Quantitative measurement of odor detection thresholds using an air dilution olfactometer, and association with genetic variants in a sample of diverse ancestry

Genetic association studies require a quantitative and reliable method for odor threshold assessment in order to examine the contribution of genetic variants to complex olfactory phenotypes. Our main goal was to assess the feasibility of a portable Scentroid air dilution olfactometer for use in such studies. Using the Scentroid SM110C and the SK5 n-butanol Sensitivity Kit (IDES Canada Inc.), n-butanol odor thresholds were determined for 182 individuals of diverse ancestry (mean age: 20.4 ± 2.5 years; n = 128 female; n = 54 male). Threshold scores from repeat participants were used to calculate a test-retest reliability coefficient, which was statistically significant (r = 0.754, p < 0.001, n = 29), indicating that the Scentroid provides reliable estimates of odor thresholds. In addition, we performed a preliminary genetic analysis evaluating the potential association of n-butanol odor thresholds to six single-nucleotide polymorphisms (SNPs) putatively involved in general olfactory sensitivity (GOS). The results of multiple linear regression analysis revealed no significant association between the SNPs tested and threshold scores. However, our sample size was relatively small, and our study was only powered to identify genetic markers with strong effects on olfactory sensitivity. Overall, we find that the Scentroid provides reliable quantitative measures of odor detection threshold and is well suited for genetic studies of olfactory sensitivity.

1   **Quantitative measurement of odor detection thresholds using an air dilution olfactometer,**

2   **and association with genetic variants in a sample of diverse ancestry.**

3   Gillian R. Cook, S. Krithika, Melissa Edwards, Paula Kavanagh, Esteban J. Parra

4   *Department of Anthropology, University of Toronto at Mississauga, ON, Canada.*

5   *Correspondence to be sent to: Esteban Parra, Department of Anthropology, University of*

6   *Toronto at Mississauga, Mississauga, Canada, 905-828-3889, esteban.parra@utoronto.ca.*

7   **Introduction**

8   Human scent perception abilities are highly variable. It is well documented that variation

9 exists between individuals and between sexes (Hoover 2010; Keller et al. 2007). Similarly,

10 several studies have identified inter-population differences in olfactory abilities (Eibenstein et al.

11 2005; Sorokowska et al. 2013; McRae et al. 2013). Matching the high degree of diversity in

12 scent perception phenotypes, there is a correspondingly large amount of genetic variation in

13 olfactory genes. Ongoing research is attempting to link variation at the genetic level to observed

14 phenotypic variation, gaining a deeper understanding of olfactory genetics, and of the genetic

15 contribution to highly personalized scent perception phenotypes (see Mainland et al. 2014;

16 Schütz et al. 2014; McRae et al. 2013). The majority of studies have focused on the impact of

17 genetic variants in olfactory receptor (OR) genes, although there have also been efforts to

18 examine the potential role of variants in auxiliary olfactory genes (Keydar et al. 2013). In

19 addition, gene expression analysis has shown that inter-individual differences in olfactory

20 abilities may be due to differential expression of functional OR genes in the olfactory mucosa

21 (Verbeurgt et al. 2014).

22   A major bottleneck in the study of olfactory sensitivity has been a lack of instrumentation

23 for accurately measuring smell. Unlike for vision and hearing, there is no standardized test for

24 olfactory sensitivity in either the clinical or research setting (Eibenstein et al. 2005). Numerous

25 psychophysical methods exist, all of which present olfactory stimuli and allow the test subject to

26 report on their experience (Doty 2006). These tests of olfactory sensitivity evaluate detection or

27 perception thresholds, as well as odor discrimination, identification, memory, intensity and

28 pleasantness (Doty et al. 1995; Eibenstein et al. 2005). The majority of these tests are qualitative,

29 such as the University of Pennsylvania Smell Identification Test (UPSIT), or are not precise

30 enough for the purposes of genetic association studies (Doty et al. 1995; Kremer et al. 1998).

31 The most quantitative measurements are gained through odor threshold testing, where the lowest

32 concentration of an odor that the subject can detect is determined without requiring recognition of

33 the smell (Eibenstein et al. 2005).

34   While similar in principle, individual threshold tests vary in their design. Common

35 differences include the method of odor delivery, and the order in which dilutions are presented

36 during testing. The most simple odor presentation strategy is the ascending method of limits

37 (AML), whereby the series of diluted odorants is always presented from lowest to highest

38 concentration. With an AML strategy, the threshold is estimated from the transition point at

39 which the subject reports odor detection (Doty 2006). Threshold tests can be either forced choice

40 or non-forced choice in design. Forced choice tests offer one or more blank stimuli. A non-

41 forced choice design requires presentation of a single odor stimulus, such that the test subject is

42 required to respond whether or not they smell the odor from a single sample without blanks

43 presented for comparison (Doty et al. 1995).

44   Common threshold tests employed today include the Connecticut Chemosensory Clinical

45 Research Center Test (CCCRC), Sensonic Inc.'s Smell Threshold Test (STT), and a component of

46 the Sniffin' Sticks test battery (Cain et al. 1983; Doty 2006; Hummel et al. 1997). These three

47 tests are characterized by the use of liquid odorant dilutions, which are delivered in plastic

48 squeeze bottles or modified felt tip pens. Despite the convenience of liquid dilution, air dilution

49 methods are preferable for evaluating olfactory response. Air dilution olfactometry is more

50 precise than liquid dilution, and circumvents issues of odor intensity loss in volatile aqueous

51 solutions (Eibenstein et al. 2005; Kremer et al. 1998). However, preparation and administration

52 is generally more expensive and time consuming for air dilutions than for liquid dilutions.

53 Therefore, a portable, affordable and reliable air dilution olfactory threshold test that can be

54 administered in a short amount of time is needed.

55   The main goals of our study were to 1) assess the suitability of a portable dynamic

56   olfactometer, the Scentroid SM110C (IDES Canada Inc.), for olfactory threshold studies, and 2)

57   carry out a pilot study to examine the potential association of a selected number of GOS genetic

58   variants and n-butanol thresholds in a sample of diverse ancestry. We evaluated the test-retest

59   reliability of the Scentroid SM110C using n-butanol as an odorant (SK5 n-butanol Sensitivity Kit,

60   IDES Canada Inc). The odorant n-butanol is widely used in olfaction studies, in part due to its

61   variable odor detection threshold (Doty et al. 1995; Hummel et al. 1997). We surveyed the

62   olfactory thresholds for n-butanol in a multi-ethnic population, and genotyped the participants at

63   six missense single-nucleotide polymorphisms (SNPs) located in putative auxiliary olfactory

64   genes. The markers were chosen for their potential involvement in general olfactory sensitivity

65   (GOS), a phenotype characterized by correlation of sensitivities to multiple odorants (Keydar et

66   al. 2013). Genetic variants involved in GOS affect the olfactory thresholds of numerous

67   odorants, making them easier to detect than OR variants when the odor threshold is measured for

68   a single odorant.

69   We genotyped five SNPs in three genes that had been identified in a database of candidate

70   auxiliary olfactory genes (http://genome.weizmann.ac.il/GOSdb): rs17132289 and rs17712299 in

71   the ATP-binding cassette, sub-family A, member 13 gene (*ABCA13*); rs2889732 and rs13036385

72   in the BPI fold containing family B, member 4 gene (*BPIFB4*); and rs6746030 in the sodium

73   channel, voltage-gated, type IX, alpha subunit gene (*SCN9A*) (Keydar et al. 2013). The *SCN9A*

74   variant rs6746030 has previously been implicated as having an effect on olfactory function

75   (Heimann et al. 2013; Weiss et al. 2011). In addition to genes identified in the GOS database

76   (GOSdb), we genotyped the SNP rs6265 located in the brain-derived neurotrophic factor gene

77   (*BDNF*), which has been linked to olfactory ability in previous studies (Hedner et al. 2010;

78   Tonacci et al. 2013).

79   **Materials and methods**

80   *Subjects*

81   182 participants were recruited on the University of Toronto at Mississauga campus and

82   ranged in age from 18 to 32 years old (mean age: 20.4 ± 2.5 years; n = 128 female; n = 54 male).

83   60 individuals were of East Asian ancestry (Chinese, Japanese, Korean, and Taiwanese), 55 were

84   of European ancestry, 58 were of South Asian ancestry (Bangladeshi, Indian, Pakistani, and Sri

85   Lankan), and nine were of mixed or other ancestry. Table 1 describes the main characteristics of

86   the sample. Prior to testing, participants were asked not to eat or drink for twenty minutes. All

87   the individuals gave written informed consent prior to participating in the study. The research

88   protocol was approved by the University of Toronto Research Ethics Board. This study complies

89   with the Declaration of Helsinki for Medical Research involving Human Subjects.

90   *Olfactory threshold testing*

91   Olfactory thresholds for n-butanol were assessed using the Scentroid SM110C

92   olfactometer and the SK5 n-butanol Sensitivity Kit (IDES Canada Inc.), shown in Fig. 1. This

93   instrument complies with the European Standard EN13725:2003 for dynamic olfactometers.

94   Additionally, results obtained with the Scentroid SM110 have previously been shown to correlate

95   well with those from established odor assessment techniques. Bokova (2012) reported very good

96   correlation between the results obtained with the Scentroid SM110 and traditional odor

97   evaluations using a dynamic olfactometer. Szyłak-Szydłowski (2014) reported very strong

98   correlations between the odor concentrations estimated by the Scentroid SM-100 and the Nasal

99   Ranger for Tetrahydrothiophene and Hydrogen sulphide (r values 0.98 and 0.93, respectively), for

100   field tests on a water treatment plant (r=0.89).

101   The odor was presented birhinally using a non-forced choice strategy that consisted of

102   fifteen steps with dilution ratios ranging from 200 to 3600. A fresh dilution of n-butanol was

103   prepared daily in a stainless steel evaporation chamber by combining 0.5 µl of aqueous n-butanol

104   with compressed air, equivalent to a concentration of 20 ppm, and was subsequently stored in a 1

105   L Tedlar bag (Fig. 2). Using the Scentroid's standardized restriction plate two in the flow

106   regulator allowed for the 20 ppm sample of n-butanol to be further diluted within a 5.5 to 100 ppb

107   range. Subjects were presented with the lowest concentration of n-butanol first and, following an

108   AML strategy, were exposed to successively increased concentrations. A metal cone expelling air

109   at a constant flow of 20 L/min and velocity of 2.5 m/s was presented to each subject for

110   approximately 2 seconds per dilution step, followed by a brief stimulus break (Fig. 3). Subjects

111   were asked to verbally respond whether or not they could detect the odor at each step. The

112   olfactory threshold was determined from the transition point at which the subjects reported odor

113   detection, and calculated as the mean of the scores from three consecutive tests. Each individual

114   threshold test took approximately 1 to 3 minutes to complete.

115   A replication sample consisting of 29 participants was tested 14 to 18 weeks after their

116   original testing date. The olfactory threshold test protocol remained unchanged.

117   *Genotyping*

118   Saliva was collected with the Oragene DNA OG-500 (DNA Genotek Inc.) for genomic

119   DNA extraction following recommended protocols. Samples were sent to LGC Genomics for

120   genotyping by means of the KASP genotyping assay. Genotyping was performed for six SNPs

121   with potential involvement in GOS phenotypes: rs6265, rs2889732, rs6746030, rs13036385,

122   rs17132289, and rs17712299.

123   *Statistical analyses*

124   The olfactory threshold scores were found to be positively skewed and a log

125   transformation was applied. Using the log-transformed data, an ANOVA was run to examine

126   differences in n-butanol olfactory thresholds between ancestry groups. Test-retest reliability was

127   evaluated using correlation tests and Bland-Altman plots, based on the olfactory threshold scores

128   obtained from a subset of participants who were measured twice within an interval of 14 to 18

129   weeks. Associations between SNPs and olfactory sensitivity were analyzed through multiple

130   linear regression, with age and sex included as covariates. The association tests were done

131   individually for each ancestry group and also for the full sample, with ancestry as an additional

132   covariate. For the association tests, we used a genotypic model, where the effects of one of the

133   homozygous categories and the heterozygous category are reported in relation to the remaining

134   homozygous category, which is used as a reference. The statistical analyses were carried out with

135   IBM SPSS Statistics v.20. For all analyses the level of significance was set to alpha = 0.05.

136   Genotype data was checked for deviations from Hardy-Weinberg equilibrium, using exact tests

137   available at http://ihg.gsf.de/cgi-bin/hw/hwa1.pl. Post-hoc power analysis was performed with

138   the program Quanto (http://biostats.usc.edu/Quanto.html), using several inheritance models

139   (additive, dominant and recessive) and allele frequencies ranging from 0.05 to 0.50. Parameters

140   were set based on observed n-butanol threshold scores in ppb (mean = 22, SD = 14), with a

141   sample size of 60 and allelic effect sizes ranging from 1 to 10 beta.

142   **Results**

143   *Analysis of olfactory thresholds in individuals of diverse ancestry*

144   The n-butanol olfactory threshold was evaluated in 182 participants of diverse ancestry

145   using the Scentroid SM110C olfactometer.   Figure 4 shows the distribution of olfactory threshold

146   in the total sample. The distributions observed in each ancestry group are provided as

147   supplementary information (Supplemental Fig. S1). The average (± SD) odor threshold

148   concentration in the full sample was 22.5 ± 13.7 ppb. The average concentrations in the

149   European, East Asian and South Asian groups were 20.9 ± 13.0, 24.4 ± 16.2 and 22.1 ± 11.8 ppb,

150   respectively. Odor thresholds were non-normally distributed, and were log-transformed prior to

151   statistical analysis. Using one-way ANOVA, we examined the impact of age, sex and ancestry on

152   log-transformed n-butanol threshold scores. All three factors were not significant ($p = 0.615$ age;

153    $p = 0.053$ sex; $p = 0.586$ ancestry).

154   In order to assess the reliability of the Scentroid olfactometer and our protocol for

155   determining olfactory threshold, the thresholds from returning participants were used to calculate

156   a test-retest reliability coefficient (Pearson correlation coefficient) (Fig. 5). The resulting value of

157   0.754 was statistically significant ($p < 0.001$; $n = 29$). We also evaluated reliability using Bland-

158   Altman plots, which are shown in Fig. 6. This plot revealed three distinct outliers, whose

159   removal resulted in an improved test-retest reliability coefficient of 0.880. Furthermore, the

160   mean olfactory thresholds (ppb) did not differ significantly between time points, as evidenced by

161   the results of a repeated measures ANOVA ($p = 0.731$, $n = 29$).

162   *Association of putative GOS SNPs with olfactory thresholds*

163   Six SNPs that have been putatively linked with GOS were genotyped using the KASP

164   assay. The concordance rate observed for blind duplicates was 100%. The genotype distributions

165   of all six SNPs met the assumptions of Hardy-Weinberg equilibrium ($p > 0.05$).

166   Using multiple regression analysis, each SNP was tested for association with olfactory

167   threshold. The results are depicted in Table 2. The analysis for rs6265, rs2889732, rs6746030,

168   rs13036385, rs17132289 and rs17712299 yielded no significant results, either in the individual

169   ancestry groups or the total sample.

170   **Discussion**

171   *Threshold test*

172   We measured the n-butanol olfactory threshold in 182 subjects, in order to assess the

173   feasibility of using the Scentroid SM110C air dilution olfactometer to test olfactory sensitivity.

174   Reliability for this instrument was established using return participant test scores, from which a

175   Pearson correlation coefficient was calculated. The derived test-retest reliability coefficient,

176   representing the linear relationship between the first and second test values, was found to be high

177   ($r = 0.754$, $p < 0.001$). Therefore, the Scentroid air dilution olfactometer, when used in

178   conjunction with a non-forced choice AML strategy, produces reliable n-butanol olfactory

179   thresholds, and compares favorably with many other n-butanol threshold tests (Table 3), for

180   which the correlation coefficients range from 0.26 to 0.86 (Hummel et al. 1997; Linschoten et al.

181   2001; Lam et al. 2006; Doty et al. 1995).

182   Currently, the two most common methods for measuring olfactory thresholds are Sniffin'

183   Sticks, and versions of the CCCRC. The Sniffin' Sticks olfactory threshold test is applied

184   birhinally, using n-butanol as the odorant in a triple forced-choice strategy using a staircase

185   presentation method. Staircase strategies differ from the AML in the presentation order of the

186   dilution series, and therefore in how thresholds are estimated (see Doty 2006). Unique to Sniffin'

187   Stick is the application of felt tipped pens for odor presentation. The felt pens are easy to

188   administer, making this particular test highly popular as a result (Hummel et al. 1997; Kobal et al.

189   2000). In the CCCRC threshold test, subjects are presented with two plastic squeeze bottles, one

190   containing a blank and the other a set dilution of aqueous n-butanol. The basic procedure is two-

191   alternate forced-choice with an AML presentation of the dilution series. Between nine and 13

192   dilutions are used, with varying n-butanol concentrations (Cain et al. 1983). Various olfactory

193   tests based on the CCCRC also exist, including the Combined Olfactory Test (COT) in China. It

194   includes both identification and threshold tests, the former of which is culturally adapted. The

195   threshold test component is indistinguishable from that of the CCCRC, yet threshold scores

196   reported in the literature vary (Lam et al. 2006).

197   Variability in the olfactory threshold scores of individual tests can be attributed to

198   differences in experimental design and the study sample. The age of the subjects tested is a

199   significant factor in evaluation of olfactory sensitivity due to the degradation of the sense of smell

200   with age (Hoover 2010). The mean age of the individuals who participated in the studies listed in

201   Table 3 ranged from 31.6 to 49.5 years (Linschoten et al. 2001; Hummel et al. 1997). The mean

202   age of our sample is 20.4 years, which is between 10 and 30 years less than those previously

203   cited. In determining a test's reliability, the time between retests is an important aspect of the

204   research protocol that can impact the results. Our test-retest interval of 14 to 18 weeks is long

205   relative to the studies listed in Table 3, whose intervals range from one day to three weeks, with

206   an average of two weeks. Past research was unable to find support for a relationship between

207   test-retest scores and the time interval between tests (Doty et al. 1995). However, this was based

208   on the examination of relatively short intervals of less than two weeks. Linschoten et al. (2001)

209   included retests performed up to three weeks after initial testing and identified a general trend,

210   whereby the correlation of threshold scores decreased as the test interval increased. This reflects

211   the influence of environmental factors, which act alongside genetics, and impact olfactory

212   sensitivity (Doty 2006; Hasin-Brumshtein et al. 2009).

213   As highlighted previously, the staircase odor presentation strategy of the Sniffin' Sticks

214   test differs from the AML presentation used in the CCCRC and our protocol. Likewise, the mode

215   of stimulus delivery varies from felt-tipped pens, to plastic bottles, to the Scentroid air dilution

216   olfactometer. Yet, the Scentroid as a method for odor delivery is distinctive. A single sample of

217   the odorant needs to be prepared, and using compressed air, the instrument then further dilutes

218   that stock sample for presentation to the test subject. The test administrator can easily switch

219   between set dilutions, thus altering the compressed air to sample ratio.

220   Here, we measured n-butanol olfactory thresholds using the Scentroid SM110C air

221   dilution olfactometer. The resulting test-retest reliability coefficient indicates that the Scentroid

222   produces reliable n-butanol olfactory thresholds. While we employed the AML strategy in a non-

223   forced choice manner, adjustments to this protocol could improve the accuracy of odor thresholds

224   obtained with a Scentroid. Multiple-forced choice designs are less subject to response biases,

225   increasing the reliability of resultant threshold scores (Doty et al. 1995). Moreover, the AML

226   odor presentation strategy is less reliable than simple staircase methods, which in turn are

227   associated with higher error rates than the maximum-likelihood adaptive staircase (ML-PEST)

228   (Linschoten et al. 2001). However, such changes in experimental design will increase the test's

229   administration time and complexity.

230   *Olfactory SNPs*

231   In addition to testing the reliability of the Scentroid SM110C olfactometer, we carried out

232   a pilot study in which we genotyped six SNPs in genes potentially involved in GOS phenotypes,

233 aiming to investigate genetic associations with n-butanol olfactory thresholds. Our goal was to

234 examine variants that may contribute to overall olfactory sensitivity.

235 Studies have shown that individuals with heightened or decreased sensitivity to one odor

236 tend to display similar sensitivities to multiple odorants (Cain & Gent 1991). This has been

237 explained by variation in auxiliary olfactory genes, which are involved in aspects of olfaction

238 other than direct odorant binding, influencing neuronal development or relaying the signal

239 generated by odorant-receptor interaction (Keydar et al. 2013; Hasin-Brumshtein et al. 2009).

240 From the GOS database of candidate auxiliary olfactory genes developed by Keydar et al.

241 (2013), we chose SNPs from three genes. Our regression analysis revealed no association

242 between olfactory threshold and genotype for the variants examined in *ABCA13* and *BPIFB4*.

243 There has been no direct evidence of these genes having an olfactory effect in humans. *ABCA13*

244 and *BPIFB4* were included in the GOSdb based on RNA sequences in humans and mice, as well

245 as rat proteome data. Conversely, *SCN9A* has been associated with human olfactory function in

246 two separate studies, in addition to being included in the GOSdb. The voltage-gated sodium

247 channel encoded by *SCN9A* is expressed in both nociceptive and olfactory sensory neurons, and

248 is linked to altered pain and olfactory perception phenotypes. Individuals with loss-of-function

249 mutations in *SCN9A* cannot feel pain, and exhibit general anosmia (Weiss et al. 2011). An

250 investigation of gain-of-function mutations in this ion channel found that haplotypes carrying the

251 minor alleles of the rs6746030 and rs41268673 polymorphisms were associated with increased

252 pain sensitivity and general olfactory acuity (Heimann et al. 2013) with respect to the wild-type

253 haplotypes. In our study, we did not observe a significant effect of this polymorphism in

254 olfactory detection thresholds, either in the full sample or individual ancestry groups.

255 The Val66Met polymorphism in *BDNF*, rs6265, has been linked to age-related decline in

256 olfactory sensitivity (Hedner et al. 2010). Initially, the effect was only seen among subjects

257   between 70 and 90 years of age. However, a study investigating the effects of Val66Met in

258   younger individuals found an association with olfactory function for a sample with a mean age of

259   38.7 years (Tonacci et al. 2013). Our study failed to replicate these results, though this may be

260   due to the relatively low mean age of our sample (mean age: 20.4 years).

261   We performed a small-scale genetic association test for potential GOS variants, utilizing

262   n-butanol olfactory thresholds. No significant results were found in this pilot study. For some

263   variants, this may be due to lack of involvement in GOS. However, several of the SNPs

264   examined have been linked to human olfactory sensitivity previously. Our investigation was

265   hindered by a small sample size, which was further reduced through independent analysis of each

266   ancestry. Our post-hoc power analyses indicate that our study is only adequately powered to

267   identify alleles with strong effects on olfactory sensitivity. For example, assuming an additive

268   model of inheritance (Supplementary Table S1), we estimated that we have 80% power to

269   identify common alleles (allele frequencies (0.35 – 0.50) with effect sizes of 7 ppb or higher.

270   However, our power decreases substantially for alleles with lower allele frequencies and/or

271   smaller effect sizes. Therefore, it is possible that the SNPs examined here have minor effects on

272   inter-individual differences in olfactory sensitivity, and thus should continue to be candidates in

273   future studies.

274   **Conclusion**

275   In summary, in this study we show that the Scentroid SM110C provides reliable

276   quantitative estimates of odor thresholds, and would be an adequate olfactometer to explore the

277   genetic basis of olfactory sensitivity. Air dynamic olfactometers, which allow a continuous

278   control of the concentration of odorant, have advantages over methods based on liquid dilutions

279　　in bottles, which may loose their smell intensity with time (Eibenstein et al. 2005; Kremer et al.

280　　1998). A distinct advantage of the Scentroid SM110C with respect to other olfactometers is that it

281　　is portable, conferring flexibility in terms of the sampling location. Adequate phenotype

282　　characterization is a critical step in the success of genome-wide association (GWA) studies. For

283　　traits with an underlying quantitative distribution, using quantitative measures, instead of

284　　threshold-based dichotomous outcomes, enhances statistical power because of the larger

285　　information content (Plomin et al. 2009; Smith and Newton-Cheh, 2009). However, it is also

286　　important to consider the precision of the estimates, as it has been shown that measurement error

287　　reduces statistical power and can have a strong effect on the profile of SNPs identified in GWA

288　　studies (Barendse, 2011; Liao et al. 2014). Measurement errors can be reduced by taking multiple

289　　measurements (Barendse, 2011; Liao et al. 2014). In our study, we took three measurements per

290　　individual, and used the mean value as the dependent variable in our association tests. Using the

291　　AML strategy employed in this study, it was possible to get reliable, quantitative measures of

292　　odor threshold in less than 5 minutes per participant. This is particularly important for genome-

293　　wide association studies focusing on olfactory sensitivity, which ideally should include thousands

294　　of participants in order to identify markers with relatively small effects on olfactory sensitivity.

295　　Here, we have primarily focused on odor threshold as a measure of olfactory sensitivity. Other

296　　types of test have been developed to evaluate olfactory sensitivity, such as tests for odor

297　　discrimination, odor identification, odor memory, and suprathreshold scaling of odor intensity

298　　and pleasantness (Doty, 1995). All these tests capture different aspects of olfactory performance.

299　　**Acknowledgements**

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Figure 1

Scentroid SM110C olfactometer used in measuring n-butanol olfactory thresholds.

Figure 2

Stainless steel evaporation chamber used for transferring diluted n-butanol to a Tedlar bag.

Figure 3

Olfactory threshold testing using the Scentroid SM110C olfactometer and SK5 n-butanol Sensitivity Kit.

Figure 4

Distribution of n-butanol olfactory threshold scores for 182 participants of diverse ancestry.
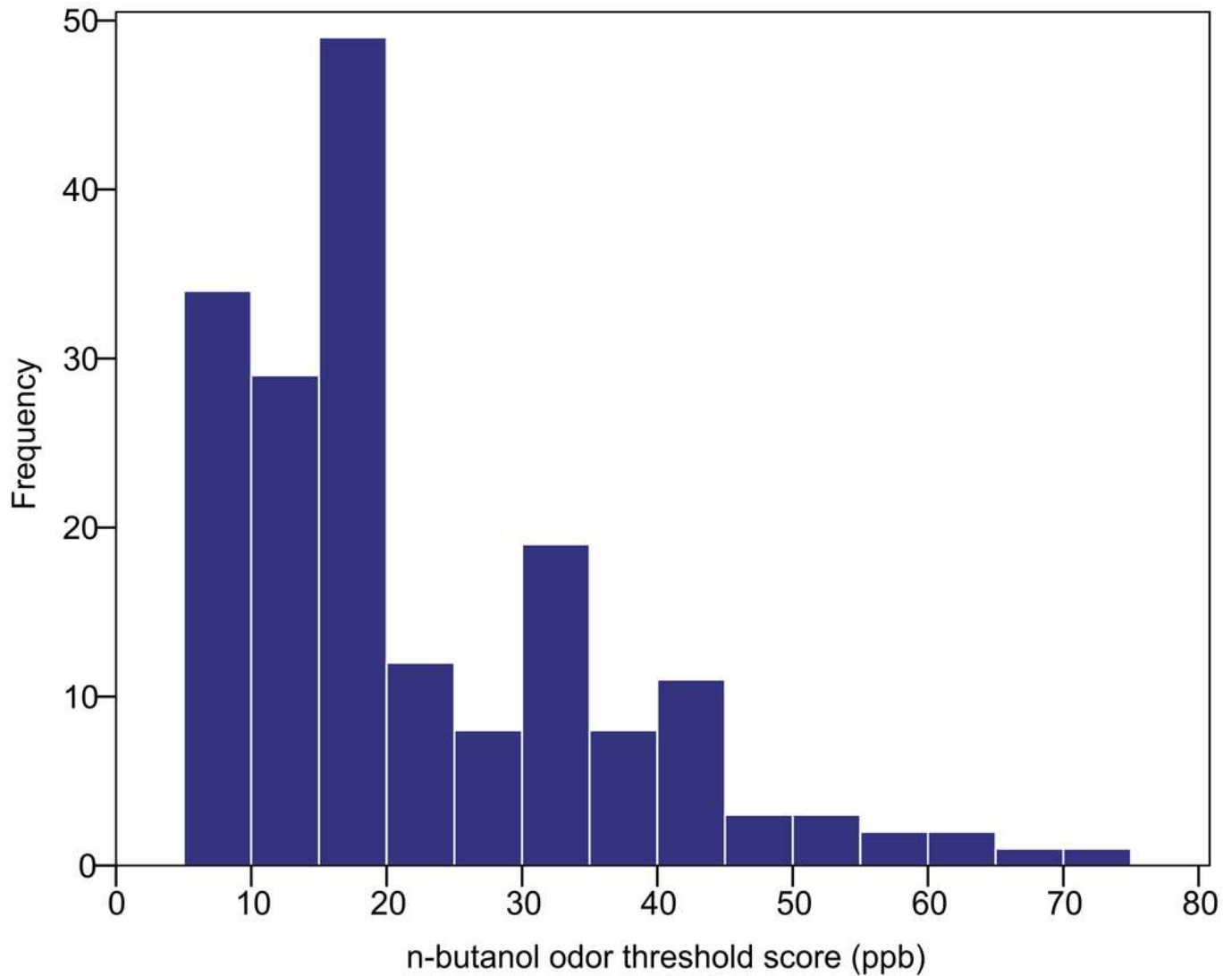
Figure 5

Correlation of n-butanol olfactory threshold scores obtained from participants tested twice, 14 to 18 weeks apart.
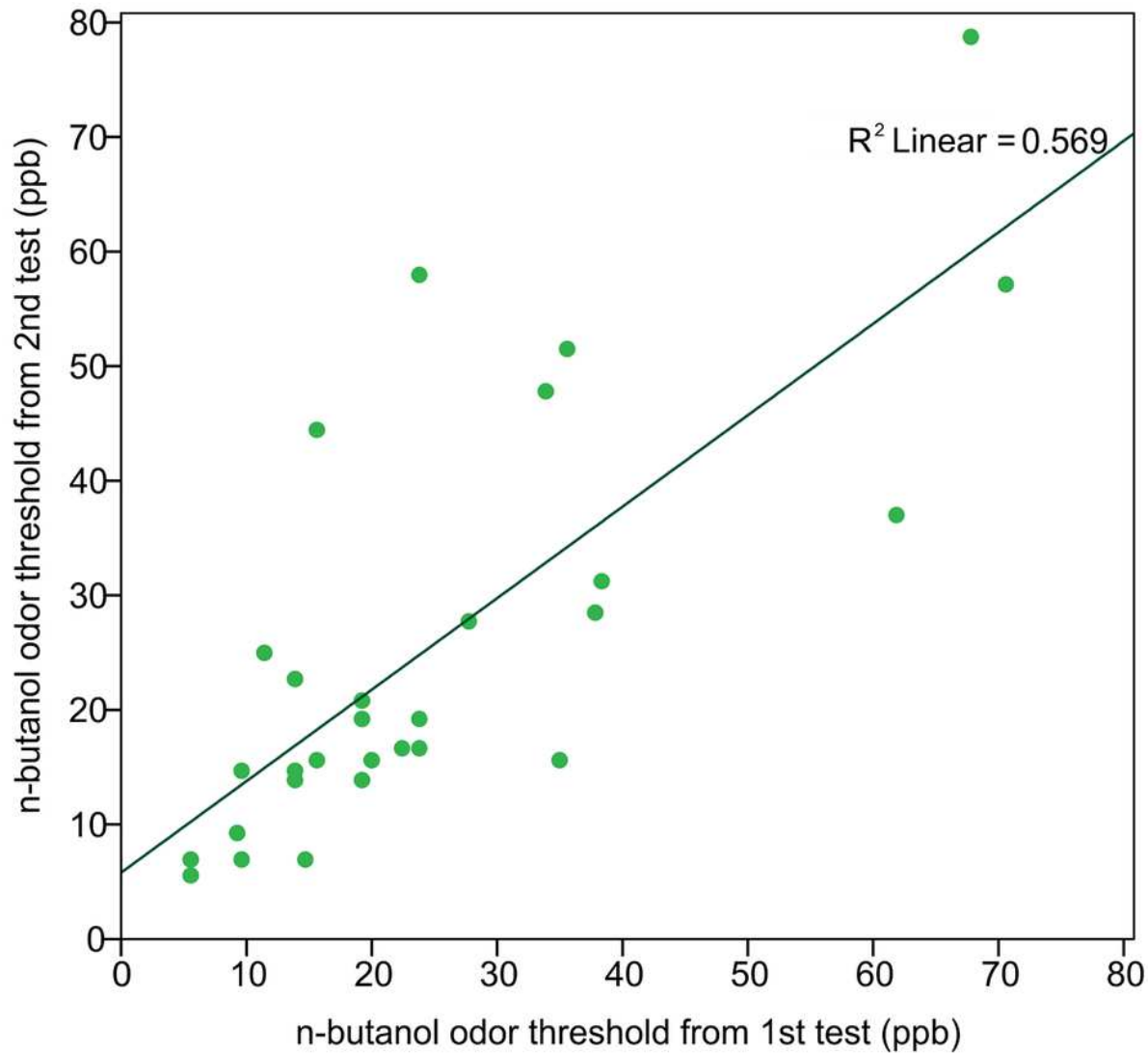
Figure 6

Bland-Altman plot comparing the n-butanol olfactory threshold scores obtained from participants tested twice, 14 to 18 weeks apart
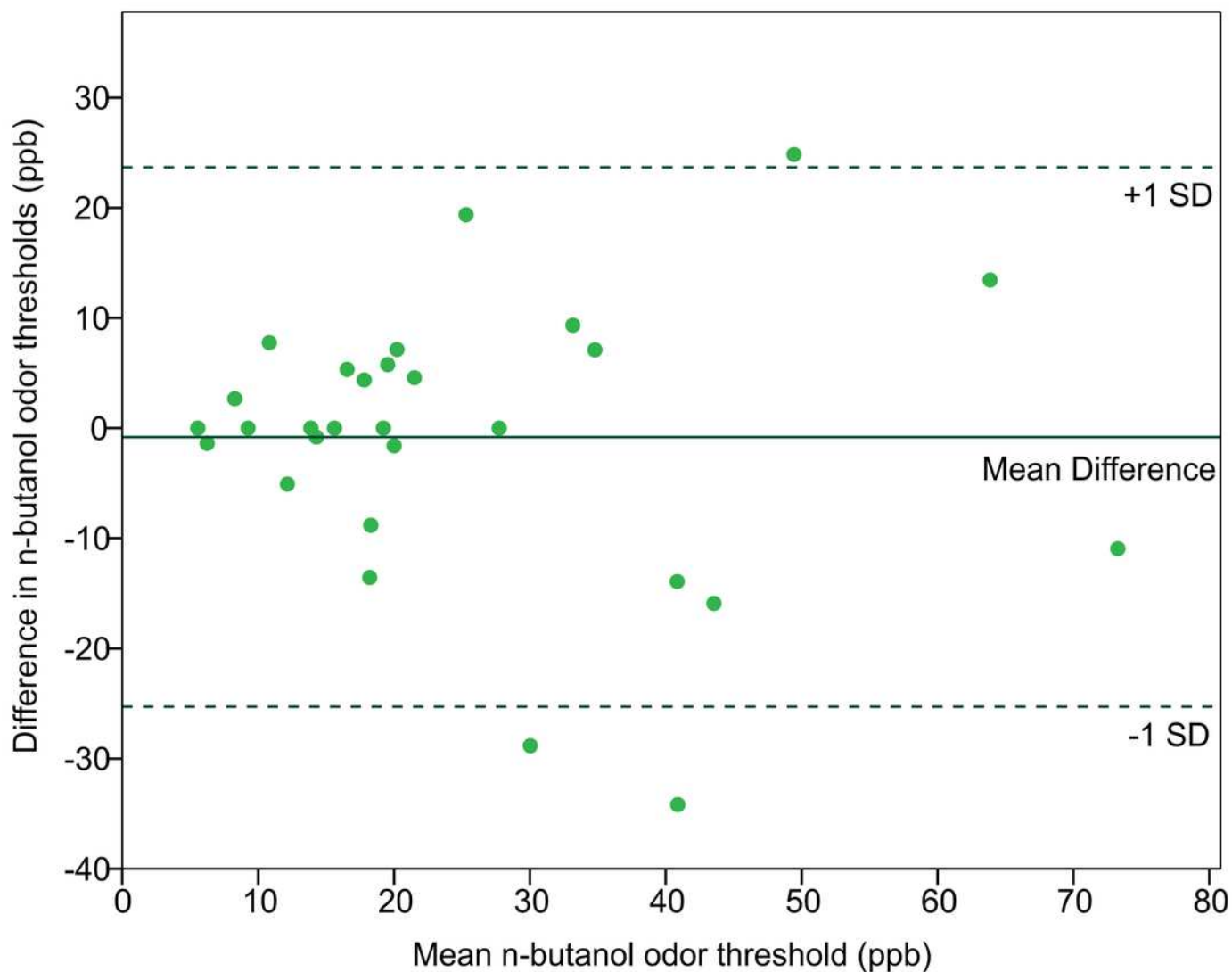
Table 1(on next page)

Sample characteristics

Table 1. Sample characteristics by ancestry.

	Total Sample	East Asian	European	South Asian	Other
N (M, F)	182 (54, 128)	60 (15, 45)	55 (18, 37)	58 (17, 41)	9 (4, 5)
Mean age ± SD	20.4 ± 2.5	20.8 ± 2.5	21.0 ± 2.7	19.6 ± 2.1	19.7 ± 1.3

Table 2 (on next page)

Results of linear regression tests evaluating association of genetic markers with n-butanol thresholds

Table 2. Results of multiple linear regression analysis using a genotypic model, organized by ancestry. None of the markers are significant at a p - value threshold of 0.05.

SNP	Reference Genotype	Genotype	East Asian		European		South Asian		Total Sample	
			Beta	p - value	Beta	p - value	Beta	p - value	Beta	p - value
1s6265	TT	CC	0.121	0.453	-0.501	0.256	-0.098	0.698	-0.670	0.596
		TC	0.085	0.594	-0.420	0.342	-0.103	0.684	-0.005	0.965
rs2889732	AA	CC	-0.138	0.702	-0.173	0.208	-0.183	0.270	-0.069	0.525
		AC	-0.283	0.431	-0.024	0.876	-0.076	0.640	-0.132	0.202
rs6746030	GG	AA								
		GA	0.078	0.566	0.201	0.141	0.139	0.313	0.120	0.131
rs13036385	GG	CC	-0.026	0.841	0.025	0.860	-0.064	0.697	0.016	0.846
		GC	-0.126	0.340	0.017	0.904	0.097	0.494	-0.016	0.841
rs17132289	AA	TT	-0.004	0.978					-0.005	0.946
		AT	-0.049	0.718	-0.173	0.208			-0.107	0.182
rs17712299	TT	CC	-0.001	0.994					0.001	0.990
		TC	-0.008	0.953	-0.104	0.459	0.222	0.111	0.035	0.654

Table 3(on next page)

Test-retest reliability coefficients of various n-butanol olfactory threshold tests

Table 3. Test-retest reliability coefficients of various n-butanol olfactory threshold tests.

Olfactory Test	Test-retest Reliability Coefficient	Sample Size	p - value	Reference
Connecticut Chemosensory Clinical Research Center (CCCRC)	0.36	104	<0.001	(Hummel et al. 1997)
	0.49	57	<0.001	(Doty et al. 1995)
CCCRC modification using ML-PEST	0.26 to 0.86 a	27	0.190 to <0.001	(Linschoten et al. 2001)
CCCRC done as part of the Combined Olfactory Test (COT)	0.85	35	<0.001	(Lam et al. 2006)
Sniffin' Sticks	0.61	104	<0.001	(Hummel et al. 1997)
Scentroid SM110C	0.76	29	<0.001	

a Variation in test-retest reliability coefficients due to time between retests