

# The promise of disease gene discovery in South Asia

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43 **The more than 1.5 billion people who live in South Asia are correctly viewed**  
44 **not as a single large ethnic group, but as many small endogamous groups. We**  
45 **assembled genome-wide data from over 2,800 individuals from over 275**  
46 **distinct South Asian groups. We document shared ancestry across groups that**  
47 **correlates with geography, language, and religious affiliation. We characterize**  
48 **the strength of the founder events that gave rise to many of the groups, and**  
49 **identify 14 groups with census sizes of more than a million that descend from**  
50 **founder events significantly stronger than those in Ashkenazi Jews and Finns,**  
51 **both of which have high rates of recessive disease due to founder events.**  
52 **These results highlight a major and under-appreciated opportunity for**  
53 **reducing disease burden among South Asians through the discovery of and**  
54 **testing for recessive disease genes. As a proof-of-principle, we show that it is**  
55 **possible to localize genes for progressive pseudorheumatoid dysplasia and**  
56 **mucopolysaccharidosis type IVA, two recessive diseases prevalent in South**  
57 **India, using the founder event disease gene mapping approach introduced**  
58 **here.**

59  
60 South Asia is a region of extraordinary cultural, linguistic, and genetic diversity, with  
61 a conservative estimate of over 4,600 anthropologically well-defined groups, many  
62 of which are endogamous communities with significant barriers to gene flow due to  
63 sociological and cultural factors that restrict marriage between groups<sup>1</sup>. Of the small  
64 fraction of South Asian groups that have been characterized using genome-wide  
65 data, many exhibit large allele frequency differences from geographically close  
66 neighbors<sup>2-4</sup>, indicating that they have experienced strong founder events, whereby  
67 a small number of ancestors gave rise to many descendants today<sup>4</sup>. The pervasive  
68 founder events in South Asia present a major opportunity for reducing disease  
69 burden in South Asia. The promise is highlighted by studies of founder groups of  
70 European ancestry – including Ashkenazi Jews, Finns, Amish, Hutterites, Sardinians,  
71 and French Canadians – which have resulted in the discovery of dozens of recessive  
72 disease causing mutations in each group. Prenatal testing for these mutations has  
73 substantially reduced recessive disease burden in all of these communities<sup>5,6</sup>.

74  
75 To characterize the medically relevant founder events in South Asia, we carried out  
76 new genotyping of 1,663 samples from 230 endogamous groups in South Asia on the  
77 Affymetrix Human Origins single nucleotide polymorphism (SNP) array<sup>7</sup>. We  
78 developed an algorithm to measure the strength of an endogamous group's founder  
79 event, aiming to genotype at least five samples per group as our power calculations  
80 indicated that this was sufficient to detect a founder event stronger than that in  
81 Finns with high confidence (Supplementary Figure 1 and Supplementary Table 1).  
82 We combined the new data we collected with previously reported data, leading to  
83 four datasets (Figure 1a). The Affymetrix Human Origins SNP array data comprised  
84 1,955 individuals from 249 groups in South Asia, to which we added 7 Ashkenazi  
85 Jews. The Affymetrix 6.0 SNP array data comprised 383 individuals from 52 groups  
86 in South Asia<sup>4,8</sup>. The Illumina SNP array data comprised 188 individuals from 21  
87 groups in South Asia<sup>9</sup> and 21 Ashkenazi Jews<sup>9,10</sup>. The Illumina Omni SNP array data  
88 comprised 367 individuals from 20 groups in South Asia<sup>11</sup>. We merged 1000

89 Genomes Phase 3 data<sup>12</sup> (2,504 individuals from 26 different groups including 99  
90 Finns) with each of these datasets. We performed quality control to remove SNPs  
91 and individuals with a high proportion of missing genotypes or that were outliers in  
92 Principal Component Analysis (PCA).

93

94 We performed PCA on each of the three different datasets along with European  
95 Americans (CEU), Han Chinese (CHB), and West Africans (YRI), and found that the  
96 Siddi are strong outliers as previously reported (Supplementary Figure 2)<sup>4,13,14</sup>. We  
97 next removed YRI, Siddi and indigenous Andamanese (another known outlier) from  
98 the datasets and repeated PCA (Figure 1b, Supplementary Figure 3). Similar to past  
99 studies, the PCA documents three broad genetic groupings<sup>4,8,11</sup>. First, almost all  
100 South Asian groups speaking Indo-European and Dravidian languages lie along the  
101 “Indian Cline,” with different proportions of Ancestral North Indian (ANI) ancestry  
102 related to Europeans, Central Asians, and Near Easterners, and Ancestral South  
103 Indian (ASI) ancestry that is as different from ANI as Europeans and East Asians are  
104 from each other<sup>4</sup>. The second major cluster includes groups that speak Austroasiatic  
105 languages, as well as some non-Austroasiatic speaking groups that have similar  
106 ancestry possibly due to gene flow with Austroasiatic speaking neighbors or due to  
107 a history of language shift. This set of groups cluster together near the ASI end of the  
108 Indian cline, likely reflecting a large proportion of ASI-like ancestry as well as a  
109 distinct ancestry that has some affinity to East Asians. The Tibeto-Burman speaking  
110 groups and other groups with high proportions of East Asian related ancestry such  
111 as the Bengali and Austroasiatic speaking Khasi form a gradient of ancestry relating  
112 them to East Asian groups such as Han Chinese. These groupings are also evident in  
113 a neighbor-joining tree based on  $F_{ST}$  (Supplementary Figure 4). We confirmed the  
114 East Asian related mixture in some groups by observing significantly negative  
115  $f_3(\text{Test}; \text{Mala, Chinese})$  statistics<sup>7</sup> (Supplementary Table 2).

116

117 We devised an algorithm to quantify the strength of the founder events in each  
118 group based on Identity-by-Descent (IBD) segments, large stretches of DNA shared  
119 from a common founder in the last approximately one hundred generations (Figure  
120 2). We computed an “IBD score” as a measure for the strength of the founder event  
121 in each group’s history: the average length of IBD segments between 3-20  
122 centimorgans (cM) shared between two genomes normalized to sample size. Since  
123 we are interested here in recessive diseases that do not owe their origin to  
124 consanguineous marriages of close relatives, we filtered the data to minimize this  
125 effect by computing IBD between all pairs of individuals in each group and removing  
126 one individual from the pairs with outlying numbers of IBD segments. We validated  
127 the effectiveness of this procedure for removing close relatives by simulation  
128 (Supplementary Table 2; Online Methods).

129

130 We expressed IBD scores for each group as a fraction of the IBD scores of the 1000  
131 Genomes Project Finns merged into each respective dataset. Due to the fact that all  
132 the arrays we analyzed included more SNPs ascertained in Europeans than in South  
133 Asians, the sensitivity of our methods to founder events is expected to be greater in  
134 Europeans than in South Asians, and thus, our estimates of founder event strengths

135 in South Asian groups is likely to be a conservative underestimate relative to that in  
136 Europeans (Supplementary Figure 5 demonstrates this effect empirically and shows  
137 that it is less of a bias for the strong founder events that are the focus of this study).  
138 We computed standard errors for these ratios by a weighted Block Jackknife across  
139 chromosomes and declared significance where the 95% confidence intervals did not  
140 overlap with 1. Our simulations suggest that for sample sizes of 4, the algorithm's  
141 sensitivity was greater than 95% for determining that a group with two times the  
142 bottleneck strength as Finns would have an IBD score significantly greater than that  
143 of Finns, while specificity was perfect in all the simulations we performed  
144 (Supplementary Figure 1 and Supplementary Table 1). We also used two other  
145 methods for measuring founder events that did not require phasing or IBD  
146 detection. First, we computed  $F_{ST}$  between each group and every other group with  
147 similar ancestry sources. Second, for groups on the Indian Cline we fit a model of  
148 population history using *qpGraph*<sup>7</sup> and measuring the founder event as the group-  
149 drift after admixture (Supplementary Figure 6 and Online Data Table 1). The results  
150 of both methods were highly correlated to that of the IBD-based method for all cases  
151 where a comparison was possible (Pearson correlation  $r=0.82-0.98$ ).

152  
153 The IBD analyses suggest that 29% of the South Asian groups we analyzed (96 out  
154 of 327) have significantly stronger founder events than those in both Finns and  
155 Ashkenazi Jews (Figure 3). The South Asian groups with evidence of strong founder  
156 events include diverse tribe, caste, and religious groups, and our analysis identifies  
157 14 groups with strong founder events census sizes of over a million (Figure 3; Table  
158 1). The groups with smaller census sizes are also medically significant. Study of  
159 small census size groups with extremely strong founder events such as Amish,  
160 Hutterites, and the people of the Saguenay Lac-St. Jean region have led to the  
161 discovery of dozens of novel disease variants specific to each group<sup>6</sup>, which  
162 highlights the potential of similar studies in South Asian groups. In addition to these  
163 analyses, we measured IBD across groups – searching for cases in which the across-  
164 group IBD score is at least a third of the within-group IBD score of Ashkenazi Jews –  
165 and found many cases of relatedness, which typically follow geography, religious  
166 affiliation (e.g. Catholic Brahmins), or linguistic grouping (particularly Austroasiatic  
167 speakers) (Supplementary Table 3).

168  
169 Our documentation that medically significant founder events affect a large fraction  
170 of South Asian groups presents an opportunity for decreasing disease burden. This  
171 source of risk for recessive diseases is very different from that due to marriages  
172 among close relatives, which is also a major cause of recessive disease, especially in  
173 southern India. In the case of recessive diseases arising due to founder events, there  
174 are mutations that occur recurrently across members of a group (due to deeply  
175 shared founders), and these can be mapped at a group level and easily tested.

176  
177 As proof-of-principle, we highlight two examples. The first concerns the Vysya, a  
178 group with a census size of more than 3 million that we identified as having a  
179 founder event about 1.2-fold stronger than that in Finns (Figure 3). The Vysya have  
180 a 100-fold higher rate of butyrylcholinesterase deficiency than other Indian groups,

181 and Vysya ancestry is a known counter-indication for the use of muscle relaxants  
182 such as succinylcholine or mivacurium that are given prior to surgery<sup>15</sup>.

183

184 The second proof-of-principle, we newly genotyped 18 patients from India, 12 of  
185 whom had progressive pseudorheumatoid dysplasia (PPD), a disease known to be  
186 caused by mutations in the gene *WISP3*<sup>16,17</sup>, and 6 of whom had  
187 mucopolysaccharidosis type IVA, known to be caused by mutations in the gene  
188 *GALNS*<sup>18</sup>. Though we lacked ethnic group information for most of the 18 patients, 6  
189 of the PPD patients carried Cys78Tyr mutations, and 5 of these 6 were from non-  
190 consanguineous marriages. We found a much higher fraction of IBD at the disease  
191 mutation site than in the rest of the genome (Supplementary Figure 7a). Thus,  
192 Cys78Tyr in PPD is a mutation that owes its origin to a founder event<sup>16,17</sup>. The 6  
193 other PPD patients carried Cys337Tyr mutations, and 6 of 6 were from  
194 consanguineous marriages, while the 6 patients with MPS carried Cys79Arg  
195 mutations, and 4 of 6 were from consanguineous marriages. These patients did not  
196 have IBD at the disease mutation site that was detectable using our conservative  
197 settings, but we were still able to map the disease locus using homozygosity  
198 mapping (Supplementary Figures 7b and 7c) similar to methods used by others<sup>19,20</sup>.  
199 When we examined the haplotypes at the disease loci, we found that each mutation  
200 group had high sharing of unique haplotypes (Supplementary Figure 8), but the PPD  
201 Cys337Tyr and MPS Cys79Arg haplotypes were smaller than the PPD Cys78Tyr  
202 haplotypes. This suggests that these 2 mutations are at high frequency due to older  
203 founder events than the one that occurred for the PPD Cys78Tyr mutations, which  
204 could explain why they were not discovered by IBD (which is most sensitive for  
205 young founder events) and also why they are present primarily in individuals  
206 descending from consanguineous marriages (because they may be sufficiently rare  
207 that they do not come together at an appreciable rate except in the context of a  
208 consanguineous marriage). Beyond the new genotyping we performed here, another  
209 study this year demonstrated that an Indian founder mutation in *ISCA1* mutation  
210 causes predisposition to a severe mitochondrial dysfunction syndrome.<sup>21</sup>

211

212 These observations highlight how systematic studies of South Asian founder groups  
213 are likely to be an effective approach for discovering mutations that cause recessive  
214 disease. Identification of pathogenic mutations responsible for such founder  
215 diseases is straightforward. All that is required is collection of DNA samples from a  
216 small number of affected individuals and their families, usually followed by whole-  
217 exome sequencing to discover the causal changes. Once group specific founder  
218 event disease mutations are discovered, they can be tested for prenatally. Mapping  
219 of recessive disease mutations may be particularly important in traditional  
220 communities practicing arranged marriages, which is common in India. An example  
221 of the power of this approach from outside India is given by *Dor Yeshorim*, a  
222 community genetic testing program among religious Ashkenazi Jews<sup>22</sup>, which visits  
223 schools, screens students for common recessive disease causing mutations  
224 previously identified to be segregating at a higher frequency in the target group, and  
225 enters the results into a confidential database. Match-makers query the database  
226 prior to making suggestions to the families and receive feedback about whether the

227 potential couple is “incompatible” in the sense of both being carriers for a recessive  
228 mutation at the same gene. Given that approximately 95% of community members  
229 whose marriages are arranged participate in this program, recessive diseases like  
230 Tay-Sachs have virtually disappeared in these communities. A similar approach  
231 should work as well in Indian communities where arranged marriages are common.  
232 Given the potential for saving lives, this or similar kinds of research could serve as  
233 an important investment for future generations<sup>23</sup>.

234

235 This study of more than 275 distinct groups represents the first systematic survey  
236 for founder events in South Asia, and to our knowledge also presents the richest  
237 dataset of genome-wide data from anthropologically well-documented groups  
238 available from any region in the world. Despite the breadth of this data, the groups  
239 surveyed here represent only about 5% of the well-documented endogamous  
240 groups in South Asia, and extensions of the survey to all groups would make it  
241 possible to identify large numbers of additional founder groups susceptible to  
242 recessive diseases. To take advantage of the unique population structure of South  
243 Asia to improve health, we propose to reverse the standard approach to recessive  
244 disease gene discovery. Instead of focusing entirely on collecting cases in tertiary  
245 medical centers and mapping diseases in a group of individuals found to have the  
246 same phenotype while blinded to information about their caste or tribal status, we  
247 propose that medical geneticists should adopt a parallel strategy of working with  
248 community doctors, medical workers, and social workers to identify recessive  
249 diseases that occur at a high rate in endogamous groups with founder events, and  
250 then go out into the community to identify cases. Once a small number of cases are  
251 sampled, it is straightforward to map the causal variants. This approach was  
252 pioneered in 1950s by the work of Victor McKusick and his colleagues studying the  
253 Old Order Amish in Pennsylvania U.S.A., a founder population of approximately  
254 100,000 individuals in whom many dozens of recessive diseases were mapped, a  
255 research program that was crucial to founding modern medical genetics and was of  
256 extraordinary health benefit to that community<sup>24</sup>. Our study suggests that the  
257 potential for disease gene mapping in India would be orders of magnitude greater.

258

259  
260 **Supplementary Data:**

261 Supplementary Data include an excel spreadsheet detailing all groups and their  
262 scores on the IBD,  $F_{ST}$ , and group-specific drift analyses. Also included are 8  
263 supplementary figures and 5 supplementary tables.  
264

265  
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278 corresponding authors.

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365

Group	Sample Size	IBD Score	IBD Rank	F <sub>ST</sub> Rank	Drift Rank	Census Size	Location
Gujjar	5	11.6	19	20	46	1,078,719	Jammu and Kashmir
Baniyas	7	9.6	24	24	18	4,200,000	Uttar Pradesh
Pattapu_Kapu	4	9.5	25	24	21	13,697,000	Andhra Pradesh
Vadde	3	9.2	26	34	26	3,695,000	Andhra Pradesh
Yadav	12	4.4	48	102	67	1,124,864	Puducherry
Kshatriya_Aqnikula	4	2.4	75	154	NA	12,809,000	Andhra Pradesh
Naga	4	2.3	76	NA	NA	1,834,483	Nagaland
Kumhar	27	2.3	77	222	197	3,144,000	Uttar Pradesh
Reddy	7	2.0	84	133	106	22,500,000	Telangana
Brahmin_Nepal	4	1.9	86	95	141	4,206,235	Nepal
Kallar	27	1.7	94	95	73	2,426,929	Tamil Nadu
Brahmin_Manipuri	17	1.6	99	NA	NA	1,544,296	Manipur
Arunthathiyar	18	1.3	108	133	81	1,192,578	Tamil Nadu
Vysya	39	1.2	110	55	35	3,200,000	Telangana

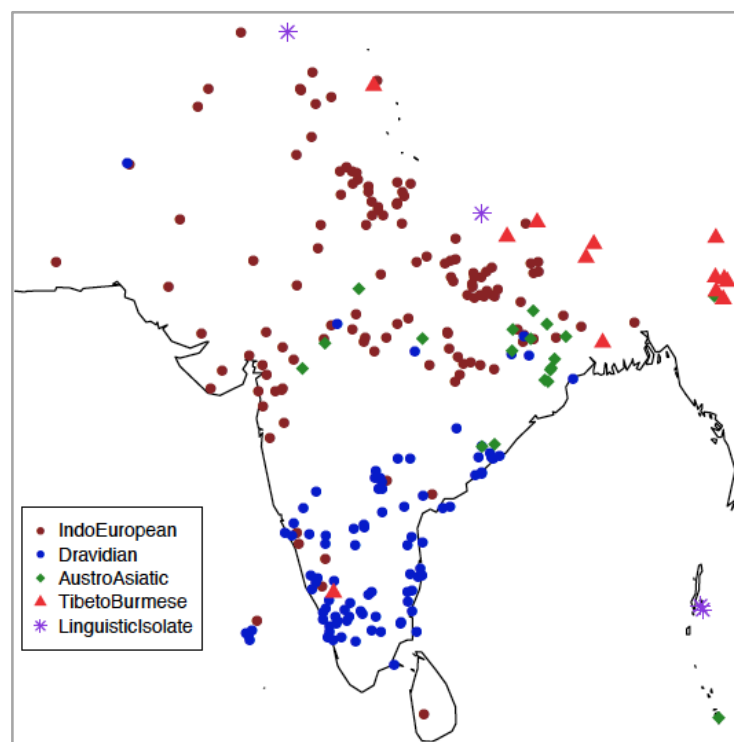
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367 **Table 1. South Asian groups with census sizes over 1 million and IBD scores greater than those of Ashkenazi Jews**

368 **and Finns.** Fourteen South Asian groups with IBD scores significantly higher than that of Finns, census sizes over 1  
369 million, and sample sizes of at least 3 that are of particularly high interest for founder event disease gene mapping studies.  
370 For reference, Finns and Ashkenazi Jews (on Human Origins) would have IBD scores of 1.0 and 0.9, IBD ranks of 121 and  
371 135, and F<sub>ST</sub> ranks of 133 and 154, respectively (the group-specific drift is difficult to compare for groups with  
372 significantly different histories, so they were not calculated for Finns or Ashkenazi Jews).

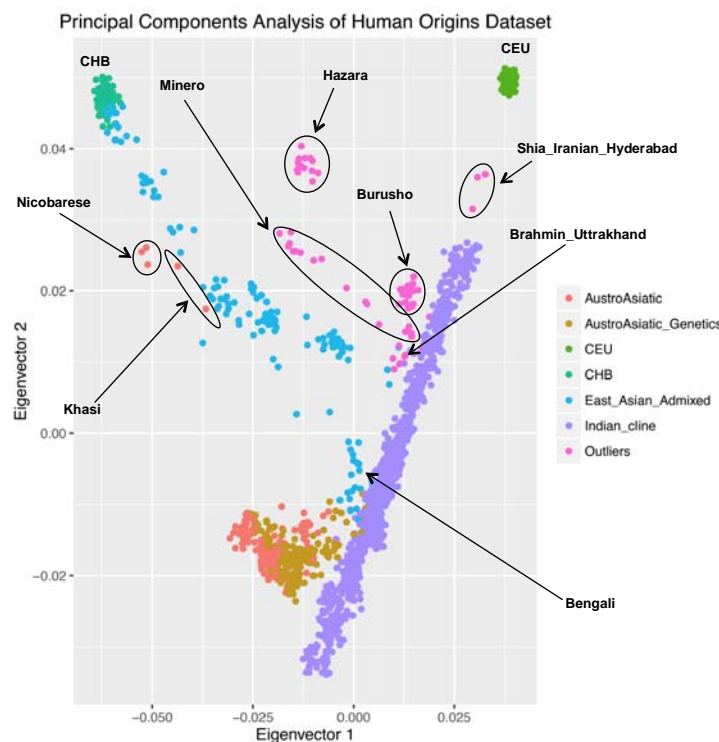
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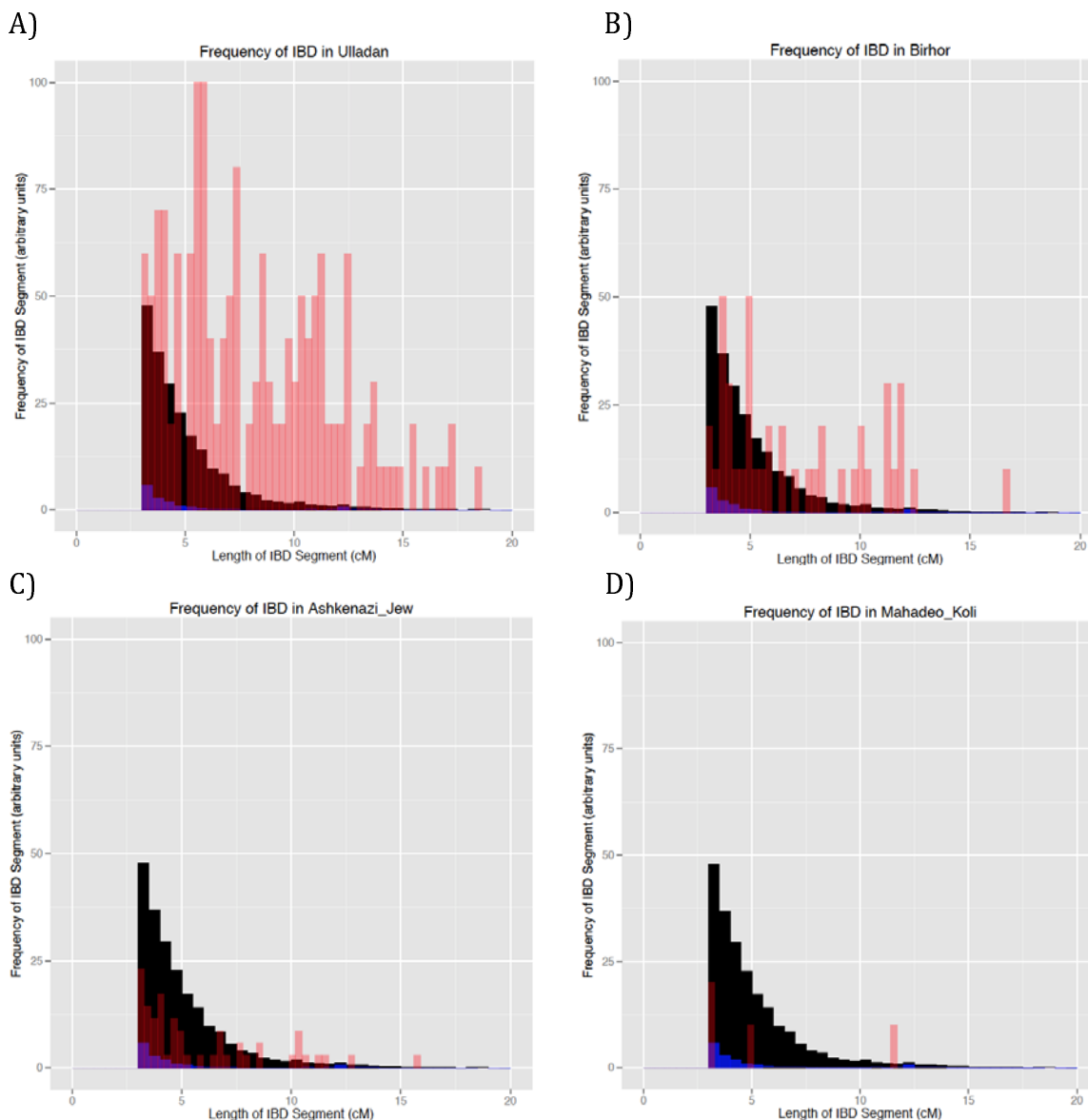


377

378 **Figure 1. Dataset overview.** (a) Sampling locations for all analyzed groups. Each  
379 point indicates a distinct group (random jitter was added to help in visualization at  
380 locations where there are many groups). (b) PCA of Human Origins dataset along  
381 with European Americans (CEU) and Han Chinese (CHB).

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388 **Figure 2. Example histograms of IBD segments to illustrate the differences**

389 **between groups with founder events of different magnitudes:** These histograms

390 provide visual illustrations of differences between groups with different IBD scores.

391 As a ratio relative to Finns (FIN; black), these groups (red) have IBD scores of: (A)

392 ~26 in Ulladan, (B) ~3 in Birhor, (C) ~0.9 in Ashkenazi Jews, and (D) ~0.1 in

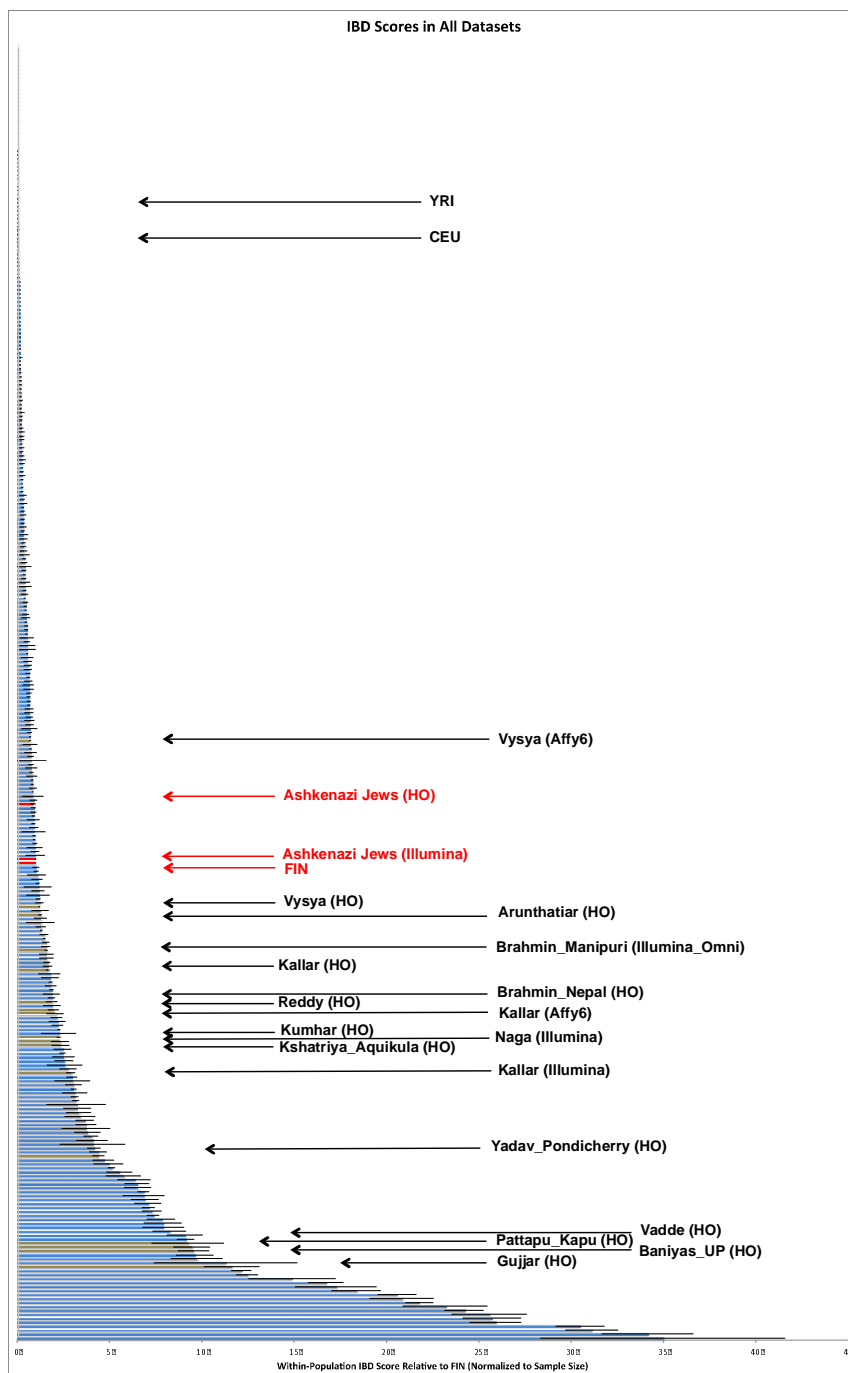
393 Mahadeo\_Koli. In each plot, we also show European Americans (CEU) with a

394 negligible founder event in blue. Quantification of these founder events is shown in

395 Figure 3 and Online Table 1. The IBD histograms were normalized for sample size

396 by dividing their frequency by  $\left\{ \binom{2n}{2} - n \right\}$ , where  $n$  is the number of individuals in

397 the sample. All data for the figure are based on the Human Origins dataset.



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**Figure 3. IBD scores relative to Finns (FIN).** Histogram ordered by IBD score, which is roughly proportional to the per-individual risk for recessive disease due to the founder event. (These results are also given quantitatively for each group in Online Table 1.) We restrict to groups with at least two samples, combining data from all four genotyping platforms onto one plot. Data from Ashkenazi Jews and Finns are highlighted in red, and from Indian groups with significantly stronger founder events than Finns and census sizes of more than a million in brown. Error bars for each IBD score are standard errors calculated by weighted block jackknife over each chromosome. YRI= Yoruba (West African); CEU= Northern European.

## 408 **Online Methods:**

### 409 **Data Sets:**

410 We assembled a dataset of 1,955 individuals from 249 groups genotyped on the  
411 Affymetrix Human Origins array, of which data from 1,663 individuals from 230  
412 groups are newly reported here (Figure 1a). We merged these data with the dataset  
413 published in Moorjani *et al.*<sup>8</sup>, which consisted of 332 individuals from 52 groups  
414 genotyped on the Affymetrix 6.0 array. We also merged it with two additional  
415 datasets published in Metspalu *et al.*<sup>9</sup>, consisting of 151 individuals from 21 groups  
416 genotyped on Illumina 650K arrays as well as a dataset published in Basu *et al.*<sup>11</sup>,  
417 consisting of 367 individuals from 20 groups generated on Illumina Omni 1-Quad  
418 arrays. These groups come from India, Pakistan, Nepal, Sri Lanka, and Bangladesh.  
419 All samples were collected under the supervision of ethical review boards in India  
420 with informed consent obtained from all subjects.  
421  
422

423  
424 We analyzed two different Ashkenazi Jewish datasets, one consisting of 21  
425 individuals genotyped on Illumina 610K and 660K bead arrays<sup>10</sup> and one consisting  
426 of 7 individuals genotyped on Affymetrix Human Origins arrays.  
427

428 Our “Affymetrix 6.0” dataset consists of 332 individuals genotyped on 329,261 SNPs,  
429 and our “Illumina\_Omni” dataset consists of 367 individuals genotyped on 750,919  
430 SNPs. We merged the South Asia and Jewish data generated by the other Illumina  
431 arrays to create an “Illumina” dataset which consists of 172 individuals genotyped  
432 on 500,640 SNPs. Finally, we merged the data from the Affymetrix Human Origins  
433 arrays with the Ashkenazi Jewish data and data from the Simons Genome Diversity  
434 Project<sup>25,26</sup> to create a dataset with 4,402 individuals genotyped on 512,615 SNPs.  
435 We analyzed the four datasets separately due to the small intersection of SNPs  
436 between them and possible systematic differences across genotyping platforms. We  
437 merged in the 1000 Genomes Phase 3 data<sup>27</sup> (2504 individuals from 26 different  
438 groups; notably, including 99 Finnish individuals) into all of the datasets. We used  
439 genome reference sequence coordinates (hg19) for analyses.  
440

### 441 **Quality Control:**

442 We filtered the data at both the SNP and individual level. On the SNP level, we  
443 required at least 95% genotyping completeness for each SNP (across all  
444 individuals). On the individual level, we required at least 95% genotyping  
445 completeness for each individual (across all SNPs).  
446  
447

448 To test for batch effects due to samples from the same group being genotyped on  
449 different array plates, we studied instances where samples from the same group  $A$   
450 were genotyped on both plates 1 and 2 and computed an allele frequency difference  
451 at each SNP,  $Diff_A^i = (Freq_{PopA,Plate1}^i - Freq_{PopA,Plate2}^i)$ . We then computed the  
452 product of these allele frequencies averaged over all SNPs for two groups  $A$  and  $B$   
453 genotyped on the same plates,  $\frac{1}{n} \sum_{i=1}^n (Diff_A^i)(Diff_B^i)$ , as well as a standard error

454 from a weighted Block Jackknife across chromosomes. This quantity should be  
455 consistent with zero within a few standard errors if there are no batch effects that  
456 cause systematic differences across the plates, as allele frequency differences  
457 between two samples of the same group should be random fluctuations that have  
458 nothing to do with the array plates on which they are genotyped. This analysis  
459 found strong batch effects associated with one array plate, and we removed these  
460 samples from further analysis.

461  
462 We used EIGENSOFT 5.0.1 smartpca<sup>28</sup> on each group to detect PCA outliers. We also  
463 developed a procedure to distinguish recent relatedness from founder events so  
464 that we could remove recently related individuals. We first identified all duplicates  
465 or obvious close relatives by using Plink “genome” and GERMLINE<sup>29</sup> to compute IBD  
466 (described in more detail below) and removed one individual from all pairs with a  
467 PI\_HAT score greater than 0.45 and the presence of at least 1 IBD fragment greater  
468 than 30cM. We then used an iterative procedure to identify additional recently  
469 related individuals. For sample sizes above 5, we identified any pairs within each  
470 group that had both total IBD and total long IBD (>20cM) that were greater than 2.5  
471 SDs and 1 SD, respectively, from the group mean. For sample sizes 5 or below, we  
472 used modified Z scores of  $0.6745 * (\text{IBD\_score} - \text{median}(\text{score})) / \text{MAD}$ , where MAD is  
473 the median absolute deviation, and identified all pairs with modified Z scores  
474 greater than 3.5 for both total IBD and total long IBD as suggested by Iglewicz and  
475 Hoaglin<sup>30</sup>. After each round, we repeated the process if the new IBD score was at  
476 least 30% lower than the prior IBD score. Simulations showed that we were always  
477 able to remove a first or second cousin in the dataset using this method  
478 (Supplementary Table 5). Together these analyses removed 53 individuals from the  
479 Affymetrix 6.0 dataset, 21 individuals from the Illumina dataset, 43 individuals from  
480 the Illumina Omni dataset, and 225 individuals from the Human Origins dataset.

481  
482 After data quality control and merging with the 1000 Genomes Project data, the  
483 Affymetrix 6.0 dataset included 2,842 individuals genotyped on 326,181 SNPs, the  
484 Illumina dataset included 2,662 individuals genotyped on 484,293 SNPs, the  
485 Illumina Omni dataset included 2,828 individuals genotyped on 750,919 SNPs, and  
486 the Human Origins dataset included 4,177 individuals genotyped at 499,158 SNPs.

#### 487 488 **Simulations to Test Relatedness Filtering and IBD Analyses**

489  
490 We used ARGON<sup>31</sup> to simulate groups with different bottleneck strengths to test the  
491 IBD analyses, relatedness filtering, and founder event dating algorithms. We used  
492 ARGON’s default settings, including mutation rate of  $1.65 * 10^{-8}$  per base pair (bp) per  
493 generation and a recombination rate of  $1 * 10^{-8}$  per bp per generation and simulated  
494 22 chromosomes of size 130 Mb each. We pruned the output by randomly removing  
495 SNPs until there were 22,730 SNPs per chromosome to simulate the approximate  
496 number of positions in the Affymetrix Human Origins array. For the IBD analyses,  
497 we simulated groups to have descended from an ancestral group 1,800 years ago  
498 with  $N_e=50,000$  and to have formed two groups with  $N_e=25,000$ . These groups  
499 continued separately until 100 generations ago when they combined in equal

500 proportions to form a group with  $N_e=50,000$ . The group then split into 3 separate  
501 groups 72 generations ago that have bottlenecks leading to  $N_e$  of either 400, 800, or  
502 1600. The 3 groups then exponentially expanded to a present size of  $N_e=50,000$ .  
503 We designed these simulations to capture important features of demographic  
504 history typical of Indian groups as detailed by Moorjani *et al.*<sup>8</sup> and Reich *et al.*<sup>4</sup> We  
505 chose the bottleneck sizes because they represent founder events with  
506 approximately the strength of Finns (the bottleneck to 800), and twice as strong  
507 (400) and half as strong (1600) as that group. We then performed the IBD analyses  
508 described below with 99 individuals from the group with bottleneck strength  
509 similar to that of Finns (198 haploid individuals were simulated and merged to  
510 produce 99 diploid individuals) and different number of individuals from the other  
511 groups. These analyses demonstrate that with only 4-5 individuals we can  
512 accurately assess the strength of founder events in groups with strong founder  
513 events (Supplementary Figure 1 and Supplementary Table 1). Weaker founder  
514 events are more difficult to assess, but these groups are of less interest for founder  
515 event disease mapping, so we aimed to sample ~5 individuals per group.

516  
517 We wrote custom R scripts to carry out forward simulations for creating first and  
518 second cousins. We took individuals from the bottleneck of size 800 and performed  
519 “matings” by taking 2 individuals and recombining their haploid chromosomes  
520 assuming a rate of  $1 \times 10^{-8}$  per bp per generation across the chromosome and  
521 combining one chromosome from each of these individuals to form a new diploid  
522 offspring. The matings were performed to achieve first and second cousins. We then  
523 placed these back into the group with group of size 800, and ran the relatedness  
524 filtering algorithms to evaluate whether they would identify these individuals.

### 525 526 **Distance-Based Phylogenetic Tree:**

528 We calculated genetic differentiation ( $F_{ST}$ ) between all pairs of groups using  
529 EIGENSOFT *smartpca* and created a neighbor-joining tree using PHYLIP<sup>32</sup> with  
530 Yoruba as the outgroup. We used ItoI<sup>33</sup> to display the tree.

### 531 532 **Phasing, IBD Detection, and IBD Score Algorithm:**

533  
534 We phased all datasets using Beagle 3.3.2 with the settings *missing=0; lowmem=true;*  
535 *gprobs=false; verbose=true*<sup>34</sup>. We left all other settings at default. We determined IBD  
536 segments using GERMLINE<sup>29</sup> with the parameters *-bits 75 -err\_hom 0 -err\_het 0 -*  
537 *min\_m 3*. We used the genotype extension mode to minimize the effect of any  
538 possible phasing heterogeneity amongst the different groups and used the  
539 HaploScore algorithm to remove false positive IBD fragments with the  
540 recommended genotype error and switch error parameters of 0.0075 and 0.003<sup>35</sup>.  
541 We chose a HaploScore threshold matrix based on calculations from Durand *et al.*  
542 for a “mean overlap” of 0.8, which corresponds to a precision of approximately 0.9  
543 for all genetic lengths from 2-10cM. In addition to the procedure we developed to  
544 remove close relatives (Quality Control section), we also removed segments longer  
545 than 20cM as simulations showed that this increased sensitivity of the analyses  
546 (Supplementary Table 4). We computed “IBD score” as the total length of IBD



547 segments between 3-20cM divided by  $\left\{ \binom{2n}{2} - n \right\}$  where  $n$  is the number of  
548 individuals in each group to normalize for sample size. We then expressed each  
549 group's score as a ratio of their IBD score to that of Finns and calculated standard  
550 errors for this score using a weighted Block Jackknife over each chromosome with  
551 95% confidence intervals defined as IBD score  $\pm 1.96 * s.e.$

552  
553 We also repeated these analyses with FastIBD<sup>36</sup> for the Affymetrix 6.0 and Illumina  
554 datasets and observed that the results were highly correlated ( $r > 0.96$ ) (data not  
555 shown). We chose GERMLINE for our main analyses, however, because the FastIBD  
556 algorithm required us to split the datasets into different groups, since it adapts to  
557 the relationships between LD and genetic distance in the data, and these  
558 relationships differ across groups. We used data from several different Jewish  
559 groups and all twenty-six 1000 Genomes groups to improve phasing, but of these  
560 groups we only included results for Ashkenazi Jews and two outbred groups (CEU  
561 and YRI) in the final IBD score ranking.

562

### **Disease patient analyses:**

563  
564  
565 We use Affymetrix Human Origins arrays to genotype 15 patients with progressive  
566 pseudorheumatoid dysplasia (PPD) and 6 patients with mucopolysaccharidosis  
567 (MPS) type IVA, all of which had disease mutations previously determined<sup>16-18</sup>, and  
568 3 of which (MPS patients) are newly reported here. After quality control, 6 of the  
569 PPD patients with Cys78Tyr mutations, 6 of the PPD patients with Cys337Tyr  
570 mutations, and 6 of the MPS patients with Cys78Arg mutations remained. We  
571 measured IBD as described above and also detected homozygous segments within  
572 each individual by using GERMLINE with the parameters *-bits 75 -err\_hom 2 -err\_het*  
573 *0 -min\_m 0.5 -homoz-only*.

574

575 Haplotype sharing was assessed by analyzing phased genotypes for each mutation  
576 group. At each SNP, we counted the number of identical genotypes for each allele  
577 and calculated the fraction by dividing by the total number of possible haplotypes (2  
578 times the number of individuals), then took the larger value of the two possible  
579 alleles (thus the fraction range was 0.5-1). We then averaged these values over  
580 blocks of 10 or 25 SNPs and plotted the averages around the relevant mutation site.

581

### **Between-Group IBD Calculations:**

582  
583  
584 We determined IBD using GERMLINE as above. We collapsed individuals into  
585 respective groups and normalized for between-group IBD by dividing all IBD from  
586 each group by  $\left\{ \binom{2n}{2} \right\}$  where  $n$  is the number of individuals in each group. We  
587 normalized for within-group IBD as described above. We defined groups with high  
588 shared IBD as those with an IBD score greater than three times the founder event  
589 strength of CEU (and  $\sim 1/3$  the event strength of Ashkenazi Jews).

590

### **$f_3$ -statistics:**

591  
592

593 We used the  $f_3$ -statistic<sup>7</sup>  $f_3(\text{Test}; \text{Ref}_1, \text{Ref}_2)$  to determine if there was evidence that  
594 the *Test* group was derived from admixture of groups related to *Ref*<sub>1</sub> and *Ref*<sub>2</sub>. A  
595 significantly negative statistic provides unambiguous evidence of mixture in the  
596 *Test* group. We determined the significance of the  $f_3$ -statistic using a Block Jackknife  
597 and a block size of 5 cM. We considered statistics over 3 standard errors below zero  
598 to be significant.

#### 600 **Calculating Group Specific Drift:**

602 We used ADMIXTUREGRAPH<sup>7</sup> to model each Indian group on the cline as a mixture  
603 of ANI and ASI ancestry, using the model (YRI, (Indian group, (Georgians, ANI)),  
604 [(ASI, Onge)]) proposed by Moorjani *et al.*<sup>8</sup> This approach provides estimates for  
605 post-admixture drift in each group (Supplementary Figure 6), which is reflective of  
606 the strength of the founder event (high drift values imply stronger founder events).  
607 We only included groups on the Indian cline in this analysis, and we removed all  
608 groups with evidence of East Asian related admixture because this admixture is not  
609 accommodated within the above model.

#### 611 **PCA-Normalized F<sub>ST</sub> Calculations:**

613 To account for intermarriage across groups, we used clusters based on PCA to  
614 estimate the minimum F<sub>ST</sub> for each South Asian group (Supplementary Figure 6).  
615 Specifically, we computed the F<sub>ST</sub> between each group and the rest of the individuals  
616 in their respective cluster based on EIGENSOFT *smartpca*. For these analyses we  
617 only included groups on the Indian Cline and those with Austroasiatic-related  
618 genetic patterns (groups clustering near Austroasiatic speakers on the PCA). For  
619 Ashkenazi Jews and Finns, we used the minimum F<sub>ST</sub> to other European groups.

#### 621 **Code Availability:**

623 Code for all calculations available upon request.