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Age Dependent Association of Inbreeding with Risk for Schizophrenia in Egypt

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Abstract

Background—Self-reported consanguinity is associated with risk for schizophrenia (SZ) in several inbred populations, but estimates using DNA-based coefficients of inbreeding are unavailable. Further, it is not known whether recessively inherited risk mutations can be identified through homozygosity by descent (HBD) mapping.

Methods—We studied self-reported and DNA-based estimates of inbreeding among Egyptian patients with SZ (n=421, DSM IV criteria) and adult controls without psychosis (n=301), who were evaluated using semi-structured diagnostic interview schedules and genotyped using the Illumina Infinium PsychArray. Following quality control checks, coefficients of inbreeding (F) and regions of homozygosity (ROH) were estimated using PLINK software for HBD analysis. Exome sequencing was conducted in selected cases.

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CONTRIBUTORS

HM, BD, MT, and VN designed the study. HM, WF, II, NI, AE, WE, ST, HES, AY, EE, HS, AA, and HEB conducted interviews and recruited subjects. LM, HM, CK, SE, MT, and AM carried out experiments. LM, LK, BD, and VN drafted the manuscript. LK, LM, JW, AM, SW, and SE analyzed data. *All authors provided input to the revisions to the manuscript and approved the final version.*

CONFLICT OF INTEREST

The Authors have declared no conflicts of interest in relation to the subject of this study.

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Results—Inbreeding was associated with schizophrenia based on self-reported consanguinity ($\chi^2=4.506$, 1 df, $p=0.034$) and DNA-based estimates for inbreeding (F); the latter with a significant F x age interaction ($\beta=32.34$, $p=0.0047$). The association was most notable among patients older than age 40 years. Eleven ROH were over-represented in cases on chromosomes 1, 3, 6, 11, and 14; all but one region is novel for schizophrenia risk. Exome sequencing identified six recessively-acting genes in ROH with loss-of-function variants; one of which causes primary hereditary microcephaly.

Conclusions—We propose consanguinity as an age-dependent risk factor for SZ in Egypt. HBD mapping is feasible for SZ in adequately powered samples.

Keywords

genotyping; psychosis; statistical genetics; consanguinity

INTRODUCTION

Schizophrenia (SZ) is a life-long and potentially disabling illness with a 1% lifetime morbid risk, detectable across the globe. Treatment is symptomatic and palliative, failing in over half of patients (Lieberman et al., 2005; Stroup et al., 2006). In view of the unsatisfactory treatment, it is important to understand etiology and pathogenesis of SZ. The relatively high heritability for SZ (twin studies~0.70; single nucleotide polymorphisms, SNP~0.45), motivated a variety of gene mapping efforts (Bulik-Sullivan et al., 2015; Gottesman, 1991; McGue et al., 1983; Zheng et al., 2017). Genome-wide association studies (GWAS) conducted by the Psychiatric Genomics Consortium identified 108 SZ risk loci (Schizophrenia Working Group of the Psychiatric Genomics, 2014); further enlargement of study samples has enabled the identification of even more risk variants (Pardiñas et al., 2018). Rare variants, including copy number variants (CNVs) and de novo mutations have also been implicated (Consortium, 2008; Sebat et al., 2009). Additional risk undoubtedly remains to be identified (So et al., 2011), thus motivating additional genetic studies for this multifactorial, polygenic disorder.

While most GWAS have sought to identify additive risk variants, recessively inherited risk factors have also been proposed for SZ (Gottesman and Shields, 1973). It is relatively easy to map recessively inherited monogenic disorders in populations with historically high rates of inbreeding, because relatively large genomic segments flanking disease mutations are likely to be inherited identical by descent among cases. Homozygous by descent (HBD) analysis was used to map mutations successfully for several hundred rare Mendelian diseases (Alkuraya, 2010; Botstein and Risch, 2003). Although HBD analysis has been used for mapping typical monogenic diseases, it could also be useful for diseases with polygenic or multifactorial inheritance. A linkage study of intellectual disability in consanguineous pedigrees, followed by focused sequencing in regions with suggestive linkage, identified 50 novel mutations and confirmed 23 known disease genes (Najmabadi et al., 2011). This study included a heterogeneous set of families, suggesting success can be achieved despite genetic heterogeneity (Lezirevitz et al., 2008). Thanks to densely spaced genetic markers and innovative analyses, it is no longer necessary to access family members for mapping recessive diseases, because HBD genomic segments can be identified among ostensibly

unrelated cases, particularly within inbred populations (Browning and Browning, 2010; Gusev et al., 2009; Purcell et al., 2007). Thus, large inherited homozygous deletions at *PCDH10* and *DIA1* were reported in one study of autism (Morrow et al., 2008). HBD analysis was used for genetically heterogeneous diseases including non-syndromic intellectual disability associated with variable postnatal microcephaly (Mochida et al., 2009), non-syndromic deafness, and patent ductus arteriosus (Mani et al., 2002; Schraders et al., 2010).

These successes motivated us to test HBD mapping for SZ. Reports from the Middle East, Dagestan, Croatia, Cuba, and India show increased risk for SZ and mood disorders among inbred populations, suggesting the presence of DNA variants acting recessively or have dosage effects (Abaskuliev and Skoblo, 1975; Britvic et al.; Bulaeva et al., 2003; Chaleby and Tuma, 1987; Dobrusin et al., 2008; Ewald et al., 2003; Gindilis et al., 1989; Lerer et al., 2003; Mansour et al., 2010; Rudan et al., 2003). In a consanguineous sample from Egypt, SZ was the most commonly reported chronic psychosis (Okasha, 2004). Approximately one-fifth of marriages in rural regions of Egypt in the Nile Delta are between first cousins (Settin and Algelani, 1997). High population inbreeding rate, along with association between consanguinity and SZ risk we identified in a small sample, suggested genomic regions of homozygosity are more prevalent among consanguineous Egyptian SZ patients versus comparable groups (Mansour et al., 2010). Here, we extend prior analyses by investigating a three-fold larger Egyptian case-control sample, characterizing the full sample using a genome-wide SNP array and a subset of the sample by exome sequencing.

2.0 METHODS

2.1 Research Participants

Egyptian patients and controls were recruited and evaluated as described previously (Mansour et al., 2010). Briefly, apparently unrelated cases were ascertained at Mansoura University Hospital (MUH) Psychiatry outpatient clinics based on clinical diagnosis of SZ (DSM-IV), blind to history of consanguinity. Participants were interviewed by psychiatrists using Arabic version of the Schedule for Clinical Assessment in Neuropsychiatry (Wing et al., 2001). Family histories of consanguinity were obtained using the Arabic version of the Family Interview for Genetics Studies (Mansour et al., 2009). Control individuals (persons without reported psychosis) of similar age to the cases and from the same geographical region were recruited without knowledge of their family structure during the same period as the cases. Individuals (or those with first-degree relatives) with a history of psychosis or bipolar 1 disorder were excluded as controls. Consensus diagnoses were established by 2 psychiatrists following a thorough review of available data. Some participants in the present report participated in our earlier study (74 cases, 124 controls) (Mansour et al., 2010).

The study was approved by the Mansoura University Ethics Committee and the University of Pittsburgh Institutional Review Board (IRB). Participants provided written informed consent. Relevant samples and data have been deposited with the NIMH data repository (<https://www.nimhgenetics.org>), following approval by the IRBs at Mansoura University and the University of Pittsburgh, as well as approval by relevant authorities from both universities.

2.2 Genotyping and Identifying Runs of Homozygosity (ROH)

DNA was extracted from venous blood at the University of Pittsburgh or at the Rutgers University Cell and DNA Repository (RUCDR Infinite Biologics, Piscataway, NJ, USA), using the phenol/chloroform method. Participants were genotyped using the Infinium PsychArray-24 v1.1 BeadChip Kit (“PsychArray”; Illumina Inc., San Diego, CA, USA). The PsychArray includes 571,054 genome-wide markers and ~50,000 markers associated with psychiatric disorders. All statistics were performed using R, unless otherwise stated.

2.2.1 Genotyping Quality Control—Genotypes from SZ (n=421) and adult controls (n=301) were called using GenomeStudio 2.0.1 (Illumina Inc.). Quality control was performed using PLINK v1.9. SNPs were removed from analysis if mapped to non-autosomal regions, were monomorphic, had a non-call rate of >0.05, had a minor allele frequency (MAF)<0.05, violated Hardy-Weinberg expectations in controls (p<0.005), or were not represented on the GRCh37/hg19 human genome reference sequence. Individuals were excluded from analyses when genotyping call rate was <95%, chromosome-X discordant homozygosity rate, or were duplicated. After, there remained 280,746 SNPs and n=674 subjects (n=398 SZ cases and n=276 controls).

2.2.2 Inbreeding Coefficient (F)—The inbreeding coefficient (F) quantifies the expected portion of the genome homozygous by descent. For example, for the progeny of full siblings, F=1/4; for the progeny of first cousins, F=1/8. F was estimated on the entire cohort using PLINK and observed regions of homozygosity (ROH) were identified across the genome.

The association between the genetically determined inbreeding values and SZ was determined using logistic regression of SZ on predictors F, age, sex, and the interaction of age by inbreeding. Furthermore, subjects who had an F > 0.05 were identified, conservatively approximating second cousins or closer relationships, the most common form of consanguineous relationships in this cohort (henceforth referred to as “consanguineous”).

2.2.3 The Effect of Inbreeding on Hardy-Weinberg Expectations—For a SNP with minor allele frequency p and major allele frequency $q = 1-p$ in a population, the Hardy-Weinberg principle states the expected genotype frequencies are p^2 (minor allele homozygote), q^2 (major allele homozygote), and $2pq$ (heterozygote). These genotype frequencies remain stable across generations in the absence of specific perturbation factors. Non-random mating of an inbred population, however, is one such factor. Deviation from Hardy-Weinberg expectations (HWE) occur due to increased homozygosity of consanguineous individuals, which increases the observed frequencies of the homozygous genotypes in the population relative to HWE expectation (e.g., Table 14.2(Falconer, 1981)).

Population genetic studies routinely remove SNPs that fail HWE because it is likely genotyping errors cause these deviations. To evaluate how this common quality control procedure would affect our results, we investigated the impact of inbreeding for SNPs deviating significantly from HWE utilizing three simulation scenarios that were generated using the attributes of this cohort. For each scenario, HWE tests were performed on different subsets of data, defined by the estimated value of F of the subjects in the Epyt data and a

threshold for the maximum F value (maxF, ranging from 0.001 to 0.30). The allele frequencies in the observed Egyptian data were used to simulate genotypes.

Scenario 1 used the estimated F for each individual from the Egyptian sample (for subjects with an estimated $F < 0$, F was set to zero). Scenario 2 was designed to show the effect of sample size on the number of SNPs significantly departing from HWE by keeping the fraction of consanguineous individuals (12.6%) constant as sample size increased. The individuals considered consanguineous were assigned $F=0.025$, 0.05 or 0.10, while the remaining samples (87.4%) had an assigned $F=0$. Each scenario was simulated 100 times.

2.2.4 Mapping Regions of Homozygosity (ROH)—SNPs in regions inherited identical-by-descent are homozygous. ROH regions vary in size by locus and individual, although consanguineous individuals are expected to harbor more ROH, on average, than outbred individuals (Mansour et al., 2010). ROH were identified across autosomes using a multi-step process. SNPs passing quality control (280,746) were imputed up to 47,072,716 SNPs on the 1000G reference panel (3.v5) using Michigan Imputation Server (Das et al., 2016). The imputed genotypes were subsequently reduced to 4,914,617 bi-allelic SNPs, following selection for: i) imputation $R^2 > 0.81$; ii) MAF > 0.05 . To avoid redundancy in genotypes, the remaining SNPs were pruned in PLINK with the setting 50 kilobase (kb) window, five SNP sliding window, and maximum linkage disequilibrium (LD) $r^2 < 0.9025$. This resulted in 1,091,357 SNPs available to calculate ROH.

ROH were determined using the “--homozyg” command (PLINK), which utilizes a sliding window approach, searching for homozygous sections across the genome. Our ROH criteria were: 1000 kb in length, 100 SNPs, and at least one SNP was present per 50 kb. The fraction of the genome covered by ROH was 2,659 megabase pairs. To identify overlapping ROH in cases and controls, consensus ROH must contain at least five overlapping ROH segments and at least 95% of the alleles matched (using jointly non-missing/homozygous sites).

After mapping ROH, common and shared haplotypes were identified among consanguineous cases ($F = 0.05$). ROH containing haplotypes far more common in SZ than the controls were identified using these criteria: ROH that were at least 500 kb and contained 45 SNPs; the ROH were shared by at least eight cases and no controls or at least 12 cases and one control (unadjusted Fisher’s exact test, “FET”, $p < 0.05$); furthermore, these regions had to have at least half (3) of the cases presenting with the predominant haplotype (excess haplotype sharing was assessed using FET without correction for multiple testing). The flow chart in Figure 1 depicts data analysis methodology.

2.2.5 Concordance Between Self-Reported Consanguinity and Inbreeding Coefficient—The concordance between self-reported consanguinity and F was compared using Cohen’s Kappa statistic (κ), in SZ and controls.

2.3 Whole Exome Sequencing

Whole exome sequencing (WES) was performed on genomic DNA from a subset of cases. We first sought ROH regions, which were in notable excess in cases versus controls,

containing at least 25 SNPs and at least 500 kb using two criteria (unadjusted FET $p < 0.05$): i) ROH observed in 8 cases and zero controls or ii) ROH observed in 12 cases and one control sample; 11 regions identified. We then selected 12 consanguineous individuals diagnosed with SZ and six non-consanguineous individuals diagnosed with SZ whose genomes matched these criteria for at least one of these regions.

WES was performed in two batches at the Broad Institute (Cambridge, MA, USA) using the Illumina HiSeq 2500 and the Illumina HiSeq 4000, generating an average of 53.8 million paired-end reads of 76bp (14 individuals) and 34.5 million paired-end reads of 151bp (four individuals) per library, respectively. Sequence reads were aligned to human reference genome, GRCh37/hg19, using BWA aln (v0.5.9) with the following parameters: `-q5-l32-k2-o1` (Li and Durbin, 2010). On average, 91% and 93% of the targeted bases in the WES had 20X coverage, respectively. Joint variant calling on these libraries was performed by GATK (v3.1-1) (Miller et al., 2010) following best practices (DePristo et al., 2011; Van der Auwera et al., 2013), which yielded 133,300 and 79,897 high-quality variants, respectively, using VCFtools v0.1.11 (Danecek et al., 2011) with the following parameters: `--max-alleles2--max-missing-count2--min-alleles2--min-mean-depth20--minQ50`. Variants were annotated using Variance Effect Predictor (VEP; Ensembl)(McLaren et al., 2016).

2.3.1 Prioritizing Exome Sequencing Variants—In the 12 consanguineous cases with WES data, we sought to characterize deleterious exome variants mapping into ROH of interest. To begin, we extracted WES variants with a mean coverage 20X, followed by annotation using VEP (selecting those in canonical transcripts and predicted as “deleterious”). SNPs assigned with multiple consequences were prioritized using the most severe, in the following order: i) stop-gain, ii) frameshift, iii) splice-donor, iv) splice-acceptor, v) start-loss, vi) stop-loss, vii) missense, and viii) all other categories. Variation categories i-vi were referred as protein truncating variants (PTV) and category vii as missense variants (MIS). Next, PTV or MIS variants were identified in genes, which were predicted to be more intolerant to loss-of-function variation (PTV), using the pLI score (Karczewski et al., 2019). pLI reflects how observed genetic variation, which is found within the exons, compares to that expected based on an evolutionary model and the observed variation in the population. Hence, pLI provides a gene-level score of evolutionary constraint of genes. Finally, for each remaining variant, the most severe type was selected based on the following criteria in this order: i) PTV more severe than MIS; ii) pLI; and iii) consequence. The MIS variants were ordered based on a missense variant constraint metric (“MPC”, where a higher value indicates increased gene constraint)(Samocha et al., 2017) or, if MPC values were not available, PolyPhen-2 scores were used as a substitute (“PPH”; PolyPhen is a prediction tool assessing the possible impact of an amino acid substitution on the structure and function of the resulting protein; higher values indicate more severe consequences)(Adzhubei et al., 2010).

Separately, we identified rare WES variants ($MAF < 0.01$ in gnomAD) mapping to recessively-acting genes ($n=1,183$)(Berg et al., 2013; Blekman et al., 2008), reasoning consanguineous individuals may harbor deleterious variants within haplosufficient loci.

3.0 RESULTS

3.0.1 Demographic Features

There were significantly more males in the SZ than in the control group (69% vs 55%, respectively; $\chi^2=12.1$, $df=1$, $p=0.0005$); the mean age for the cases were younger than the controls (31.4 years, $SD=9.79$ vs 33.0 years, $SD=9.44$, respectively; $t=2.2$, $df=667$, $p=0.027$). Among the cases, the age at onset was not significantly different between the consanguineous and the non-consanguineous subjects (21.7 years, $SD=6.1$ vs 22.2 years, $SD=6.9$, respectively; $t=(-0.49)$, $df=373$, $p=0.62$; Table 1). The prevalence of self-reported consanguinity was greater among cases ($\chi^2=4.9$, $df=1$, $p=0.027$). There was no significant difference between SZ and control subjects regarding rural/urban residence ($p>0.05$; Table 1).

3.0.2 Identification of Regions of Homozygosity (ROH)—To determine the utility of PLINK's method of moments approximation for each individual's inbreeding coefficient, we compared it to another estimator based on the genomic fraction that is ROH. The correlation of these measures was high ($r=0.97$; Supplemental Figure 1). Thus, the moment approximation for F from PLINK analysis was used for the remainder of this report.

3.0.3 Inbreeding Coefficient (F)—There were 128 cases (32%) and 73 controls (26%) considered to be consanguineous; i.e., second cousins or closer, clinical genetic definition ($F>0.0156$)(Bittles, 2001). Regression analysis with case status as the outcome indicated significant associations with the SNP-based estimate for F, age, sex, and a significant interaction effect between age and F (Table 2). In view of the significant age by F interaction, the cases and controls were partitioned by age quartiles and the mean F was determined per quartile; there were proportionally more younger cases ($\chi^2=2.86$, $df=3$, $p=0.0049$; Supplemental Table 1a). Furthermore, the mean F per age quartile was similar for controls but fluctuated in the cases. Specifically, the youngest cases (under 25 years) had lower mean F, and the eldest cases (over 39 years of age) had a mean F comparable to the controls. In addition, the mean F was computed per age quartile in males and females; however, there was no difference in the distribution of individuals per age quartile ($\chi^2=1.2$, $df=3$, $p=0.75$; Supplemental Table 1b).

3.0.4 Concordance Between Self-Reported Consanguinity and the Inbreeding Coefficient—The concordance between the self-reported measure of consanguinity (yes/no) and the genetically determined inbreeding ($F > 0.05$) was compared in SZ and controls, separately. Among cases, there was 85% agreement between the self-reported consanguinity and estimated F ($z=7.46$, $\kappa=0.54$, 95% $CI=0.43-0.65$, $p=4.46 \times 10^{-14}$). Among controls there was an 88% agreement between the self-report and the estimated F ($z=4.94$, $\kappa=0.52$, 95% $CI=0.36-0.67$, $p=3.82 \times 10^{-07}$), indicating no significant case-control differences for self-reported consanguinity.

3.0.5 The Effect of Inbreeding on Hardy-Weinberg Expectations—The effect of inbreeding on genotype frequencies deviating from Hardy-Weinberg expectations was explored in this cohort. While it is expected that some SNPs will depart from HWE due to

chance or to genotyping error, loci will also depart from HWE due to inbreeding. Larger sample sizes would be better powered to detect such an effect; smaller sample sizes like ours can detect only larger deviations. This is a challenging problem because it is not possible to know exactly which SNPs are departing HWE due to inbreeding, genotyping error, random chance, or some combination. To explore this challenging problem, we performed three simulation scenarios using parameters of the Egyptian cohort. Genotype frequencies were simulated for each scenario and SNPs were tested for departure from HWE. SNPs with HWE $p < 0.0005$ were considered significant.

Scenario 1 was designed to evaluate random error of observed genotype frequencies by simulating data using the estimated values of F per Egyptian subject and the estimated MAF for the population. If deviations from HWE observed in the Egyptian sample were only due to inbreeding and random sampling, the number of SNPs departing HWE should roughly match what is observed in the simulation. Instead, while the general pattern mirrors the results obtained in the actual data, the counts from Scenario 1 are uniformly lower than expected, presumably due to genotyping error (notably, if we were to simulate data under a no-inbreeding scenario, the number of SNPs departing HWE follows that expected by chance [data not shown]; Supplemental Figure 2a). Scenario 2 highlights the statistical phenomenon of power for detecting SNPs departing HWE due to inbreeding. This scenario tested increasing number of subjects where the proportion of consanguineous individuals was kept constant (12.6%, which was derived from the number of consanguineous individuals we detected in this particular cohort who had $F > 0.05$). Clearly, when the sample size increases, the number of SNPs detected as departing HWE also increases, while the proportion of consanguineous individuals remained constant (Supplemental Figure 2b).

By contrasting results from these scenarios to the actual data (Supplemental Figure 2), we conclude that SNPs depart from HWE due to all of the mechanisms described previously; however, it is not possible to know exactly which SNP(s) are departing HWE due to inbreeding or some other reason. Of note, we did not find SNPs departing HWE that mapped into an ROH listed in Tables 3 and 4.

3.0.6 ROH Analysis in Consanguineous Subjects—There were 29 controls and 56 SZ cases with SNP-based $F > 0.05$ and thus were considered “consanguineous” using our definition. Among these individuals, 847 consensus ROH loci were identified, of which 53% ($n=448$) spanned least 500 kb and were covered by at least 25 SNPs. Furthermore, 66% ($n=561$) of the consensus ROH spanned at least 100 kb and were covered by 10 SNPs. Eleven ROH mapping to chromosomes 1, 3, 6, 11, and 14, were identified as having an excess of ROH in cases versus controls, by our criteria (Table 3). We next investigated whether the 143 autosomal SZ risk loci reported in a GWAS by Pardiñas and colleagues localized to the ROH (Pardiñas et al., 2018). One SNP (rs61937595) mapped into an ROH on chromosome 12. To determine significance, permutation analysis was conducted where regions were identified, randomly and with replacement, in the genome with the same size characteristics as the 11 ROH regions in Table 3. The number of times a region contained one of the 143 SZ risk loci was tabulated. The procedure was repeated 1000 times and an empirical p -value was computed for the simulation data. These ROH loci did not show an excess enrichment for the 143 SZ GWAS loci ($p=0.584$). The full list of GWAS-significant

findings performed in prior mental health studies that map into these 11 ROH are provided in Supplemental Table 2 (Davies et al., 2015; Davies et al., 2018; Goes et al., 2015; Hill et al., 2019; Ikeda et al., 2018; Jones et al., 2019; Lam et al., 2017; Lee et al., 2018; Li et al., 2017; Stahl et al., 2019). GWAS findings were obtained from the National Human Genome Research Institute-European Bioinformatics Institute (NHGRI-EBI) catalog of published GWAS (date accessed 2019-09-17; www.ebi.ac.uk/gwas/).

3.0.7 ROH Haplotype Analysis—We further examined ROH regions (Table 3) with at least half of the cases ($n = 3$) shared a haplotype. Shared haplotypes were identified if they were ≥ 50 kb and included ≥ 10 SNPs. Two loci, within 500 kb of one another on 1p22.2, were 170 kb and 191 kb, contained Protein Kinase-N2 (*PKN2*) and a cluster of Guanylate Binding Protein genes (*GBP1*, *GBP2*, *GBP4*, and *GBP7*). A 76 kb region on 1q21.1 contained Flavin-Containing Monooxygenase 5 (*FMO5*) and Chromodomain Helicase DNA-Binding Protein 1-Like (*CHD1L*). Two loci on 14q12, within 1000 kb of each other, were 220 kb and 181 kb, containing Protein Kinase D1 (*PRKDI*), G2/M-Phase Specific E3-Ubiquitin Protein Ligase (*G2E3*) and Sec1 Family-Domain Containing (*SCFD1*). On chromosome 12p13.31, there was a 122 kb region containing Activation Induced Cytidine Deaminase (*AICDA*) and Microfibril-Associated Protein-5 (*MFAP5*; Table 4).

3.1 Whole Exome Sequencing (WES)

3.1.1 Variant Calls, Annotation, and Prioritization—We sequenced 12 consanguineous individuals diagnosed with SZ, prioritizing ROH regions (see Methods), identifying 119,295 unique variants (present in ≥ 1 individual). Summary metrics of variants generated by PLINK/SEQ (<https://atgu.mgh.harvard.edu/plinkseq/>) are provided in Supplemental Tables 3a and 3b. The versions of databases used by VEP for annotations are provided in Supplemental Table 3c. There were 118,026 variants present in VEP, mapping to 28,033 canonical gene transcripts. There were 25,246 PTV/MIS variants (9,787 genes) annotated with pLI scores and subsequently reduced to one annotation per variant (Supplemental Table 4a). The PTV/MIS variants were next delineated, using scores for gene constraint (pLI), missense deleteriousness (MPC), or impact of altered amino acid on the resultant protein (PPH). There were 18 PTV mapped into highly constrained genes (pLI>0.995), 118 predicted deleterious MIS variants (MPC>2), and 73 MIS variants predicted as protein-deleterious (PPH>0.9; Supplemental Table 4b).

3.1.2 Exome Variants in ROH loci—Exome variants mapped into ROH established in the consanguineous cohort were identified. Homozygous alternative genotypes localizing to loci within ROH were identified in 12 consanguineous cases. There were 33 unique variants identified with homozygous alternative allele genotypes. Thirty variants were MPC1 and three variants were MPC2 missense variants. The three MPC2 variants map to *OSTM1* and *SESNI* (described above), along with *PVRL4* (the protein product contains two immunoglobulin-like C2-type domains and one immunoglobulin-like V-type domain). The *PVRL4* variant has a global allele frequency of 0.08 (gnomeAD) and is tolerant to mutations (pLI<0.5). Autosomal recessive ectodermal dysplasia-syndactyly syndrome type-1 can result from gene mutations in *PVRL4*; however, this variant was not reported as pathogenic in the ClinVar database (Supplemental Table 5).

Next, we identified exome variants localized to genes implicated in autosomal recessive conditions. From 1,183 curated recessive gene list (Berg et al., 2013; Blekman et al., 2008), we observed 2,265 variants (760 genes) in 12 subjects. Of these, we focused on rare (MAF<0.01), deleterious (PTV or MPC >2) variants. One subject identified with a homozygous genotype for a variant in *ASPM* (abnormal spindle homolog, microcephaly-associated). Primary hereditary microcephaly is associated with homozygous mutations in *ASPM*; however, microcephaly was not reported for this individual.

Furthermore, we sought any variant in the consanguineous (n=12) or the non-consanguineous (n=6) cases with WES data, likely to be deleterious (PTV/MPC>2). We detected a mean of 6.0 (SD=3.7) and 5.9 (SD=3.1) variants homozygous for the alternate allele in the non-consanguineous and consanguineous cases, respectively (W=37.5, p=0.46; not significant, Wilcoxon rank sum test); however, the number of heterozygous deleterious variants were significantly lower in consanguineous cases (mean=22.8, SD=3.9) than non-consanguineous cases (mean=37.7, SD=14.6; W=60, p=0.014).

4.0 DISCUSSION

We find inbreeding is associated with risk for SZ in Mansoura, Egypt, using self-reported consanguinity, and SNP-based estimates of inbreeding. Furthermore, we detect a significant interaction of the coefficient of inbreeding (F) with age. These results are consistent with our prior analyses that used a subset of these subjects and short tandem repeat polymorphisms (STRPs) to determine F (Mansour et al., 2010). Other groups report associations between autozygosity (alleles/regions HBD by consanguineous matings), and an increased risk for SZ, autism, and depression/suicidal behavior (Gamsiz et al., 2013; Keller et al., 2012; Maguire et al., 2018; Melhem et al., 2017; Wang et al., 2010). Previous work on either inbred or outbred cohorts report conflicting results. A whole-genome heterozygosity association study in 178 unrelated Caucasian SZ and 144 controls indicated ROH on chromosomes 1q23.3, 2q31.1, 17q21.31, and 18q12.3 were more common in cases (Lencz et al., 2007). Another study, of two independent samples of Ashkenazi Jewish and Japanese ancestry, indicated consistent association between SZ risk and homozygous fragments in the HLA region on chromosome six (Mukherjee et al., 2014). An increased prevalence of SZ subtypes were reported in genetically-isolated communities in Daghestan, Russia, where investigators demonstrated significant linkage on chromosomes 22q11 (LOD=4.4) and 17p11-p12 (LOD=3.7) (Bulayeva et al., 2007). Conversely, a recent study reported on 39,830 Caucasian individuals, selected without regard to self-reported consanguinity, did not identify excess ROH in these regions (Johnson et al., 2016). The discordant results from prior studies suggest well-powered analyses need conducted in inbred populations.

In our sample, the relationship between inbreeding and SZ risk is age-dependent; to our knowledge, this relationship has not been reported. In our previous study using a subset of the current study's subjects, the mean age was balanced (Mansour et al., 2010). Nevertheless, we examined the mean age of individuals from the earlier study versus independent individuals from the current study, and found the subjects from the current study were significantly older (earlier study mean age=27 years, SD=7 and current study mean age=32 years, SD=9; t=(-8.1), df=442, p<0.0001). In all samples, age quartiles were

examined in cases and controls separately. The controls have a consistent level of inbreeding across age quartiles; however, younger controls (< 25 years) exhibit the lowest mean F (Supplemental Table 1), which may reflect falling rates of consanguinity in Egypt in recent years, especially in urban areas (Shawky et al., 2011). Cases over age 40 have a mean inbreeding coefficient similar to controls, which was unexpected. The apparently low levels of inbreeding could reflect inadequate sample size, higher mortality in the children of consanguineous families, or it could be explained by increased mortality of consanguineous individuals diagnosed with SZ. Public health programs for psychiatric patients were instituted in Egypt only in the 1990s (Okasha, 2004), and these programs likely benefit younger cases more than older cases. Additionally, there was a hepatitis C viral epidemic in Egypt during the 1950s-1980s (Elgharably et al., 2017); conceivably, older, consanguineous individuals diagnosed with both SZ and hepatitis C could have even higher mortality.

Consanguinity is present in a major proportion of the world's population. It is important to investigate whether the risk of polygenic diseases like SZ, conferred by inbreeding, could be attributed to inherited DNA variation. We therefore tested the feasibility of HBD analyses in a case-control sample from Egypt, reasoning family-based linkage analyses might not enable sufficient power to definitively identify risk-conferring loci. In our sample, inbreeding was determined using over one-million informative genetic markers; the estimate of inbreeding levels of controls is consistent with prior estimates of self-reported average consanguinity in the Nile Delta region (Mansour et al., 2010; Settin and Algelani, 1997). Among the associated risk loci, we find chromosome 1q23.3 overrepresented in the cases (Table 3). We also identified one consanguineous patient harboring a homozygous variant in *ASPM* (abnormal spindle homolog, microcephaly-associated). Variations in *ASPM* are a common cause of autosomal recessive primary microcephaly (Nicholas et al., 2009). *ASPM* exhibits significant allelic heterogeneity; there are 209 pathogenic variations in HGMD professional (2019.1). Among deleterious, exome variants, we found less heterozygous variants in the consanguineous subjects, as is expected for individuals who have a higher degree of consanguinity.

Some limitations of the study should be noted. Some ROH could be due to structural variations masquerading as homozygous genotypes. However, there was a 97% correlation between the SNP-based estimate of F and the ROH fraction (Supplemental Figure 1); thus, we do not expect this to be a substantial factor. Furthermore, in view of the relatively young age of the controls, not all individuals passed the age of risk for SZ (Ochoa et al., 2012). Still, not more than three controls are likely to be misclassified thus; moreover, misclassification would underestimate the observed associations with consanguinity. The current study utilized a subset of earlier samples (74 cases and 124 controls) along with newly recruited subjects, the present results do not represent a replication of our prior report; similarly, independent replication of the F x age interaction need to be sought.

In conclusion, we report consanguinity is an age-dependent risk factor for SZ in the Northern Delta region of Egypt. HBD analysis using larger samples may enable fine mapping for chromosomal regions contributing to the observed risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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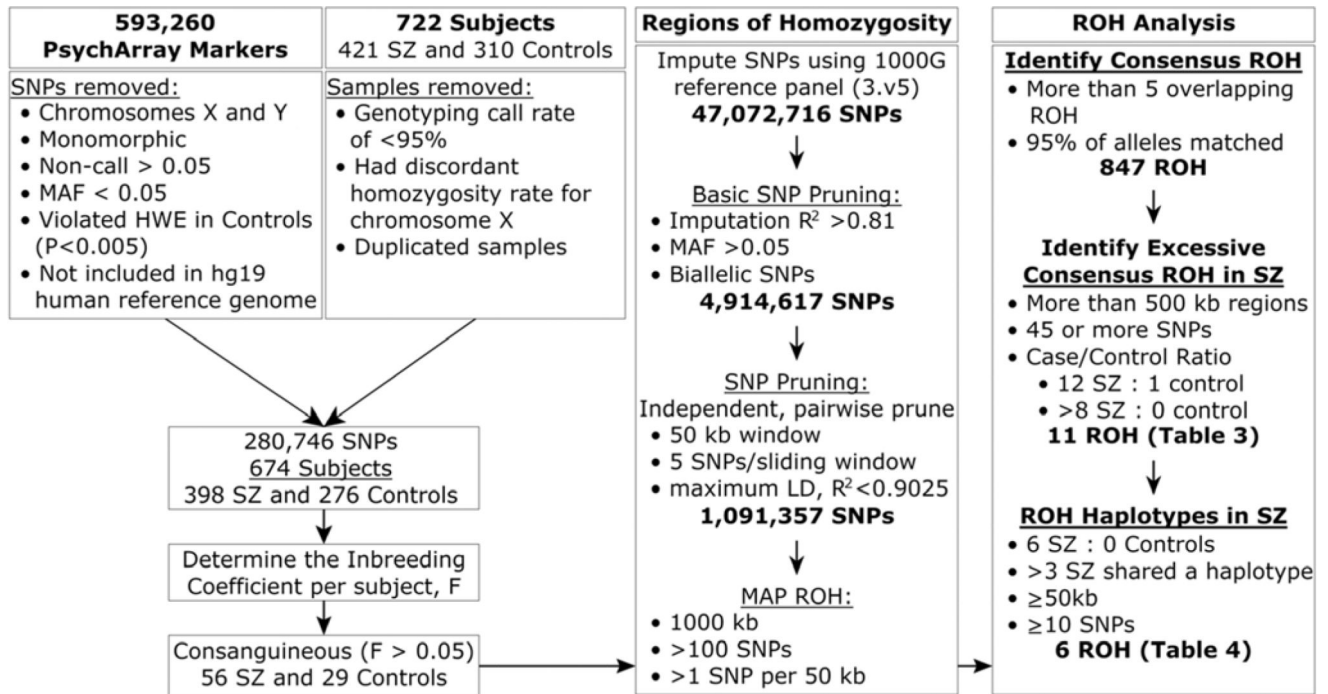


Figure 1. Procedures used to identify significant regions of homozygosity in consanguineous individuals.

TABLE 1.

Demographic features of cases and controls

	Schizophrenia		Controls		<i>t</i>	df	p
	n	mean (SD)	n	mean (SD)			
Age (years)	393	31.4 (9.8)	276	33.0 (9.4)	2.2	667	0.027
Age at onset (years) ²							
consanguineous (F>0.05)	53	21.7 (6.1)	-	-	-0.49	373	0.62
not consanguineous (F<0.05)	323	22.2 (6.9)	-	-			
<hr/>							
	Schizophrenia		Controls		Chi-square ³	df	p
	n	percent	n	percent			
Sex Ratio (Male)	268	68.70%	153	55.40%	12.1	1	0.0005
Geographic Region (Urban)	115	30.40%	68	24.70%	2.7	1	0.1
Self-reported Consanguinity (Yes)	101	36.70%	50	18.20%	4.9	1	0.027

¹Two-sided Student's t test.

²Count for age at onset does not equal total cases, due to missing data in 23 individuals (Three consanguineous cases; 20 non-consanguineous cases).

³Chi-square test corrected for sex, residential setting, and self-reported familial consanguinity.

F=inbreeding coefficient; n=subject count; SD=standard deviation; df=degrees of freedom; *t*=test statistic; p=probability.

TABLE 2.

Age-dependent association of schizophrenia with inbreeding.

	β (SE)	OR	95% CI (OR)	z	p
Inbreeding Coefficient (F)	32.39 (11.44)	1.17×10^{14}	2.13×10^4 – 6.44×10^{23}	2.83	0.0046
Age (Years)	-0.0067 (0.009)	0.99	0.97–1.01	-0.76	0.58
Sex	-0.62 (0.116)	0.54	0.39–0.74	-3.78	0.00016
Inbreeding Coefficient \times Age	-0.87 (0.34)	0.42	0.22–0.80	-2.61	0.0091

β =beta regression coefficient, SE=standard error, OR=odds ratio, 95% CI (OR)=the 95% confidence interval of the odds ratio, z=test statistic, p=probability.

TABLE 3.

Regions of homozygosity over-represented in schizophrenia cases.

Region	Chr	Location (base position)		Chromosome Band	Number of samples		Length (kb)	Total SNPs	p	Significant GWAS gene, phenotype ¹
		Start	End		SZ cases	Controls				
1	1	108,860,573	110,323,150	1p13.3	12	1	13	1462.58	<0.05	<i>SYPL2</i> , cognitive ability <i>GPR61</i> , chronotype
2	14	32,438,841	32,964,338	14q12	10	0	10	525.50	<0.05	<i>AKAP6</i> , SZ, BP, cognition
3	1	89,045,338	89,735,314	1p22.2	9	0	9	689.98	<0.05	none
4	1	161,957,719	163,494,784	1q23.3	9	0	9	1537.07	<0.05	none
5	1	165,625,023	166,337,053	1q24.1	9	0	9	712.03	<0.05	<i>FAM78B</i> , cognition
6	14	29,895,001	31,237,170	14q12	9	0	9	1342.17	<0.05	none
7	1	154,791,128	156,378,425	1q21.3-q22	8	0	8	1587.30	<0.05	<i>KCNN3-PMVK</i> , SZ, BP <i>ZBTB7B</i> , SZ <i>RIT1</i> , SZ
8	1	160,513,183	161,361,979	1q23.3	8	0	8	848.80	<0.05	none
9	3	190,779,327	191,862,817	3q28	8	0	8	1083.49	<0.05	<i>AC073365.1</i> , SZ
10	6	108,323,759	109,351,125	6q21	8	0	8	1027.37	<0.05	<i>AFGL</i> , cognition <i>FOXO3</i> , SZ, cognition, neuroticism
11	11	22,141,171	22,647,550	11p14.3	8	0	8	506.38	<0.05	none

Regions of homozygosity were identified that were over-represented in consanguineous schizophrenia cases. SZ=schizophrenia; BP=bipolar disorder; Chr=chromosome; kb=kilobases; p=equivalent to an unadjusted p-value using Fisher's exact test. Genomic locations based on GRCh37/hg19.

¹ Genome-wide association studies (GWAS) with significantly associated SNPs identified in prior studies per ROH region. Detailed information for these GWAS is provided in Supplemental Table 2.

Table 4. Regions of homozygosity having shared haplotypes that are over-represented in schizophrenia cases.

Chromosome	Position	Band	Size (kilobases)	SNP count	Case :		Major Haplotype		Gene
					Control	N	Control	Symbol	
1	89,116,300–89,286,673	1p22.2	170.4	11	9:0	3	7:0	<i>PRKN</i> ^a	Protein Kinase N2
1	89,530,860–89,721,862	1p22.2	191	14	9:0	5	5:0	<i>GBP1^b, GBP2, GBP4, GBP7</i>	Guanylate binding proteins
14	30,070,659–30,290,431	14q12	219.8	19	9:0	3	7:0	<i>PRKD1^c</i>	Protein kinase D1
14	30,991,559–31,172,582	14q12	181	14	9:0	4	6:0	<i>G2E3 SCFD1^d</i>	G2/M Phase-specific E3 Ubiquitin Ligase SEC1 Family Domain Containing 1
1	146,660,395–146,736,137	1q21.1	75.7	11	7:0	4	4:0	<i>FMO5^e CHD1L^f</i>	Flavin Containing Monooxygenase 5 Chromodomain Helicase DNA-binding Protein 1 Like
12	8,698,471–8,820,124	12p13.31	121.7	13	6:0	4	3:0	<i>AICDA MFAP5</i>	Activation-induced Cytidine Deaminase Microfibrillar-associated Protein 5

^aROH in *PRKN2* (NM_006256) contained 16 of 22 exons.

^bROH in *GPBI* (NM_002053) contained one of 11 exons.

^cROH in *PRKD1* (NM_002742) contained 13 of 18 exons.

^dROH in *SCFD1* (NM_016106) contained 16 of 25 exons.

^eROH in *FMO5* (NM_001461) contained 8 of 9 exons.

^fROH in *CHD1L* (NM_004284) contained 7 of 23 exons.