reduction between *benign* and *probably damaging* POLYPHEN groups, i.e., 34% compared to 54–60% for the other pathways (Supplementary Table 5). This result suggests a lower effect of negative selection on this pathway.

By further parsing this pathway into the different sub-pathways, we could show that this high frequency of deleterious nsSNPs is due to the olfactory sub-pathway (KEGG number 4740). Indeed, on this particular sub-pathway there is not a significant difference between *deleterious* and *tolerated* SNPs predicted by SIFT (Fig. 2d, one-tailed Mann–Whitney U-test p-value = 0.26);

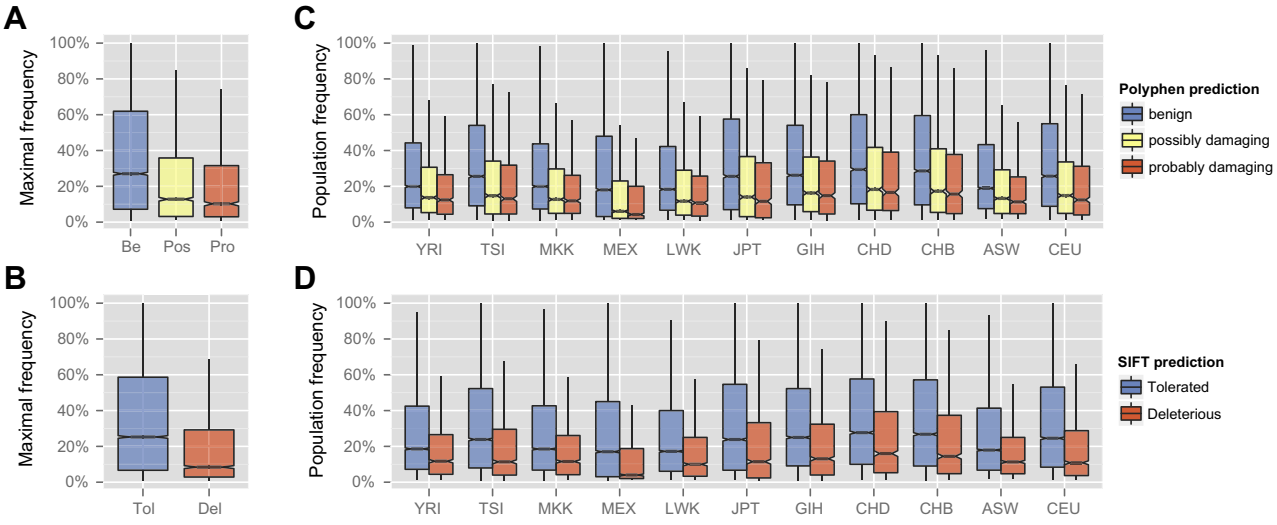

Fig. 1. Boxplots presenting the dispersion of maximal frequency of nsSNPs according their effect prediction status. Notches drawn on each side of the boxes of all figures give roughly a 95% confidence interval for the difference in two medians. (A) Predicted status based on POLYPHEN. Be, benign; Po, possibly damaging; Pr, probably deleterious. (B) Predicted status according to SIFT. (C) Dispersion of nsSNP frequency in different HAPMAP populations according to POLYPHEN predictions. Abbreviations as in A. (D) Predicted status based on SIFT; T, tolerated; D, deleterious.

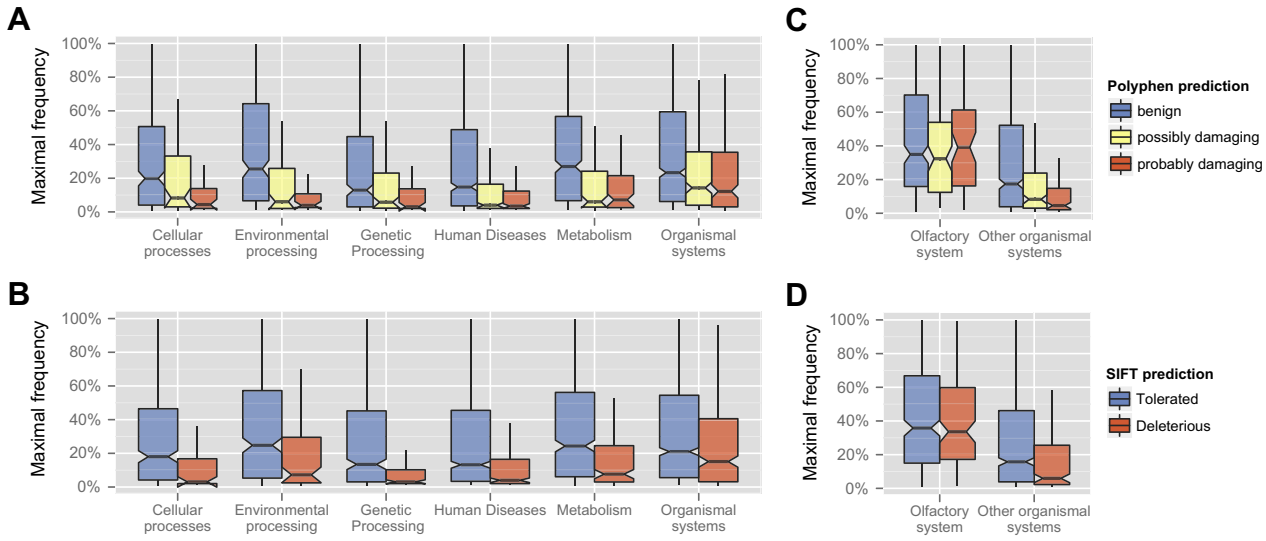

Fig. 2. Boxplots presenting the dispersion of maximal frequency of nsSNPs in different Kegg major pathways according their effect predictions. (A) Predicted status based on POLYPHEN. Abbreviations as in Fig. 1A legend. (B) Status based on SIFT; T, tolerated; D, deleterious. (C) Dispersion of maximal frequency of nsSNPs in comparing olfactory transduction and other organismal systems according their POLYPHEN predictions. (D) Predicted status based on SIFT; T, tolerated; D, deleterious.

by contrast, we have observed a very significant difference between the deleterious and tolerated SNPs belonging to the "organismal pathway" but not to the "olfactory pathway" (Fig. 2d, one-tailed Mann–Whitney U-test p-value $< 5 \times 10^{-16}$). We observed similar results based on POLYPHEN prediction: for pathway 4740 we observed no significant difference between *possibly damaging* and *benign* (Fig. 2c, one-tailed Mann–Whitney U-test p-value = 0.053), nor any significant difference between *probably damaging* and *benign* (Fig. 2c, one-tailed Mann–Whitney U-test p-value = 0.37), whereas for the other polymorphisms belonging to "organismal pathway" we observed a very significant difference between *possibly damaging* or *probably damaging* and *benign* (Fig. 2c, one-tailed Mann–Whitney U-test p-value $< 5 \times 10^{-12}$). It was also possible to obtain very similar results for the 11 HAPMAP populations based on SIFT or POLYPHEN predictions (Fig. S2).

The olfactory sub-pathway (KEGG 4740) contains a significantly higher proportion of deleterious common polymorphisms (with a frequency >15%) than any other pathway (chi-square one-tailed p-value $< 5 \times 10^{-8}$, Supplementary Table 6, Fig. S3). This result was found very significant using both maximal frequency data and population frequency and using both SIFT and POLYPHEN data (chi-square one-tailed p-value $< 5 \times 10^{-8}$, Supplementary Table 7).

We considered whether the high observed frequencies of sub-pathway 4740 could represent positive selection. However, the list of iHS p-values on the locus carrying nsSNPs classified by SIFT as *deleterious* and by POLYPHEN as *probably damaging* in pathway 4740 failed to show any significant sign of positive selection (Table 1). This result suggests that the high observed frequencies are not due to any positive selection but instead are due to a relax-

Table 1
Frequencies of common deleterious mutation on the olfactory receptor genes and iHS p-value associated in three HAPMAP populations. p-values are based on the number of SNPs with an extreme iHS (Integrated Haplotype Score).

SNP	Gene	NP	a.a. change	% Max	Pop max	CEU (n = 110–113)		CHB (n = 83–84)		YRI (n = 111–113)	
						%	iHS p-value	%	iHS p-value	%	iHS p-value
rs1030726	OR51F1	NP001004752	D294Y	99.4	CHD	83.6	0.23	NA	0.27	43.8	0.64
rs7397032	OR56B1	NP001005180	C106R	85.8	CEU	85.8	0.21	70.8	0.75	67.7	0.31
rs1378739	OR51G1	NP001005237	Y125S	81.5	LWK	NA	0.53	70.2	0.36	77.2	0.19
rs4075258	OR8S1	NP001005203	L82P	77.3	JPT	55.9	0.53	76.8	0.13	45.6	0.47
rs10838852	OR4X1	NP001004726	P282S	62.0	MEX	54.9	0.09	48.8	0.03	16.8	0.06
rs12150427	OR1A2	NP036484	W293C	60.0	CHD	32.7	0.10	57.7	0.42	3.1	0.76
rs17277221	OR11H6	NP001004480	Y236H	56.2	YRI	49.6	0.79	17.5	0.24	56.2	0.89
rs1030723	OR51F1	NP001004752	S226F	54.9	YRI	8.8	0.23	NA	0.27	54.9	0.64
rs4057749	OR8B4	NP001005196	C178R	54.9	YRI	18.1	0.06	23.2	0.24	54.9	0.55
rs10252253	OR2A2	NP001005480	L210P	46.9	YRI	16.4	0.05	NA	0.76	46.9	0.19
rs28446289	OR13G1	NP001005487	R224C	44.6	CHD	34.5	NA	33.3	NA	31.0	NA
rs12225462	OR4C15	NP001001920	R286C	39.7	GIH	9.7	0.04	23.2	0.24	10.7	0.47
rs7116575	OR8B4	NP001005196	C140F	35.4	YRI	8.0	0.06	22.0	0.24	35.4	0.55
rs16841009	OR6K6	NP001005184	C197R	34.5	YRI	NA	0.53	NA	0.42	34.5	0.76
rs16841017	OR6K6	NP001005184	P211L	34.5	YRI	NA	0.53	NA	0.42	34.5	0.76
rs7132600	OR6C1	NP001005182	T222I	33.1	MKK	17.6	0.29	NA	0.14	18.0	0.09
rs17127947	OR8D4	NP001005197	L55R	28.8	YRI	16.8	0.53	11.9	0.14	28.8	0.55
rs4367963	OR5I1	NP006628	L50S	23.3	LWK	11.9	0.15	11.3	0.16	10.2	0.31
rs11835321	OR6C3	NP473445	M133T	22.8	LWK	NA	0.26	NA	0.14	22.1	NA
rs12070953	OR2B11	NP001004492	D300G	22.2	LWK	12.8	0.56	NA	0.76	20.4	0.55
rs11826041	OR2AG1	NP001004489	N42S	20.4	YRI	NA	0.45	NA	0.07	20.4	0.76
rs10245778	OR6V1	NP001001667	S323F	16.5	YRI	8.8	0.14	5.4	NA	16.5	0.05
rs12224086	OR5AS1	NP001001921	R122L	15.3	GIH	11.9	0.13	11.4	0.31	3.1	0.36

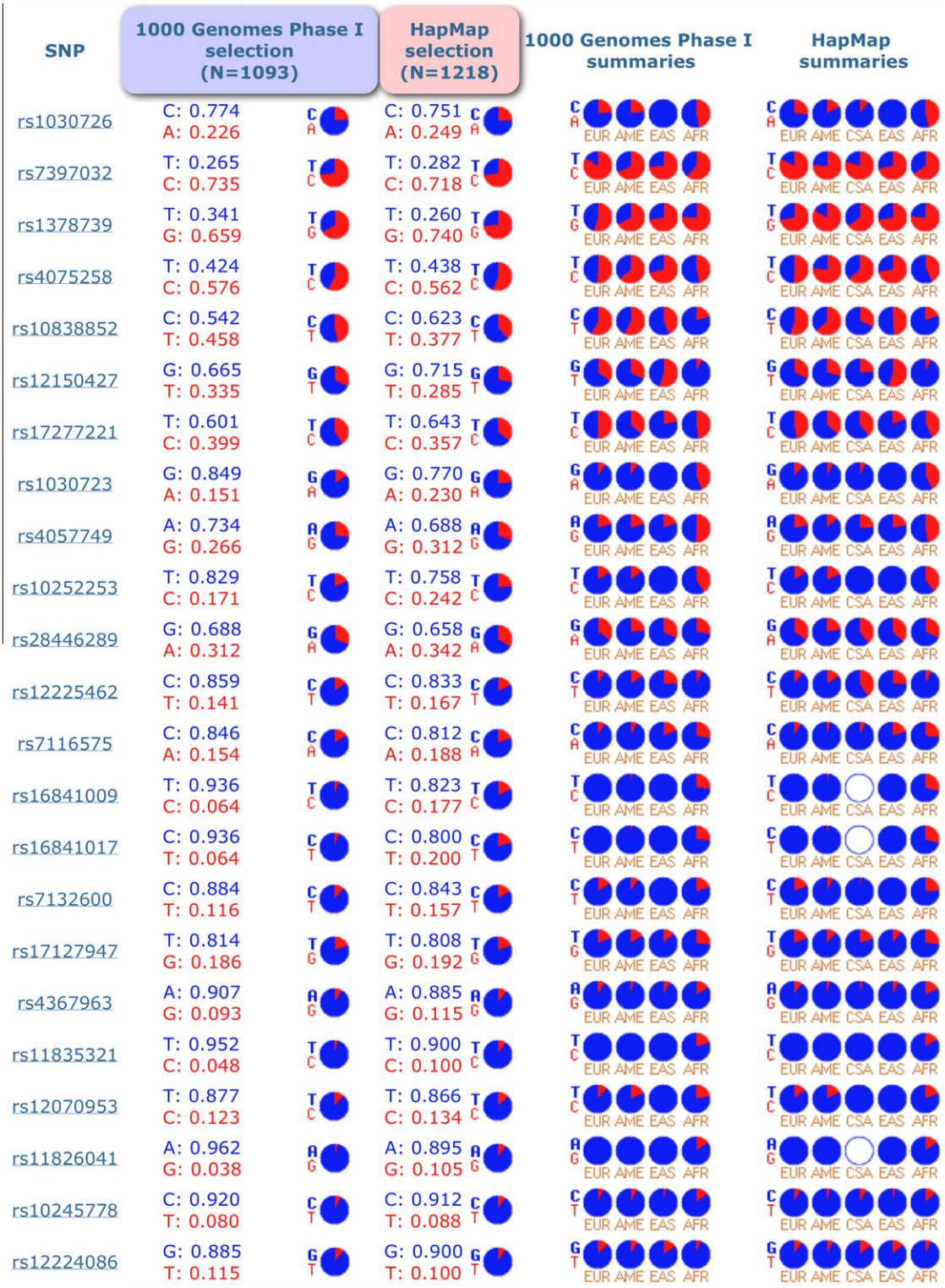

Fig. 3. SPSMART comparison of observed frequencies between the HAPMAP and 1000 genomes projects for common nsSNPs predicted as "*deleterious*" by SIFT and "*probably damaging*" by POLYPHEN. The SPSMART interface is at http://spsmart.cesga.es.

ation of the negative selection constraint. Interestingly, all these nsSNPs are located on olfactory receptors. The common origin of these genes and their strong sequence homology could explain the result in two ways: (i) a strong sequence homology could decrease the specificity of the probe used by HAPMAP; however, it was possible to exclude this technical problem by showing the HAPMAP frequency obtained for these nsSNPs is very similar to the frequency obtained by direct sequencing by the project "1000 genomes" (Fig. 3); and (ii) the high duplication rate of these genes could interfere with the algorithm used, and these positions at the particular genes may not be so conserved in primates. Again, it was possible to exclude this mismeasure of primate conservation

by showing that these human polymorphic sites are conserved in other primates species based on genomic alignment (Fig. S4).

4. Discussion

Our results show that negative selection still shapes the genetic diversity of modern humans and its action is strong enough to be seen in every population studied. We have shown that all the major pathways are subject to negative selection; however, some pathways, such as the olfactory transduction pathway, and specifically the olfactory receptor genes, are currently under a relaxation of this negative selection. Interestingly, previous studies had already shown a decreasing number of functioning olfactory receptors in primates compared to other mammals and even on humans compared to chimpanzees (Gilad et al., 2003a,b; Gilad and Lancet, 2003; Rouquier et al., 1998). Those results suggest that the role of olfactory receptors on survival and reproductive success has decreased. By showing that negative selection is still relaxed, the present result implies that no plateau of minimal function has yet been reached.

The decreased number of olfactory receptors tends to confirm the general belief that modern humans have a poor sense of smell, supported by the comparison of human nose size with the front of the snout of most of others mammals. Evolution studies have also linked the decrease of olfactory function to the adaptation to arboreal habitat and the gradual ascendance of vision, which are key features of primate evolution. Indeed, arboreal habitat has enhanced the selective pressure in favor of 3D vision; in consequence, snout volume has decreased to allow eyes to come into the middle of the face. Due to the arboreal habitat and later due to the adoption of a bipedal posture by human ancestors, the nose became farther from the ground, decreasing the variety of odors sampled and thereby decreasing the selective constraints on the rich olfactory receptors panel. However, this theory is challenged by some authors (Shepherd, 2004), who propose instead that human sense of smell could actually be better than expected due to brain evolution and enlargement, other key features of human evolution. Indeed, Shepherd argues that the decrease of nose size and olfactory receptor number have actually been compensated by a highly developed brain capable of better smell integration and memory. In this context the functional decrease could be less severe than it might appear from counting the number of functional olfactory receptors (Go and Niimura, 2008; Matsui et al., 2010). However, to our knowledge no inter-species comparison presently supports or invalidates this alternative theory.

In any case, the nature of the deleterious effects of the damaging nsSNPs identified in the present study is not yet known and will require molecular studies. The classification of their sequence changes as damaging suggests a disruption of receptor functions and thus a decrease of the odor sensing human repertoire. By suggesting that the average olfactory capability of the human species is still decreasing, these results are a first clue to the future of human evolution.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.07.032.

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