Individual olfactory perception reveals meaningful nonolfactory genetic information

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Each person expresses a potentially unique subset of ∼400 different olfactory receptor subtypes. Given that the receptors we express partially determine the odors we smell, it follows that each person may have a unique nose; to capture this, we devised a sensitive test of olfactory perception we termed the "olfactory fingerprint." Olfactory fingerprints relied on matrices of perceived odorant similarity derived from descriptors applied to the odorants. We initially fingerprinted 89 individuals using 28 odors and 54 descriptors. We found that each person had a unique olfactory fingerprint ($P < 10^{-10}$), which was odor specific but descriptor independent. We could identify individuals from this pool using randomly selected sets of 7 odors and 11 descriptors alone. Extrapolating from this data, we determined that using 34 odors and 35 descriptors we could individually identify each of the 7 billion people on earth. Olfactory perception, however, fluctuates over time, calling into question our proposed perceptual readout of presumably stable genetic makeup. To test whether fingerprints remain informative despite this temporal fluctuation, building on the linkage between olfactory receptors and HLA, we hypothesized that olfactory perception may relate to HLA. We obtained olfactory fingerprints and HLA typing for 130 individuals, and found that olfactory fingerprint matching using only four odorants was significantly related to HLA matching ($P < 10^{-4}$), such that olfactory fingerprints can save 32% of HLA tests in a population screen ($P < 10^{-6}$). In conclusion, a precise measure of olfactory perception reveals meaningful nonolfactory genetic information.

olfactory perception | HLA | MHC | olfactory genetics | autoimmunity

Humans have superb olfactory discrimination (1, 2), likely reflecting the combined output of ∼400 different subtypes of olfactory receptors (3). Specific olfactory receptors are likely responsible for specific aspects of olfactory perception (4–7). Because any two individuals differ by ∼30% of their olfactory receptor subtype genome (8), this renders a potentially unique nose for each person (4, 9). If we could capture this uniqueness with a perceptual test, a sort of perceptual olfactory fingerprint, this should then be informative on the underlying individual olfactory receptor subtype genome. The notion of a psychophysical test informing on underlying genes is of course well known from vision, where color blindness charts inform us about genes coding for different opsins in the retina (10). In olfaction, specific anosmias can also point to alterations in a specific gene (4, 11, 12). Unlike in color vision or specific anosmias, the olfactory fingerprints we propose will likely not inform on a specific gene, but they may link overall perception to overall genetic makeup.

Moreover, because the olfactory receptor genome is linked to various other genetic loci, this perceptual test may inform on genetic makeup beyond olfaction alone. For example, the olfactory receptor subtype genome is linked to MHC genetic makeup (13–15). The human counterpart of MHC is known as HLA. HLA genes encode cell-surface glycoproteins that bind short peptides and make them available to T lymphocytes. Through this mechanism, HLA genes influence immunological self/non–self-discrimination and subsequently, tissue rejection and immune recognition of infectious disease. Therefore, HLA tests are routinely conducted to match up donors and recipients of organ and bone marrow transplants. Given an olfactory receptor genome to HLA link, we hypothesized that if olfactory fingerprints mirror olfactory receptor repertoire, they should be indicative of HLA. It has long been known that information on HLA is available in body odor (16, 17). Linking HLA to the sense of smell alone (18, 19) would close a functional loop potentially subserving behavioral mechanisms of selection based on the sense of smell (20). With all this in mind, we set out to test if we could generate individual olfactory fingerprints, and whether these inform on HLA.

Results

Olfactory Fingerprints Were Based on Matrices of Perceived Odorant Similarity. We derived fingerprints using a matrix of perceived odor similarities (21). We used a palette of 28 odors (list of experiment 1A odors in Table S1) that provided for 378 pairwise similarities ($28 \times 27/2 = 378$). Such a 378-dimensional olfactory fingerprint allows for potential characterization of a practically infinite number of individuals. Rather than directly obtaining pairwise similarity estimates, we derived pairwise similarity from 54 different descriptors applied to each odorant alone (list of experiment 1A descriptors in Table S2). We opted for derived over direct similarity ratings because though the two are highly correlated (22), derived similarity is potentially much easier and faster to obtain. For example, direct similarity ratings of the 378

Significance

Cyrano de Bergerac observed that "a large nose is the mark of a witty, courteous, affable, generous and liberal man." Here we report that individual noses, not how they look but rather how they function, indeed say a lot about a person. Each person expresses a nearly unique set of different olfactory receptor genes, and therefore may have unique olfactory perception. We developed a highly sensitive perceptual test we call the "olfactory fingerprint" that captures this variability. Individual olfactory fingerprints are therefore mirrors of individual olfactory genomes. We demonstrate that such fingerprints predict genetic features linked to the olfactory system, such as aspects of immune regulation. Thus, a precise measure of olfactory perception reveals meaningful nonolfactory genetic information.

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Conflict of interest statement: The Weizmann Institute is filing for a patent on the method of olfactory fingerprinting.

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possible odorant pairs in this study would entail 756 individual odorant presentations (A vs. B × 378) each followed by one question: rate similarity. Such a large number of odorant presentations (756) are impractical in an experiment with a human subject. In contrast, 378 derived similarity ratings entail 28 odorant presentations each followed by several questions (e.g., rate how coconut, rate how lemony), and derivation of similarity from the answers; this remains a feasible experiment, and moreover, the number of questions can later be reduced based on the current analysis. Importantly, we acknowledge that the use of odorant descriptors likely entails personal (23) and cultural differences (24), yet this method does not assume or rely on any agreement across individuals in the application of a given descriptor (Fig. 1 contains a schematic of fingerprint acquisition that explains this issue). Thus, we calculated an individual olfactory fingerprint by computing all of the pairwise distances (i.e., derived similarity) between all odorants rated. For a measure of distance between odorant k and odorant m we used the following equation:

$$distance_{k,m} = \sqrt{\frac{\sum_{i=1}^{n} \left(P_i^k - P_i^m\right)^2}{n}}, \quad [1]$$

where P_i^k is the perceptual rating of odorant k using descriptor i, and P_i^m is the perceptual rating of odorant m using descriptor i. In words, the distance between odorant k and odorant m is the square root of the mean of the squared difference between all perceptual ratings for those two odorants. Once all of the pairwise distances are computed, we can notate an olfactory fingerprint in the following form:

$$FP_{k+m-1} = distance_{k,m}, \quad [2]$$

where $k = 1...N-1$ and $m = k+1...N$. In words, each element of the olfactory fingerprint is the distance between pairs of odorants, where each pairwise distance is calculated only once (because $distance_{k,m} = distance_{m,k}$), and the distance between an odorant to itself is omitted (because $distance_{k,k} = 0$). Consequently, if we use N odors to construct an olfactory fingerprint, the resulting olfactory fingerprint will have $N \times (N-1)/2$ elements. To negate the impact of variance in the number of descriptors used by subjects (e.g., one subject may have used only 50 of the 54 descriptors, and another only 45, leaving all other descriptors unrated, see Figs. S1 and S2 for descriptor use), we normalized each distance between odor pairs by the number of mutual descriptors used for this pair. For example, if odors A and B were rated along 50 descriptors by subject 1, and 45 descriptors by subject 2, dividing the distance between odor pairs by $\sqrt{50}$ for subject 1 and by $\sqrt{45}$ for subject 2 enables direct comparison of the perceived distances between odors A and B for subjects 1 and 2.

As noted, such derivations of similarity do not assume that people agree with each other along any given descriptor (e.g., how coconut?), they only assume that a person agrees with him/herself (Fig. 1). In other words, such similarity matrices are odor dependent but descriptor independent, which is here verified in experiments where fingerprints for the same individual obtained with the same odorants but different descriptors remained highly correlated. Finally, for fingerprint visualization purposes alone, we first generated a rectangular image by interpolating the 378 fingerprint values to 500 values. We then projected these values onto a 25 × 20 matrix, and conducted a 2D interpolation providing a 2 × 2 K matrix, which we then projected onto a semicircle (Fig. 2 *A*–*D*). We applied these methods in 89 subjects (40 women, mean age = 25.7 ± 3.1 y).

Olfactory Fingerprints Were Individually Specific. Previous studies implied that gross olfactory perception is similar across individuals (25), and to test whether this was true here as well, we calculated the mean rating along each descriptor for each odorant, and then calculated the correlation of each individual

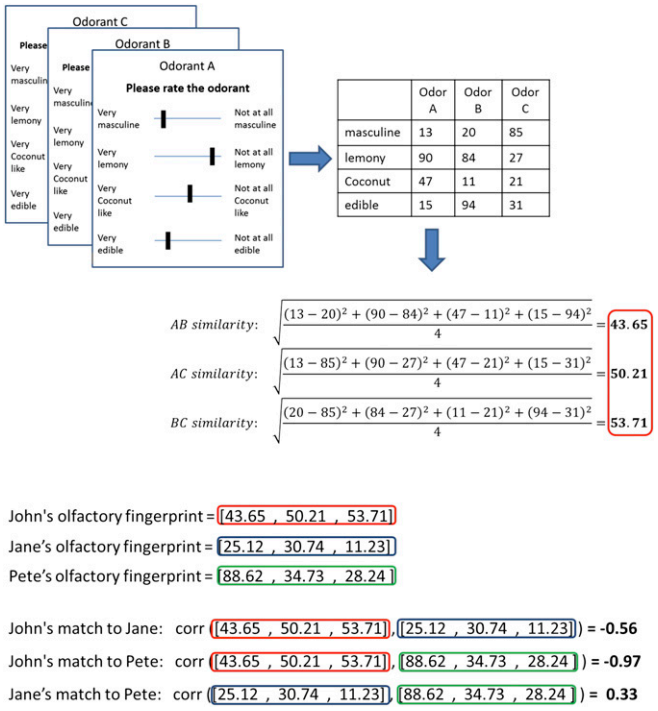

Fig. 1. A schematic for obtaining olfactory fingerprints. Odorant ratings along visual analog scales (VAS) are converted into numbers reflecting location on the VAS line. Pairwise odorant similarity is calculated as the distance across all descriptors used, and pairwise person similarity is calculated as the correlation across individuals. All this ensures that fingerprints are odorant specific but descriptor independent. For example, imagine John who was raised on an island smelling real coconuts, and Jane who knows coconut only from Bounty chocolate bars. Coconut is very different for these two individuals. John may rate odor A as 47% like coconut, odor B as 49% like coconut, and odor C as 4% like coconut. Thus, odors A and B are highly similar, and both are dissimilar from odor C. Jane may rate same odor A as 21% like coconut, odor B as 19% like coconut, and odor C as 100% like coconut. Once again, odors A and B are highly similar, and both are dissimilar from odor C. Thus, John and Jane will have very similar olfactory fingerprints derived from these three odorants and one descriptor, even though they are in total disagreement as to what coconut smell is like.

with the mean. Note that here we are not comparing fingerprints, but rather ratings along specific descriptors; this revealed that individuals are indeed similar to each other in their gross perception. For example, in all top 10 correlated descriptors the mean perception was a very good predictor of individual perception (all $r > 0.68$; Fig. 2*E*). Moreover, the primary perceptual dimension of odor, namely pleasantness (2, 26) was particularly highly correlated across individuals and odors, at $r = 0.73 \pm 0.1$ (Fig. 2*E*). In other words, the average description of an odorant using common descriptors is a pretty good estimation of what any given individual will say about that odor.

We next set out to determine whether despite this gross agreement on odor perception, our sensitive perceptual test will uncover a unique olfactory perceptual fingerprint. Consistent with our hypothesis, we found that across the 89 subjects we tested, no two subjects had the same fingerprint (e.g., Fig. 2*A* vs. Fig. 2 *D*, *F*, and *G*). We calculated all pairwise distances between olfactory fingerprints (using Pearson's correlation) and found that the average correlation across individuals (omitting self–self) was $r = 0.3 \pm 0.13$ (Fig. 2*G*). In other words, whereas gross perception was similar across individuals, a fine measure of perception revealed individual perception for each of the 89 subjects we tested.

Olfactory Fingerprints Were Independent of Descriptor Identity. The emergence of 89 individual fingerprints alone does not necessarily

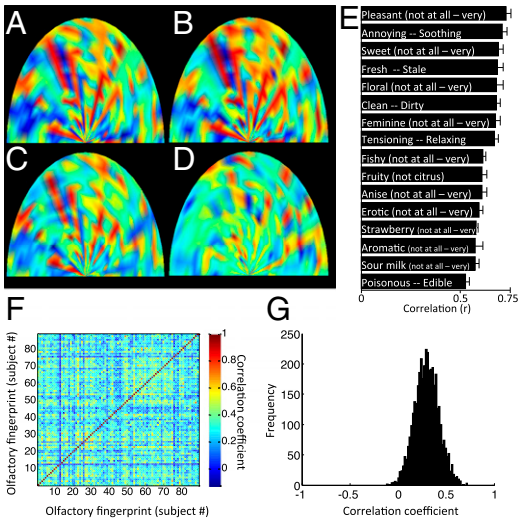

Fig. 2. Olfactory fingerprints were consistent within individuals and different across individuals. To visualize fingerprints, we interpolated the 378 pairwise similarities (*Methods*). (*A*) An example olfactory fingerprint of one individual. (*B*) The olfactory fingerprint of the same individual from *A*, but here derived using a different set of nonoverlapping descriptors. (*C*) The olfactory fingerprint of the same individual from *A* and *B*, but here obtained separately 16 d later. (*D*) The olfactory fingerprint of a different individual. Correlations: A ⇔ B, $r = 0.89$; A ⇔ C, $r = 0.61$; A ⇔ D, $r = 0.25$. (*E*) The best-correlated descriptors across all odors. (*F*) Heat-map matrix of distances between all subject pairs. (*G*) Histogram of correlation coefficients of all non-self–self pairs.

imply that we captured individual olfactory perception because one can potentially obtain such a result (89 different fingerprints) with random odor similarity ratings. To verify that olfactory fingerprints captured individual olfactory perception, for each of the 89 participants we next generated two alternative fingerprints, A and B, each using a random independent half of the descriptors used to derive similarity. We computed a matrix of all of the pairwise distances between olfactory fingerprints A and B (89 × 89) and tested whether the distance of a subject from him/herself (using different descriptors) was smaller than the distance of a subject to anyone else. In other words, whether, despite the use of different descriptors each time, a subject remained more similar to him/herself than to anyone else (distance between fingerprints was estimated by correlation, see *SI Text* and Figs. S3–S5 for impact of alternative methods); we repeated this 1,000 times, each time selecting a different set of nonoverlapping descriptors for fingerprints A and B, and assessed the distances between fingerprints. We again plotted the heat-map correlation matrix of each individual with all other individuals, this time, however, each pairwise distance is computed as the correlation between olfactory fingerprints A and B (Fig. 3*A*). We found that the distance between an individual's two fingerprints based on the same odors but different descriptors (the diagonal in Fig. 3*A*, and Fig. 2*A* vs. Fig. 2*B*) was overwhelmingly smaller than the average distance between two different individuals [nondiagonal values in Fig. 3*A* and Fig. 2*A* vs. Fig. 2*D*; mean difference between an individual's two fingerprints $r = 0.75 \pm 0.025$, mean difference between two different individuals' fingerprints $r = 0.25 \pm 0.008$, paired t test, $t(999) = 885.7$, $P < 10^{-10}$; Fig. 3*B*]. We also found that the maximum of the heat-map correlation matrix lies on the diagonal (i.e., self–self correlation). In other words, olfactory fingerprints A and B of the same individual were always more similar than olfactory fingerprints of different individuals. We repeated the analysis of this data using a permutational multivariate ANOVA (PERMANOVA) to compare the distance between an individual's two fingerprints based on the same odors but different descriptors (e.g., Fig. 2*A* vs. Fig. 2*B*) to the distance between two different individuals (e.g., Fig. 2*A* vs. Fig. 2*D*). PERMANOVA implements a flexible nonparametric distance-based analog of analysis of variance for multivariate data that provides a distribution-free means of testing differences between treatments in their multivariate profile (27). Again, the mean difference between an individual's two fingerprints was $r = 0.75 \pm 0.025$, whereas the mean difference between two different individuals' fingerprints was $r = 0.25 \pm 0.008$ (PERMNOV test, pseudo $F = 8.16$, $P < 10^{-6}$; Fig. S3*C*). Thus, the fingerprint genuinely captured personal identity, and a subject's odorant-specific olfactory fingerprint remains unique even when different descriptors are used to construct it. Once we established the main effect using PERMANOVA, we set out to extrapolate the ability of the olfactory fingerprint to identify an individual beyond our sample; for this, one needs to calculate whether a subject's correlation to him/herself (calculated between fingerprints A and B) is within the distribution of correlations of a subject to all other subjects (between fingerprint A of a subject to fingerprint A of all other subjects). In other words, to conclude that a subject has a unique fingerprint, the intersubject correlation should not belong (low probability) to the distribution of intrasubject correlations. We fitted a Gaussian to the intrasubject distances distribution, then calculated how many SDs a intersubject's score lies from the mean of the distribution of intrasubject scores (i.e., z value); from this, we determined the probability of a subject's correlations to him/herself to be within the distribution of correlations of a subject to all other subjects (i.e., P value). Finally, we averaged all of the individual z-value scores and calculated the overall P value. The distribution of correlations of a subject to all other subjects is not Gaussian, and the subject's correlation to him/herself is limited by 1 (or −1), hence other metrics for distance between subjects may yield modestly different results (see *SI Text* for caveats of the Z value and for impact of alternative methods). We repeated this procedure 1,000 times, each time randomly halving the descriptors used to derive similarity (with one half used to generate fingerprint A and the other half used to generate fingerprint B), and averaged across all iterations and all subjects. We obtained an average Z value of 4.9 that corresponds to an ability to use the 28-odor olfactory fingerprint to identify one person of ∼2 million individuals.

Fingerprint Specificity Depended on Number of Descriptors and Odors Used. In our initial experiment we used as many as 54 descriptors and 28 odors because we wanted to explore the impact of these parameters. To estimate the dependence of fingerprint discriminability on the number of descriptors and odorants used, we again generated two alternative fingerprints for each subject, A and B, each using a random independent half of the descriptors used to derive similarity. Here, however, we successively reduced the number of odorants and descriptors

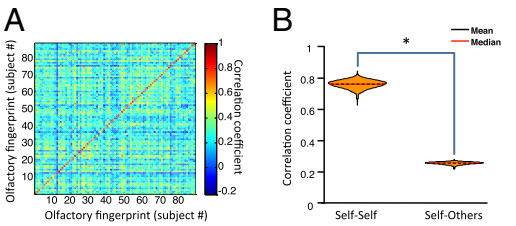

Fig. 3. Olfactory fingerprints are independent of descriptor identity. (*A*) Heat-map matrix of distances between fingerprints A and B for 89 subjects, where A and B were derived using the same odorants but different descriptors. The diagonal represents the correlation of a subject to him/herself. (*B*) Violin plots comparing correlation coefficients of all self–self pairs (using different descriptors) to all self–other pairs, the distribution of correlation coefficients of self–self and self–others are shown in orange; the mean and median of the distribution are depicted in black and red, respectively.

used. We repeated each analysis 1,000 times, each time shuffling the particular odorants and descriptors omitted. We plotted the averaged fingerprint specificity (averaging the Z score across subjects and iterations) as a function of the number of descriptors and odorants used to generate it (Fig. 4 *A* and *B*). We observed a monotonic decrease in the specificity of the fingerprint, yet even with only 7 odors and 11 descriptors, the correlation between an individual's two fingerprints based on the same odors but different descriptors was significantly above the correlation between two different individuals: $z = 1.65$, $P < 0.05$. Thus, potentially meaningful olfactory fingerprints can be obtained in under 10 min. In turn, we extrapolated to estimate how many odors and descriptors we would need to use to obtain an individual fingerprint for each of the ~7 billion people on earth, and concluded at 34 odors and 35 descriptors. We estimate that obtaining such a detailed fingerprint would take ~5 h.

Olfactory Fingerprints Remained Specific Despite Fluctuation Over Time. Olfactory perception is not only variable across individuals, it is also highly variable within individuals over time (28). This variability may reflect in part that odor perception is the combination of a given receptor activation pattern with the fluctuating homeostatic state in which it is perceived (hunger/satiety, mood, arousal, etc.) (2). To test the persistence of the olfactory fingerprint at retest, we refingerprinted 23 participants at a time ranging between 10 and 30 d following their initial fingerprinting (e.g., Fig. 2*A* vs. Fig. 2*C*). We found that the average distance of a person from him/herself remained significantly lower than the average distance between different individuals [mean difference between an individual's two fingerprints over time: $r = 0.58 \pm 0.15$; mean difference between two different individuals' fingerprints, $r = 0.31 \pm 0.076$, paired t test, $t(44) = 7.69$, $P < 10^{-8}$; Fig. 4*C*]. In other words, despite the passage of time, a person remained significantly more correlated with him/herself than with others.

Despite the above result, the slight reduction in self–self correlation over retests raises the concern that given additional retests, the self–self correlation advantage may disappear altogether. To address this concern, we refingerprinted an additional group of 18 subjects across five fingerprinting sessions that spanned 14–30 d (list of odorants and descriptors for experiment 1B in Tables S3 and S4). A repeated-measures ANOVA revealed that at each repetition (II, III, IV, and V) the average distance of a person from his/her first fingerprint remained unchanged [$F(17, 3) = 2.24$, $P = 0.09$, mean difference between an individual's two fingerprints across retests r first–second $= 0.58 \pm 0.21$, r first–third $= 0.54 \pm 0.18$, r first–fourth $= 0.54 \pm 0.2$, r first–fifth $= 0.49 \pm 0.19$; Fig. 4*D*, yellow]. Moreover, the fingerprint stability in fact improved after the first retest [$F(17, 3) = 6.08$, $P < 0.001$] such that the second to third ($r = 0.66 \pm 0.19$), third to fourth ($r = 0.68 \pm 0.20$), and fourth to fifth ($r = 0.69 \pm 0.16$) repetitions were all significantly better than the first to second [$r = 0.58 \pm 0.21$, all $t(17) > 2.67$, all $P < 0.02$; Fig. 4*D*]. Taken together, we conclude that despite the passage of time and repeated testing, a person remained significantly more correlated with him/herself than with others. We recalculated the ability of the olfactory fingerprint to identify an individual beyond our sample, this time comparing the initial and the later (a few weeks later) fingerprints with all of the other subjects, and observed a decreased yet significant discriminability ($z_{1-2} = 2.67$, $P < 0.01$, $z_{1-3} = 2.67$, $P < 0.01$, $z_{1-4} = 2.67$, $P < 0.01$, $z_{1-5} = 2.67$, $P < 0.01$; Fig. 4*D*, black and red); this amounts to an ability to use the current olfactory fingerprint to identify 1 subject of ~300 individuals. Moreover, given this variability over time, to effectively obtain long-lasting olfactory fingerprints for the entire world population we find by extrapolation that rather than 34 odors with 35 descriptors we would now need 160 odors with 35 descriptors. Note that this reduced discriminability is not only because of the extent of shift in fingerprint over time; self-correlation over time decreased from $r = 0.75$ to $r = 0.58$, which remains significantly higher than the correlation across individuals. However, because on average all subjects shifted in this way, the ability to identify one person out of a crowd is significantly reduced.

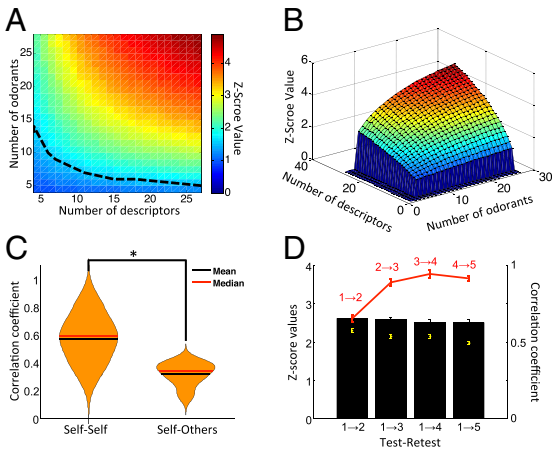

Fig. 4. Fingerprints depend on the number of odors and descriptors and the passing of time. (*A*) Heat map of fingerprint ability to distinguish self–self from self–other pairs (represented in Z-score values) as a function of number of odors and descriptors used. Dashed line represents Z-score value of 1.65 ($P = 0.05$). (*B*) 3D plot of Z-score values as a function of number of odors and descriptors used to generate a fingerprint. (*C*) First test–retest. Violin plot comparing correlation coefficients of 23 subjects refingerprinted across time. (*Left*) Correlation coefficients distribution of a subject to him/herself over time. (*Right*) Correlation coefficients distribution of a subject to other subjects over time. The mean and median of the distribution are depicted in black and red, respectively. (*D*) Second test–retest with five repetitions. Right y axis is correlation across retests (r) shown in yellow. Left y axis is the ability of the fingerprint to discriminate self from others in Z-score values when comparing the first to ensuing retests (black bars) or each two consecutive retests (red line).

Similar Olfactory Fingerprints Imply High HLA Matching. The hypothesis underlying our effort was that fingerprints would provide a unique perceptual counterpart of an individual's unique olfactory receptor subtype genome. Potentially consistent with this notion, 28-odor-based fingerprints were special to the tune of 1 in 2 million. However, whereas olfactory receptor genomes are likely stable over 10–30 d (but see refs. 29–32), fingerprints were less so. Given this temporal fluctuation, we set out to test whether olfactory fingerprints can nevertheless remain informative of genetic traits linked to olfaction, in this case HLA; to test this, we studied an additional 130 subjects (65 women, mean age $= 29.93 \pm 8.44$ y) who provided blood samples for HLA typing (*Methods*), and olfactory fingerprints using 11 odors (Table S5). Combinatorially, 130 subjects provide for 16,770 possible donor–recipient pairs, because HLA match is not symmetric, i.e., in a given pair, a subject can have a high HLA match as a donor but poor HLA match as a recipient (note that we use the terms "donor" and "recipient" to describe the directionality of HLA matching). Therefore, 130 subjects resulted in 16,770 possible pairs (130×129) and not in 8,385 ($130 \times 129/2$). For each pair we calculated an olfactory fingerprint match using Euclidean distance (see *SI Text* for impact of alternative methods) and an HLA match along a 7-point scale (0–6, 0 = no match) previously described (16). Only 65 of 16,770 possible pairs of individuals had a high HLA match of 5 or 6 (Fig. 5*A*). We found that the olfactory fingerprint match of these individuals was significantly better than the olfactory fingerprint match for poorly HLA-matched individuals [HLA 5–6: mean olfactory fingerprint match in arbitrary units (AU) of Euclidean distance $= 12.7 \pm 4.1$ AU, HLA 1–4: mean olfactory fingerprint match $= 14.6 \pm 5.3$ AU, Wilcoxon rank-sum test: $Z = 3.2$, $P < 0.0015$]. In other words, the olfactory perceptual fingerprint similarity was significantly informative on HLA matching, implying that it captured meaningful genetic information.

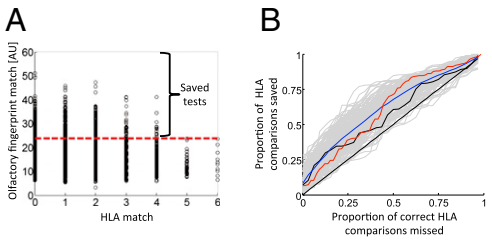

Fig. 5. Similar olfactory fingerprints imply high HLA matching. (*A*) A total of 16,770 pairwise comparisons of olfactory fingerprint distance vs. HLA match. The dotted red line reflects the cutoff for saved tests. (*B*) ROC curves of HLA comparisons saved vs. HLA matches missed. The diagonal identity line reflects no gain or loss. ROCs: red, using all 11 odors; gray, 200 testing curves using 4 odorants; black and blue, median and mean of the 4 best odorants, respectively.

To further assess the strength of the link between olfactory fingerprint match and HLA match, we asked what would happen if one used olfactory fingerprints to screen for potentially high HLA matches in the population. We calculated the percentage of high HLA matches one would potentially miss (incurred cost) vs. the percentage of matches one could identify (gain), and present this using a receiver operating characteristic (ROC) curve (Fig. 5*B*, red line). We found that all points in the ROC curve fall above the identity line; hence, olfactory fingerprints can potentially identify pairs of individuals likely to have a high HLA match. Given the extended time needed to test 11 odorants as we did in data collection, we next asked if we could optimize this test. We halved the data into training (8,387 subject pairs) and testing sets (8,385 subject pairs), each maintaining the original fractions of each level of HLA match. In the training set, we calculated olfactory fingerprints using all possible combinations of 3–11 odorants, and selected the four best-performing odorants, which we then tested in the testing set. We repeated this 200 times (Fig. 5*B*, grey lines). Taking the median score (Fig. 5*B*, black line), we found that a selection of four odors (isoamyl acetate, isovaleric acid, 2-ethyl pyrazine, and 1-hexanol) decreased the average olfactory fingerprint distances of high-HLA matched individuals to 10.8 ± 3.7 AU compared with an olfactory fingerprint distance for poorly HLA matched individuals of 13.8 ± 5.8 AU ($Z = 4.35$, $P < 0.000015$). Finally, we calculated the actual savings implicated by this: the 65 high-HLA matches in our data comprised of 45 individuals (some individuals were matched with more than one). We iteratively selected one individual from these 45 as recipient, and randomly drew donors until we encountered a high HLA match. We repeated this 10,000 times. Consistent with the expectation from chance, we had to test an average of 65.35 ± 37.5 donors to identify a match. We then repeated these exact procedures again, but rather than randomly drawing donors, we drew them in rank order in accordance with their rapidly obtainable optimized olfactory fingerprint distance, starting with the closest. We found that the average number of individuals we had to test to identify a match was 44 ± 29, implying a 32% savings [$t(64) = 5.5$, $P < 10^{-6}$]. In other words, using this brief perceptual test, one could rank order the population to save more than 30% of HLA tests.

Discussion

A recurring question in both popular and scientific settings is whether people are similar or different in their olfactory perception. This question, however, must be answered with caution, even with the current data in hand. If we look at gross olfactory perception, people remain quite similar to one another. As evidenced in Fig. 2*E*, if we take the average perception of an odor, it serves as a reasonable estimation of what any given individual will say about that odor. However, once we applied the method we developed for maximizing differences, we find that olfactory perception was highly individually specific and variable across individuals.

Notably, variability across individuals was somewhat smoothed by variability within individuals over time, which we think reflects an interaction between a given olfactory receptor subtype genome that determines the borders of individual perception, and fluctuating homeostatic state that determines position within these borders. Nevertheless, these borders remained confined enough such that despite fluctuation, individual olfactory perception meaningfully informed on nonolfactory genetic makeup, in this instance, HLA matching. As noted in the introduction, information on HLA is available through body odor (16, 17). It has been suggested that several vertebrate species use this information to select mates with dissimilar HLA (33–36), and for this to occur, the olfactory system should have access to the information of self HLA makeup. In here finding that HLA is also linked to olfactory perception alone, our results combine with a previous effort (19) to provide a path for such self-recognition, and in this close a loop likely subserving behavioral selection mechanisms that rely on the sense of smell (20).

The current effort has some clear limitations that we would like to acknowledge: the tool we developed provides a precise measure of olfactory perception. Although we speculate that individual olfactory perception reflects individual olfactory receptor subtype repertoire, we did not directly measure genetic makeup. Therefore, an obvious albeit expensive follow-up study should collect olfactory fingerprints and full olfactory receptor genomes from the same individuals. Similarly, we speculated that the olfactory fingerprint to HLA link is related to the genetic link between HLA and olfactory receptor loci. However, the fingerprint to HLA link may in fact follow some other indirect path. Specifically, the HLA mechanism both enables and depends on identification of self. In turn, the olfactory fingerprint also captures self. Given that HLA captures self and olfactory fingerprints capture self, then there will be a link between HLA and olfactory fingerprints even if they are not the result of linked genes. There is in fact a future way to test these alternatives—namely, whether the fingerprint-to-HLA link reflects linkage through the genome or not: HLA is on chromosome 6. If the fingerprint-to-HLA link reflects linkage through the genome, then olfactory fingerprints based on odors that activate olfactory receptors on chromosome 6 should be better predictors of HLA than olfactory fingerprints based on odors that activate receptors on other chromosomes. The current data, however, do not have the power to conduct such an analysis, because 6 of the 11 odorants we used do not have a known receptor, and only two of the odorants have known receptors on chromosome 6 (androstadienone and *cis*-3-hexen-1-ol) (4, 37). Notably, these two odorants did not end up in the optimized set of four odorants.

Despite these limitations, our results indicate a powerful tool whose potential usefulness goes beyond characterization of olfaction alone. In some instances, usefulness may follow linkage to the olfactory genome. In turn, various olfactory receptors, in addition to responding to odors in the nose, likely also play assorted roles in various nonolfactory organs and functions (38–40). Olfactory fingerprints may provide a measure for these receptors, and therefore serve as a predictive tool for their respective nonolfactory functions as well. Finally, olfaction is highly predictive of health status (41, 42), strongly linked to neurodegenerative diseases (43), autoimmune diseases (44), and more. With this in mind, we conclude in predicting that olfactory fingerprints can become a widely applicable tool, informative on a host of conditions in both health and disease.

Methods

Subjects. A total of 238 generally healthy subjects participated in three experiments (experiment 1A: 89 subjects, 40 women, mean age = 25.7 ± 3.1 y; experiment 1B: 18 subjects, 11 women, mean age 26.8 ± 3.4; experiment 2: 130 subjects, 65 women, mean age = 29.93 ± 8.44 y) after providing written informed consent to procedures approved by the Sheba Medical Center Helsinki Committee.

Odorants. We used two forms of odorant presentation in this study. Experiment 1A contained 24 odorants in scratch-and-sniff form provided by The

PrintBox, Inc., and four odorants presented in sniff jars. Experiment 1B contained 22 odorants presented in sniff jars. Experiment 2 contained 11 odorants all in jars; the four jar odorants from experiment 1 (isoamyl acetate, 1,8-cineole, *cis*-3-hexen-1-ol, and isovaleric acid) and seven additional odorants. All odorants and the experiments they were used in are detailed in Tables S1–S6. Because the initial test–retest experiment used mostly scratch-and-sniff odorants and the second test–retest experiment used jars, we could directly assess any difference between these methods of presentation. We found no significant difference in test–retest when comparing scratch and sniff ($r = 0.59 \pm 0.14$) to jars [r first–second $= 0.58 \pm 0.21$, $t(39) = 0.24$, $P = 0.81$].

Ratings. Each subject rated 28 odorants along 54 verbal descriptors in experiment 1A, 22 odorants along 23 verbal descriptors in experiment 1B, and 11 odorants along 57 verbal descriptors in experiment 2 (see list of odorants in Tables S1, S3, and S5 and list of descriptors in Tables S2, S4, and S6) using visual analog scales. For example, the question "to what extent does this odor smell like coconut?" above a 14-cm line ranging from "not at all" at one end to "very much so" at the other. After sniffing the odorant presented in scratch and sniff or jar, participants crossed the line at a point reflecting their perception, and the line was later parsed to 100 for analysis. Odor order was random across participants, and interodor interval was >40 s. To account for individual differences in use of scales, each subject's data were normalized by first subtracting the minimal value applied by the subject, then dividing by the maximal remaining value, and finally multiplying by 100; this generated a normalized range between 0 and 100.

HLA Typing. Blood samples were collected at the Sheba Medical Center Autoimmune and Chemical Laboratories. HLA analyses were conducted at the Sheba Medical Center Tissue Typing Unit. In brief, 5–10 mL of blood was drawn from each volunteer and kept at 4 °C until DNA was extracted. Genomic DNA Extraction was carried out from 400 μL of whole blood using the MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostics). DNA samples were stored at −20 °C. HLA typing was performed using Luminex technology and Immucor Transplant Diagnostic kits to obtain HLA A*, B*, and DRB1* loci typings at low/intermediate resolution (a list of genotypes at the three HLA loci of each of the 130 subjects is in Tables S7).

HLA Matching. We calculated HLA match using methods previously reported in ref. 16. There are three general groups of HLA: HLA-A*, HLA-B*, and HLA-DRB1*, and within each group there are different specific HLA proteins (there are 59 different HLA-A* proteins, 118 different HLA-B*, and 124 different HLA-DRB1*). Each of these HLA groups (A*, B*, and DRB1*) is notated by a two-digit numerical designation (e.g., HLA-A* 01:07, HLA-B* 15:15, HLA-DRB1* 15:33). A match is calculated by counting the number of HLA proteins (in each group separately) present in one subject that are also present in another subject. Because there are two digits for each HLA group, the count can be 0, 1, or 2. Once the count for each HLA group is obtained, a match is calculated by summing the values of all of the groups. In other words, for each donor/recipient pair we counted the number of antigens present in the donor that matched an antigen in the recipient. Homozygous antigens in the recipient that matched a donor antigen were counted as two matches. Because we measured three HLA loci, there was a potential for a maximum of seven matches. For example, if donor A has the following HLA genotype A*24,68 B*14,35 DRB1*01,11 and recipient B has the following HLA genotype A*03,23 B*41,47 DRB1*10,11, this pair (A→B) will have an HLA match score of $0 + 0 + 1 = 1$. However, if donor C has the following HLA genotype A*02, 30 B* 13, 50 DRB1* 07, 07 and recipient D has the following HLA genotype A*02, 02 B*27, 41 DRB1*07, 11 then the pair (C→D) will have an HLA match score of $2 + 0 + 1 = 3$ and the pair (D→C) will have an HLA match score of $1 + 0 + 2 = 3$ (note that even though the total HLA match score is the same, it is not symmetric between C↔D).

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