

ORIGINAL ARTICLE

Genome-wide autozygosity is associated with lower general cognitive ability

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Inbreeding depression refers to lower fitness among offspring of genetic relatives. This reduced fitness is caused by the inheritance of two identical chromosomal segments (autozygosity) across the genome, which may expose the effects of (partially) recessive deleterious mutations. Even among outbred populations, autozygosity can occur to varying degrees due to cryptic relatedness between parents. Using dense genome-wide single-nucleotide polymorphism (SNP) data, we examined the degree to which autozygosity associated with measured cognitive ability in an unselected sample of 4854 participants of European ancestry. We used runs of homozygosity—multiple homozygous SNPs in a row—to estimate autozygous tracts across the genome. We found that increased levels of autozygosity predicted lower general cognitive ability, and estimate a drop of 0.6 s.d. among the offspring of first cousins ($P=0.003$ – 0.02 depending on the model). This effect came predominantly from long and rare autozygous tracts, which theory predicts as more likely to be deleterious than short and common tracts. Association mapping of autozygous tracts did not reveal any specific regions that were predictive beyond chance after correcting for multiple testing genome wide. The observed effect size is consistent with studies of cognitive decline among offspring of known consanguineous relationships. These findings suggest a role for multiple recessive or partially recessive alleles in general cognitive ability, and that alleles decreasing general cognitive ability have been selected against over evolutionary time.

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INTRODUCTION

General cognitive ability, traditionally measured through intelligence quotient-type psychometric tests, is a composite measure

of cognition across multiple domains.^{1–3} It reliably predicts many life outcomes, such as health, longevity, social mobility and occupational success.^{4–7} Decades of behavioral genetic research

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on general cognitive ability have shown moderate to high heritability estimates across development.^{8–11} Results from GWAS and mixed linear models estimating variance components from single-nucleotide polymorphisms (SNPs) suggest that the genetic variation underlying general cognitive ability is highly polygenic and predominantly additive in nature.^{12–14} Consequently, family studies have shown that offspring of consanguineous marriages have lower cognitive performance than the general population, supporting a role for inbreeding depression on general cognitive ability.^{15–20}

The hypothesized cause of inbreeding depression, directional dominance of alleles that affect fitness, is thought to occur because selection acts more efficiently on additive effects than on recessive effects, which tends to bias deleterious effects toward a recessive mode of action.²¹ Inbreeding increases the probability that recessive/partially recessive deleterious mutations are homozygous by increasing the proportion of the genome that is autozygous (stretches of two homologous chromosomes in the same individual that are identical by descent). We denote genome-wide estimates of inbreeding as F , with the subscript denoting the method by which inbreeding is estimated (for example, F_{SNP} measures inbreeding directly from individual SNP genotypes). It is important to recognize that traits influenced by inbreeding depression are not predicted to have high levels of nonadditive genetic variation; if inbreeding depression occurs because of the effects of rare, partially recessive deleterious mutations, most of the genetic variation will be additive.^{22,23} Although highly inbred individuals are autozygous for a substantial proportion of their genome (for example, first cousin inbreeding leads to 6.25% average autozygosity genome-wide), autozygosity still occurs in outbred populations, albeit at lower levels, owing to shared distant common ancestors between mates of no known relationship. Using high-density SNP arrays, the existence of autozygosity arising from distant inbreeding can be inferred using runs of homozygosity (ROH)—multiple homozygous SNPs in a row.^{24–26} To the degree that ROHs accurately measure autozygosity, ROHs capture not only homozygosity at measured SNPs, but also homozygosity at rare, unmeasured variants that exist within ROHs.^{27,28} Thus, inbreeding estimates based on SNP-by-SNP excess homozygosity (F_{SNP}) capture the effects of homozygosity at common variants, whereas inbreeding estimates based on the proportion of the genome in ROHs (F_{ROH}) capture the effects of homozygosity at both common and rare variants.

To date, a number of studies have examined the effect of F_{ROH} burden and individual ROH regions on case/control and quantitative phenotypes, with early studies showing mixed results,²⁹ including a nonsignificant F_{ROH} -cognitive ability relationship among individuals of European ancestry ($n = 2329$).³⁰ Given the low variation in F_{ROH} among outbred samples, it is likely that these studies were underpowered.²⁸ Investigations with larger samples have been more successful, finding increased F_{ROH} burden associated with schizophrenia,³¹ height³² and personality.³³ Here we present an analysis of F_{ROH} on general cognitive ability for 4854 individuals of European ancestry from nine samples, including five samples from the COGENT consortium.³⁴ Understanding the contribution of autozygosity to individual differences in general cognitive ability can help elucidate the genetic architecture underlying this important and highly polygenic trait.

MATERIALS AND METHODS

Genetic and sample quality control

Quality control (QC) procedures focused on properties that would be appropriate across a range of genotyping platforms that differed in SNP density. The main goal—analyzing runs of homozygosity to infer autozygosity—differed from the usual goal of finding associations between individuals SNPs and a phenotype, and so the procedures

Table 1. Descriptive statistics of SNPs and ROHs across data sets

Data set	N	Region	Platform	SNPs passing QC	LD-pruned SNPs passing QC	Avg $F_{\text{ROH}} \times 100$	S.d. $F_{\text{ROH}} \times 100$	Avg ROH length (kb)	S.d. ROH length (kb)	Avg ROH count
IBG	301	Colorado, USA	Affymetrix 6.0	577 090	206 772	0.33	0.16	1264	703	7.20
GAIN NE	357	Northern Europe	Perlegen 600K	280 995	198 218	0.31	0.39	1537	1875	5.50
GAIN UK	183	United Kingdom	Perlegen 600K	242 867	174 013	0.19	0.15	1550	1133	3.39
GAIN SP	68	Spain	Perlegen 600K	312 730	174 598	0.11	0.10	1522	887	1.99
MANC	763	England	Illumina 610	470 062	250 351	0.47	0.42	1312	1480	9.91
NEWC	717	England	Illumina 610	466 613	247 990	0.44	0.35	1286	1365	9.39
LOGOS	776	Greece	Illumina OmniExpress	393 770	239 923	0.38	0.54	1780	2982	5.92
NCNG	623	Norway	Illumina 610	373 975	195 130	0.41	0.35	1682	1827	6.79
ZHH	175	New York, USA	Illumina OmniExpress	548 508	274 325	0.60	0.53	1540	1676	10.84
TOP	305	Norway	Affymetrix 6.0	578 177	237 481	0.45	0.31	1273	956	9.76
RJG	586	Germany	Illumina OmniExpress	560 407	277 024	0.48	0.53	1297	2148	10.32
Total	4854	—	—	—	—	0.41	0.42	1421	1829	8.02

Abbreviations: LD, linkage disequilibrium; ROH, run of homozygosity; SNP, single-nucleotide polymorphism; QC, quality control. F_{ROH} is calculated as proportion of an individual's genome captured in ROH.

Table 2. Descriptive statistics of general cognitive ability, age and sex across data sets

Data set	N	Region	Cognitive ability measures	Mean age, years (s.d. or range)	Male (%)	Female (%)
IBG	301	Colorado, USA	WAIS-III: 2 subtests (Ages 16+)	15.91 (1.53)	232 (77%)	69 (23%)
GAIN NE	357	Northern Europe	WISC-III: 2 subtests (Ages 8–16)	10.95 (2.57)	305 (85%)	52 (15%)
GAIN UK	183	United Kingdom	WAIS-III: 4 subtests (Ages 16+)	11.67 (2.83)	165 (90%)	18 (10%)
GAIN SP	68	Spain	WISC-III: 4 subtests (Ages 5–16)	9.40 (2.53)	62 (91%)	6 (9%)
MANC	763	England	WAIS-III: 4 subtests (Ages 16+)	64.9 (6.14)	226 (30%)	537 (70%)
NEWC	717	England	WISC-III: 4 subtests (Ages 5–16)	65.71 (6.10)	206 (29%)	511 (71%)
LOGOS	776	Greece	Cambridge NTAB: 3 subtests	22.13 (18–29)	776 (100%)	0 (0%)
			N-Back task			
			Wisconsin card sort			
			Stroop Gambling task			
			Wechsler memory scale			
NCNG	623	Norway	California Verbal Learning Test-II	NA	200 (32%)	423 (68%)
			D-KEFS Color Word interference			
			WAIS-III Matrix Reasoning subscale			
			Multiple choice reaction time task			
ZHH	175	New York, USA	MATRICES Consensus Cognitive Battery	NA	85 (49%)	90 (51%)
TOP	305	Norway	WASI: 4 subtests	NA	165 (54%)	140 (46%)
			National Adult Reading Test			
RUJ	586	Germany	WAIS-R	NA	293 (50%)	293 (50%)
Total	4854	—	—	—	2715 (56%)	2139 (44%)

Abbreviation: NA, not applicable. Data sets where age information was unavailable were not included in the regression model.

adopted were more stringent than those typically used in genome-wide association studies. Moreover, because so many SNPs (70–75% depending on the sample) were removed owing to linkage disequilibrium (LD) pruning during ROH detection (see below), we could afford to use more stringent QC procedures, because dropped SNPs were likely to be in strong LD with other nearby SNPs that were retained.

Table 1 lists the specific genotyping platforms used, with an average LD-pruned SNP density of 229 k SNPs (range: 174 k–277 k). The specific QC procedures and numbers of individuals or SNPs dropped at each step can be found in Supplementary Table S1. Individuals whose self-reported sex was discrepant from their genotypic sex were dropped, as these individuals might represent sample mix-ups. Individuals who self-identified as non-European ancestry were dropped, as both homozygosity and phenotypic measures might differ between ethnicities or across different levels of genetic admixture. We also merged the genotype data with HapMap2 reference samples,³⁵ and removed anyone clearly outside of the European ancestry cluster. Finally, we did not remove individuals with excess genome-wide homozygosity as such individuals are more likely to be inbred and therefore informative for investigating the current hypothesis. After subject QC, autosomal SNPs with MAF > 5%, successfully genotyped in at least 99.5% of individuals, and in Hardy–Weinberg equilibrium ($P > 0.001$) were retained for analysis.

Runs of homozygosity calling procedures

ROH were called in PLINK using the `--homozyg` command,³⁶ which has been found to outperform other programs in accurately identifying autozygous segments.³⁷ The current analysis incorporated the ROH tuning parameters recommended in Howrigan *et al.*³⁷ In particular, each data set was pruned for either moderate LD (removing any SNP with $R^2 > 0.5$ with other SNP in a 50-SNP window) or strong LD (removing any SNP with $R^2 > 0.9$ with other SNP in a 50-SNP window). For moderate LD-pruned SNPs, the minimum SNP length threshold was set to 35, 45, or 50 SNPs. For strong LD-pruned SNPs, the minimum SNP length threshold was set to 65 SNPs. We did not allow for heterozygote SNPs, used a window size equal to the minimum SNP threshold, and allowed for 5% of SNPs to be missing within the window.³⁷ In addition, PLINK's `--homozyg-group` and `--homozyg-match` commands were used to find allelically matching ROH that overlapped at least 95% of physical distance of the smaller ROH, and this parameter was used to define common and uncommon ROH. We chose the 65-SNP minimum pruned for strong LD, as this parameter setting has been used in previous analyses.³¹ Primary F_{roh} burden results, however, were similar for all four tuning parameters used (Supplementary Table S2).

F_{roh} genotype

Genome-wide ROH burden, or F_{roh} , represents the percent of the autosomal ROHs. F_{roh} was derived by summing the total length of autosomal ROHs in an individual and dividing this by the total SNP-mappable autosomal distance (2.77×10^9). The distribution of F_{roh} in the sample is listed in Supplementary Figure S1. F_{roh} can be affected by population stratification (for example, if background levels of homozygosity or autozygosity differ across ethnicities), low-quality DNA leading to bad SNP calls, and heterozygosity levels that differ depending on, for example, genotype plate, DNA sources, SNP calling algorithm or sample collection site. We controlled for covariates in two steps—within data set and across the combined data sets. Within each data set, we controlled for the first 10 principal components generated from an identity-by-state matrix derived from a subset of SNPs (~50 000) within each data set. We also controlled for age and age-squared within data set when provided, as age information was not available in 4 of the 11 studies (Table 2). We used the linear model residuals from within each data set as our F_{roh} genotype moving forward. Across the combined samples, we controlled for gender, data set, the percentage of missing calls—which has been shown to track the quality of SNP calls,³⁸ and excess SNP-by-SNP homozygosity (F_{snp} , from PLINK's `--het` command)—which can be used to test the effects of homozygosity at common but not rare variants.

General cognitive ability phenotype

Table 2 lists the sample characteristics and various measures of general cognitive ability employed (additional description in Supplementary Information). To reduce the bias of specific cognitive testing instruments, composite scores or measurement schemes, measures of general cognitive ability were converted into Z-scores within each data set (Supplementary Figure S2). We then controlled for potential confounds in same manner as the F_{roh} genotype, regressing out the first 10 principal components, age and age-squared within each data set, and data set, gender, SNP missingness and F_{snp} across the combined data set. By controlling for covariates in a similar manner for both the F_{roh} and general cognitive ability phenotype, we are able to assess the unique covariance between F_{roh} and general cognitive ability within a multilevel statistical framework.

F_{roh} burden analysis

To test the effect of F_{roh} burden on general cognitive ability, we examined both fixed-effects modeling (that is, `lm()` in R) and mixed-effects modeling treating data set as a random effect (that is, `lmer()` from the `lme4` package

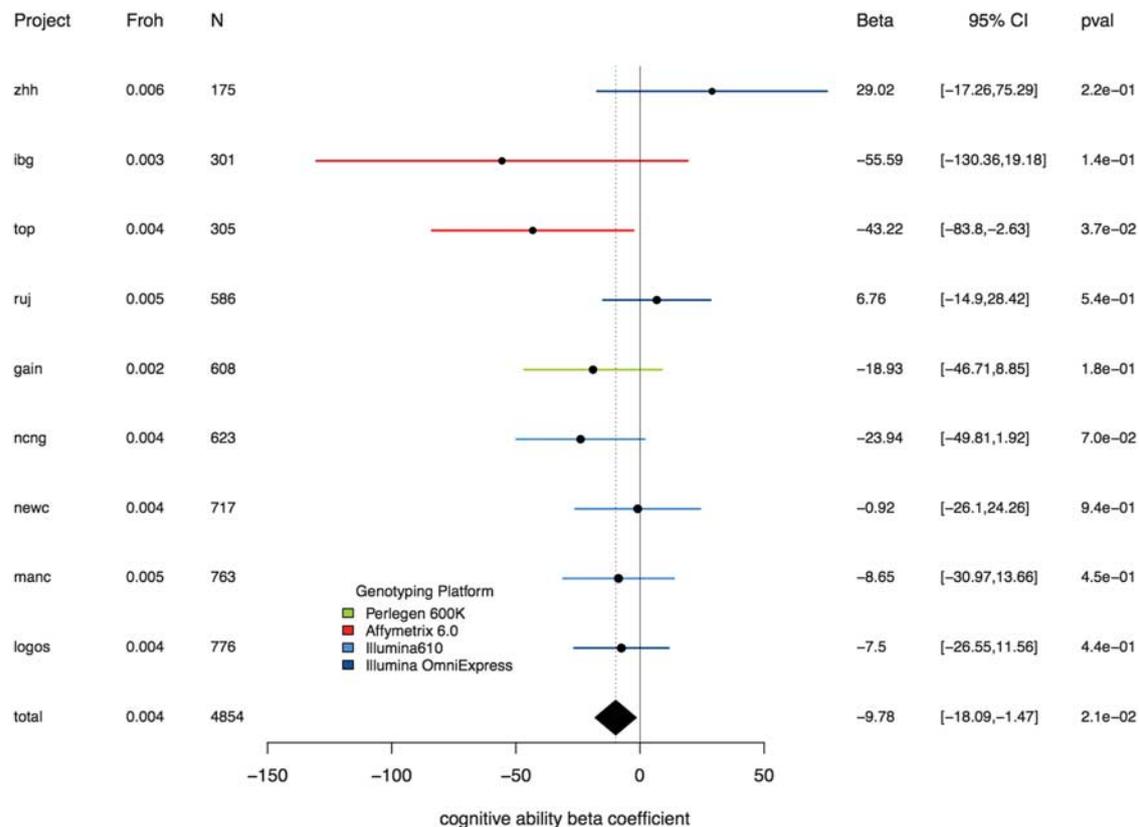


Figure 1. Forest plot of slope estimates and 95% confidence intervals of F_{roh} predicting general cognitive ability. Points represent slope estimates and bars represent 95% confidence intervals. Data sets are color coded by the genotyping platform used. The three GAIN data sets were combined for clarity.

in R). Both analyses showed very consistent results, and we used fixed-effects modeling approach for all analyses hereafter. For our primary analysis, we tested the effects of F_{roh} after controlling for F_{snp} as we have done previously,³¹ not only because this analysis provides information on the importance of rare recessive variants in particular, which are thought to be the primary cause of inbreeding depression,²¹ but also because controlling for F_{snp} can increase power to detect F_{roh} relationships in the presence of genotyping errors.²⁸ We also report the effects of F_{roh} not controlling for F_{snp} .

In follow-up analyses, F_{roh} burden was partitioned into short and long ROH, as well as common and uncommon ROH, by using a median split. Owing to the large variation in SNP density across genotyping platforms (ranging from 300 k to over 1 million SNPs), the median split for both ROH length and frequency were calculated within each data set (see Supplementary Table S3). Across all data sets, short ROH make up 34% of the total F_{roh} coverage, with 66% in long ROH. For the median split of ROH frequency, common ROH make up 38% of total F_{roh} coverage, with 62% in uncommon ROH.

ROH mapping analysis

To investigate whether specific genomic regions predicted general cognitive ability, we co-opted the rare CNV commands used in PLINK, whereby each ROH segment was tested at the two SNPs defining the start and end position. At each position, all individuals with ROH overlapping the tested SNP were included as ROH carriers. General cognitive ability residuals, after controlling for all covariates, were used as the dependent variable. We restricted ROH mapping to positions where five or more ROHs existed across the sample, and derived statistical significance at each position from one million permutations in PLINK.

To derive an experiment-wide significance threshold for multiple tests, we estimated the family-wise error rate directly from permutation. To do so, we ran 1000 permutations on the general cognitive ability phenotype and obtained empirical P -values in the same manner as above. We then

extracted the most significant P -value from each permutation, and used the 95th percentile (or 50th most significant P -value among the set) as our significance threshold ($P = 4e - 6$). Thus, under the null hypothesis, we had a 5% chance of observing a significant finding.

RESULTS

Figure 1 shows the parameter estimates of F_{roh} predicting general cognitive ability within each data set and combined across the full sample. In the combined sample, higher levels of F_{roh} were associated, albeit modestly, with lower general cognitive ability ($\beta = -9.8$, $t(4852) = -2.31$, $P = 0.02$). This estimate suggests that every 1% point increase in F_{roh} corresponds to a ~ 0.1 s.d. reduction in general cognitive ability, extrapolating to an expected ~ 0.6 s.d. reduction among the offspring of first cousins. Within each data set, only the TOP sample exhibited a significant negative relationship between F_{roh} and general cognitive ability. Nevertheless, the majority (7/9) of data sets predict lower cognitive ability and none exhibited a significant positive association. Our estimate was not driven by potential outliers in F_{roh} , as it increased when we removed the 33 individuals with no ROH calls and 5 individuals with $> 6\%$ F_{roh} ($\beta = -12.8$, $t(4814) = -2.68$, $P = 0.007$), and was insensitive to ROH calling thresholds ≥ 50 consecutive homozygous SNPs (Supplementary Figure S3). Furthermore, we found no evidence that copy number deletions reported in the literature were driving the relationship between F_{roh} and general cognitive ability (see Supplementary Information). In general, the estimate remained stable across models where covariates were removed in stepwise manner or split by age groups or sex. In particular, the estimate for F_{roh} on general cognitive ability was more significant when SNP-by-SNP

homozygosity, F_{SNP} , was removed as a covariate ($\beta = -9.9$, $t(4852) = -2.92$, $P = 0.003$), whereas F_{SNP} did not itself predict general cognitive ability ($\beta = -0.1$, $t(4852) = -0.04$, $P = 0.97$), and suggests that homozygosity at rare variants drove the observed F_{ROH} effect. Finally, contrary to a previous report,³⁰ we found no evidence for increased assortative mating or inbreeding at the upper tail of the cognitive ability distribution.

Additional analyses found that F_{ROH} from long ROH ($\beta = -9.2$, $t(4852) = -2.15$, corrected $P = 0.012$), and rare ROH ($\beta = -15.4$, $t(4852) = -2.56$, corrected $P = 0.04$) remain consistent with the overall F_{ROH} association. F_{ROH} estimates from short or common ROH, however, no longer show any association signal ($P > 0.30$ for both, see Supplementary Information for full analysis). Both short autozygous haplotypes, which arise from more distant common ancestry, and common autozygous haplotypes, which arise from chance pairing of common haplotypes segregating in the population, have had more opportunities to be subject to natural selection when autozygous. This may bias them to be less deleterious when autozygous than long or rare haplotypes.

In addition to F_{ROH} burden, we mapped individual ROH along the autosome to assess whether specific regions associate with general cognitive ability. Using PLINK, we mapped and analyzed ROH segments at their respective ends (that is, the first and last SNP in the ROH), counting all overlapping ROH incorporating that SNP as ROH carriers. We observed minimal test statistic inflation across the genome ($\lambda^{\text{GC}} = 1.02$; QQ plot shown in Supplementary Figure S5), suggesting that the integration of various subpopulations within the full sample were adequately controlled and did not inflate ROH mapping test statistics. Among mapped regions, we detected two ROH hotspots in the genome where more than 5% of individuals qualify as ROH carriers. These hotspots occur at well known regions of recent positive selection in lactase persistence on chromosome 2 (ref. 39) and the MHC region on chromosome 6 (ref. 40) with neither showing association with general cognitive ability ($P > 0.05$). Overall, we did not identify any specific ROH regions that surpassed strict genome-wide correction (Figure 2), and we highlight 16 regions with $P < 0.001$ as potential areas of interest (Supplementary Table S4). Our top association,

located on chromosome 21q21.1 ($P = 5.4e-5$, Supplementary Figure S6), predicts lower general cognitive ability and has a distinct peak over *USP25*, a ubiquitin-specific peptidase gene expressed across a variety tissues types, including brain.⁴¹

DISCUSSION

After stringent quality control and the application of preferred methods for detecting autozygosity, we observe a significant, albeit modest, trend of autozygosity burden (F_{ROH}) lowering cognitive ability among outbred populations of European ancestry. Inbreeding among first cousins leads to an average F_{ROH} burden of 6.25%, and corresponds to a predicted drop of 9.19 intelligence quotient points in the current study. This effect is consistent with previously detected effects from pedigree-based consanguineous inbreeding,¹⁵ reassuring us that our observed effect is genuine. In addition, we find that long and rare ROH are driving F_{ROH} association to general cognitive ability, as the relationship of F_{ROH} to general cognitive ability disappear when restricting to either short or common ROH, but remain when considering either long or rare ROH. At the level of individual ROH, however, we do not identify any specific autozygous loci that significantly predicted general cognitive ability after genome-wide correction.

During the acquisition of the current data set, we were aware that very large sample sizes, on the order of 20 000–60 000, were needed to obtain adequate power for detecting the effects of inbreeding depression.²⁸ This is because outbred populations generally exhibit low variation in overall autozygosity, and larger samples are necessary to detect a robust signal. In fact, a recent effort from the ROHgen consortium examining the relationship of F_{ROH} to a number of quantitative human traits robustly associated higher F_{ROH} to lower levels of general cognitive ability ($P = 2.5e-10$) in a sample of 53 300 individuals.⁴² Their sample is fully independent from the current study, and their reported effect size of F_{ROH} ($\beta = -4.6$) is attenuated relative to our observed F_{ROH} estimate ($\beta = -9.9$). The consistent direction of association between cohorts and robust significance in the fully powered

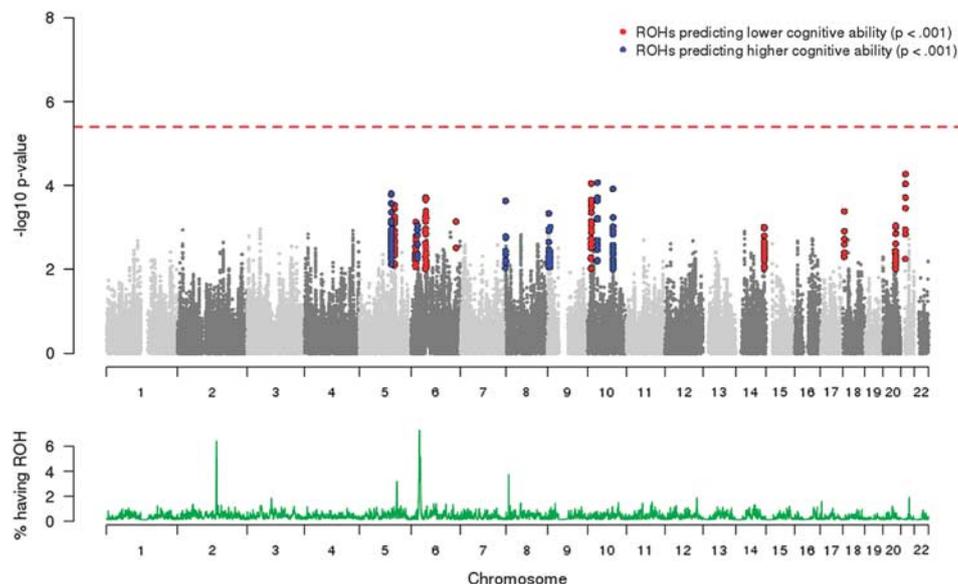


Figure 2. ROH mapping Manhattan plot predicting general cognitive ability. Top panel: $-\log_{10} P$ -values for ROH regions predicting general cognitive ability. Regions with P -values below 0.001 are flagged for predicting lower cognitive ability (red) and higher cognitive ability (blue). The red dotted line is the experiment-wide correction estimate, set at $4e-6$, which is the top 5% of minimum P -values observed across 1000 permutations. Bottom panel: ROH frequencies for each region across the autosome, with the highest frequency of ROH due to balancing selection in the MHC (chr6) and recent positive selection in lactase persistence in gene region (chr2). ROHs, runs of homozygosity.

ROHgen consortium study provides strong and replicable evidence that increased F_{roh} associates with lower general cognitive ability.

There were several limitations to the current study that were largely a consequence of combining multiple data sets together. First, the operational construct of general cognitive ability differed somewhat between data sets (see Table 2 and Supplementary Information), and statistical power can be lost as a function of the degree of phenotypic heterogeneity in measured cognitive ability across samples. Second, despite following strict QC procedures, the use of different genotyping platforms affects ROH calls across data sets. Although data set was included as a covariate, such differences add noise and reduce statistical power, and it is impossible to rule out all biases that could arise from such differences between data sets. Finally, the autozygosity—cognitive ability relationship might be mediated differentially across sites/data sets. For example, analysis of the Netherlands Twin Registry found that increased religiosity was associated with both higher autozygosity and lower rates of major depression in the Netherlands, which if unaccounted for, would have obscured the true relationship between major depression and autozygosity.⁴³ More recent evidence in the same data set found that increased parental migration mediated the relationship of education attainment to autozygosity.⁴⁴ Unfortunately, these potential confounds are often unmeasured and were unavailable in the current study. More generally, the correlational design of this study disallows causal inference. It is possible that lower general cognitive ability or some third variable associated with lower general cognitive ability leads to a reduced affinity for migration or culturally diverse mating patterns, thereby increasing the probability for distant inbreeding.

Autozygosity is the most direct measure of inbreeding at the genetic level. It can help elucidate the genetic architecture underlying heritable traits like general cognitive ability and provide clues to the evolutionary forces that acted on alleles affecting the trait. Our results suggest that alleles that decrease cognitive ability are more recessive than otherwise expected, and are consistent with the hypothesis that alleles that lead to lower cognitive ability have, on average, been under negative selection ancestrally. Moving forward, larger sample sizes, inclusion of populations with higher variance in inbreeding, ascertainment of samples toward the extreme end of cognitive ability, along with genome sequence analysis of autozygous tracts, will all help to refine to scope of inbreeding depression on general cognitive ability and provide the statistical power for more refined genetic mapping approaches.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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