Association between Autozygosity and Major Depression: Stratification due to Religious Assortment

Abdel Abdellaoui[1,2], **Jouke-Jan Hottenga**[1], **Xiangjun Xiao**[3], **Paul Scheet**[3], **Erik A. Ehli**[4], **Gareth E. Davies**[4], **James J. Hudziak**[5], **Dirk J.A. Smit**[1], **Meike Bartels**[1], **Gonneke Willemsen**[1], **Andrew Brooks**[6], **Patrick F. Sullivan**[7], **Johannes H. Smit**[8], **Eco J. de Geus**[1,2,8], **Brenda W.J.H. Penninx**[2,8], and **Dorret I. Boomsma**[1,2,8]

[1]Department of Biological Psychology, VU University Amsterdam, Amsterdam, The Netherlands [2]Neuroscience Campus Amsterdam, Amsterdam, The Netherlands [3]Department of Epidemiology, University of Texas M.D. Anderson Cancer Center, Houston, TX, USA [4]Avera Institute for Human Genetics, Avera Behavioral Health Center, Sioux Falls, SD, USA [5]University of Vermont, College of Medicine, Burlington, VT, USA [6]Department of Genetics, Rutgers, The State University of New Jersey, Piscataway, New Jersey, USA [7]Department of Genetics, University of North Carolina, Chapel Hill, North Carolina, USA [8]EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, Netherlands [9]Department of Psychiatry, VU University Medical Center, Amsterdam, Netherlands

Abstract

The effects of inbreeding on the health of offspring can be studied by measuring genome-wide autozygosity as the proportion of the genome in runs of homozygosity (F_{roh}) and relate F_{roh} to outcomes such as psychiatric phenotypes. To successfully conduct these studies, the main patterns of variation for genome-wide autozygosity between and within populations should be well understood and accounted for. Within population variation was investigated in the Dutch population by comparing autozygosity between religious and non-religious groups. The Netherlands have a history of societal segregation and assortment based on religious affiliation, which may have increased parental relatedness within religious groups. Religion has been associated with several psychiatric phenotypes, such as major depressive disorder (MDD). We investigated whether there is an association between autozygosity and MDD, and the extent to which this association can be explained by religious affiliation. All F_{roh} analyses included adjustment for ancestry-informative principal components (PCs) and geographic factors.

Religious affiliation was significantly associated with autozygosity, showing that F_{roh} has the ability to capture within population differences that are not captured by ancestry-informative PCs or geographic factors. The non-religious group had significantly lower F_{roh} values and significantly more MDD cases, leading to a nominally significant negative association between autozygosity and depression. After accounting for religious affiliation, MDD was not associated with F_{roh}, indicating that the relation between MDD and inbreeding was due to stratification.

This study shows how past religious assortment and recent secularization can have genetic consequences in a relatively small country. This warrants accounting for the historical social context and its effects on genetic variation in association studies on psychiatric and other related traits.

Please address for correspondence: Abdel Abdellaoui, VU University Amsterdam, Department of Biological Psychology, Netherlands Twin Register, Van der Boechorststraat 1, 1081 BT, Amsterdam, The Netherlands, phone: 31-20-5986316; fax 31-20-5988832; a.abdellaoui@vu.nl.

Keywords

autozygosity; runs of homozygosity; major depressive disorder; religion; population stratification; assortative mating

Introduction

There is an increasing interest in the association between runs of homozygosity (ROHs) and human disease. Alleles in long ROHs are likely to be identical-by-descent (i.e., autozygous) (Keller et al. 2011). ROHs can have harmful effects through deleterious recessive alleles that combine when related individuals mate and have offspring. Deleterious recessive alleles are usually rare because selection is unable to completely purge such mutations, since they are not damaging to heterozygote carriers; a process known as mutation-selection balance (Charlesworth and Willis 2009). The closer or more recent inbreeding is, the longer the ROHs will be, increasing the chances of combining deleterious recessive alleles in offspring (Szpiech et al. 2013). Studies on the association between autozygosity and psychiatric traits may help provide insights into both the evolutionary history and the genetic etiology of complex psychiatric disorders. Strong selection against deleterious variants should results in a bias toward both rarity and recessivity of causal variants, which in turn should increase damaging effects of inbreeding (DeRose and Roff 1999). Recent reports on autozygosity as a schizophrenia risk factor suggest that purifying (negative) selection caused dominant schizophrenia risk alleles to disappear at a faster rate over evolutionary time than recessive risk alleles (Keller et al. 2012; Lencz et al. 2007). This raises the question to which extent there may be an association between autozygosity and other psychiatric disorders. One of the goals of this study was to evaluate the relation between autozygosity and major depressive disorder (MDD), the most prevalent psychiatric disorder in adults (Kessler et al. 2005; Kessler et al. 1994). In contrast to schizophrenia, MDD is less heritable (31–42%) (Lubke et al. 2012; Sullivan et al. 2000), and has had limited success in identifying reliably associated genetic variants (Ripke et al. 2012; Sullivan et al. 2012).

Before studies on autozygosity can be successfully conducted, the main patterns of variation for autozygosity between and within populations should be well understood and accounted for. Inbreeding is a matter of degree (Keller et al. 2011), and the total length of ROHs reflects relatively recent inbreeding patterns and varies between worldwide populations (McQuillan et al. 2008; Pemberton et al. 2012). This variation is mainly due to a combination of geographic factors leading to isolation and/or bottlenecks and differences in the prevalence of consanguineous matings. Variation within populations may be driven by assortment on cultural factors and attitudes, such as religious beliefs or political preferences (Alford et al. 2011; Watson et al. 2004). Such a positive assortative mating strategy has been hypothesized to improve inclusive fitness by increasing genetic relatedness within groups, which can facilitate communication and altruism (Thiessen and Gregg 1980). Religious affiliation has been reported to facilitate genetic stratification that is detectable by principal component analysis (PCA) on genome-wide single nucleotide polymorphisms (SNPs) (Haber et al. 2013). We investigate whether religious assort may have led to increased parental relatedness by testing for systematic autozygosity differences within the Netherlands, which is a relatively small country with a high population density. If autozygosity differences exist between religious subgroups, this may affect the outcomes of autozygosity studies on traits associated with religion.

This study focuses on variation in autozygosity within a country that had a long and relatively strict segregation in society based on religious affiliation, with religious groups for example having their own political parties, unions, schools, and universities (Knippenberg

1992), inducing isolation, decreasing mating options, and potentially increasing parental relatedness. Studies on Dutch marriage records of the 19th and early 20th century show considerable religious assortment (Beekink et al. 1998; Kok 1998; Polman 1951). Religion is also associated with geography. The Netherlands can be roughly divided into three regions: (1) below the major rivers, where the population is mainly Catholic; (2) the middle-band, which is largely Orthodox-Protestant; (3) the Northern part, which consists largely of more liberal Protestants (Donk et al. 2006; Knippenberg 1992). These three regions also show subtle genetic differences previously detected by PCA on genome-wide SNPs (Abdellaoui et al. 2013). The geographic distribution of religious groups has been relatively stable for the last four centuries (Knippenberg 1992), and has only started to change in the second half of the last century, due to increasing secularization and the influx of immigrants from other parts of the world (Knippenberg 1992).

Religion shows significant associations with several traits, such as personality and psychiatric disorders (Boomsma et al. 1999; King et al. 2012; Koenig 2009; Miller et al. 2012; Willemsen and Boomsma 2007). Religion and psychiatry display complex associations, with studies showing both stress-buffering as well as depression-evoking effects of religious involvement (Braam et al. 2004; Dein et al. 2010; King et al. 2012; Koenig 2009; Miller et al. 2012; Seybold and Hill 2001). However, the majority of the many studies on the relationship between religiosity and psychiatric disease (in different settings, ethnic backgrounds, age groups, and locations) reports a protective influence of religion on psychiatric disorders (Koenig 2009).

Here, we investigated associations between autozygosity as quantified by ROHs based on genome-wide measured SNPs, major depressive disorder as assessed by DSM4 diagnoses, and self-reported religion to answer whether differences between religious and non-religious groups can lead to a false positive association between ROHs and MDD. Ancestry-informative PCs and living in an urban versus rural environment were accounted for, since ancestry-informative PCs can correlate with homozygosity (Abdellaoui et al. 2013), and larger cities show a higher prevalence for psychiatric disorders (Marcelis et al. 1998; Sundquist et al. 2004; Van Os 2004) and more intermediate values of ancestry-informative PCs due to incoming migration flows (Abdellaoui et al. 2013).

Methods

Participants

Genotyped subjects took part in the Netherlands Twin Register (NTR) biobank project (Boomsma et al. 2006; Willemsen et al. 2010) and in the Netherlands Study of Depression and Anxiety (NESDA) (Penninx et al. 2008). The NTR subjects (N=6,685; 2,678 males and 4,007 females) were randomly sampled from twin families across the Netherlands. The NESDA subjects (N=2,380; 806 males, and 1,574 females) were recruited from the general population, primary care and mental healthcare organizations. Analyses were done on unrelated individuals only. Unrelated individuals were chosen using GCTA (Yang et al. 2011), by excluding one of each pair of individuals with an estimated genetic relationship of >0.025 (i.e., more related than third or fourth cousin). There were 4,022 genotyped unrelated individuals with religious affiliation and current living address, and 2,916 genotyped unrelated individuals with religious affiliation, current living address, and MDD case/hyper-control status. Ancestry-informative PCs were computed using 5,166 unrelated subjects and were projected onto the rest of the subjects. In addition to the genotyped subjects, data on religious affiliation were available for 25,450 subjects from the total group of NTR participants. These data were used to display the geographic distribution of religious affiliations and included 3,042 spouse pairs used to test for non-random assortment between spouses on religious affiliation.

Only individuals with Dutch ancestry were included. Individuals with a non-Dutch ancestry were identified by projecting PCs from 1000 Genomes populations on the dataset, and with additional help of the birth country of the parents. This procedure is described in more detail elsewhere (Abdellaoui et al. 2013).

NTR and NESDA studies were approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Centre, Amsterdam, an Institutional Review Board certified by the US Office of Human Research Protections (IRB number IRB-2991 under Federal-wide Assurance-3703; IRB/institute codes, NTR 03-180, NESDA 03-183). All subjects provided written informed consent.

Phenotypes

Subjects were included as MDD cases when they had a lifetime diagnosis of DSM-IV MDD as determined by the Composite International Diagnostic Interview (CIDI, version 2.1) (WHO 1997). The control group consisted of screened hypercontrols who never scored high on a general factor score for anxious depression and never reported a history of MDD in any survey or at the blood sampling visit (either as a complaint for which treatment was sought, reported medication use, or via the CIDI). Further details on the collection and classification of MDD cases and controls are described elsewhere (Boomsma et al. 2008; Wray et al. 2010). There was a total of 1834 MDD cases and 2131 controls.

Religious affiliation was measured in a longitudinal questionnaire studies in the NTR and NESDA with the question "What is your religion?". Three answer categories that overlapped between NTR and NESDA were constructed: (1) none (N=2,114), (2) Roman Catholic (N=1,069), and (3) Protestant (N=839).

For the current living addresses, the postal codes were translated into geographic coordinates (longitude and latitude) for each participant using the open source 6PP database (Broek 2012) and used to create Figure 2. Population sizes of the cities were obtained from the Central Bureau of Statistics (CBS 2012) and recoded into a dichotomous variable reflecting whether a subject lives in a city with >100k inhabitants.

Genotyping, quality control (QC), and principal component analysis (PCA)

Methods for blood and buccal swab collection, genomic DNA extraction, and genotyping have been described previously (Abdellaoui et al. 2013; Scheet et al. 2012). Genotyping was performed on the Affymetrix Human Genome-Wide SNP 6.0 Array (containing ~906,600 SNP probes) according to the manufacturer's protocol.

Only autosomal SNPs were analyzed. SNPs were removed if they: (1) had probes that mapped badly against NCBI Build 37/UCSC hg19 (i.e., to a "random" region, to >1 region, or to 0 regions); (2) showed a minor allele frequency (MAF) smaller than 5%; (3) had a missing rate greater than 5%; (4) deviated from Hardy-Weinberg equilibrium (HWE) with a p-value smaller than 0.001. After QC, 498,592 SNPs remained.

Individuals were removed if they: (1) showed a Contrast QC < .4 (CQC, a quality metric from Affymetrix representing how well allele intensities separate into clusters); (2) fell outside of the main cluster of a PC reflecting a batch effect (Abdellaoui et al. 2013); (3) had a missing rate greater than 5%; (4) had excess genome-wide heterozygosity / inbreeding levels (F, as calculated in Plink on an LD-pruned set, must be greater than −0.10 and smaller than 0.10); (5) had non-European/non-Dutch ancestry (Abdellaoui et al. 2013); (6) had genotypes with inconsistencies regarding reported sex or reported relatedness within families.

PCA was conducted using Eigenstrat (Price et al. 2006). The first three PCs correlated significantly with geography: PC1=North-South PC, PC2=East-West PC, PC3=middle-band PC. The procedure for the PCA and the three ancestry-informative PCs are described in detail elsewhere (Abdellaoui et al. 2013).

ROH calling

ROHs were called in Plink (Purcell et al. 2007), which was found to optimally predict autozygous stretches in a recent study comparing several software packages designed for this goal (Howrigan et al. 2011). Howrigan et al (2011) used simulated data based on the Affymetrix 6.0 chip, making their density of SNPs in linkage disequilibrium (LD) close to the data analyzed in our study. We followed their recommendations in calling ROHs: (1) SNPs were pruned for LD (window size = 50, number of SNPs to shift after each step = 5, based on a variance inflation factor [VIF] of 2), resulting in 131,325 SNPs; (2) an ROH was defined as 65 consecutive homozygous SNPs with no heterozygote calls allowed. To calculate the proportion of the autosome in ROHs, the total length of ROHs were summed for each individual, and then divided by the total SNP-mappable autosomal distance (2.77×10^9 bases), resulting in the F_{roh} measure.

Statistical analyses

Chi-squared tests were performed in IBM SPSS Statistics 19 to test the association between religion and city size, and to test for non-random assortment between spouses on religious affiliation. All other statistical analyses were done in purpose written perl scripts. Regression analyses were computed with the help of the PDL::Stats::GLM perl module (see http://search.cpan.org/~maggiexyz/PDL-Stats-0.6.2/GLM/glm.pp), which allows for the computation of the R^2 change and its significance. The R^2 change in this study is the difference in explained variance of F_{roh} between regressions with and without the predictor of interest (religious affiliation or MDD). All regressions on F_{roh} also included as predictors: three PCs reflecting ancestry, city size (dichotomous), and Contrast QC (CQC, a quality metric from Affymetrix representing how well allele intensities separate into clusters, and known to correlate with heterozygosity).

Religion and autozygosity—R^2 change was computed between multiple regression analyses on F_{roh} with and without religious affiliation as a predictor of F_{roh}. To account for the non-normal distribution of F_{roh} (see Figure 3), F_{roh} was permuted 100,000 times.

Permutation analyses were repeated using the residual of F_{roh} as the dependent variable, and the residual of religious affiliation as the independent variable (i.e., residuals from regressions on the predictors used as control variables: PCs, city size, and CQC). We performed these analyses in order to check whether the association with the control variables caused a bias in the permutation analyses, since only F_{roh} was permuted. Results (data not shown) were similar and significant in the same directions.

Religion and MDD—A logistic regression analysis was run with MDD case/control status as the dependent variable, and as a predictors religion (religious versus non-religious), and city size (small versus large).

MDD and autozygosity—R^2 change was computed between multiple regressions on F_{roh} with and without MDD as a predictor. Analyses were permuted 100,000 times and were repeated adding religion as an additional predictor.

These permutations were also repeated using the residual of F_{roh} and the residual of MDD. The results (data not shown) were similar and significant in the same directions.

ROH mapping analysis—The --*homozygous-group* command in Plink (Purcell et al. 2007) was used to obtain ROH segments that overlap between individuals. This was done separately for analyses on religious groups (resulting in 2,506 ROH segments, in which 2,879 allelically distinct ROHs were observed in ⩾2 individuals) and MDD (resulting in 1,858 ROH segments, in which 1,773 allelically distinct ROHs were observed in ⩾2 individuals). Table 3 (column "*All subjects*") and Table 4 (row "*All subjects*") show the sample sizes for these analyses. Two genome-wide association analyses were conducted: (1) logistic regressions for each ROH segment, where a dichotomous independent variable indicated the presence of an ROH for each individual, which may or may not allelically match; (2) logistic regressions for each allelically distinct ROH, where a dichotomous independent variable indicated the presence of an ROH for each individual. These analyses were done for four dichotomous phenotypes: (1) Catholics versus others, (2) Protestants versus others, (3) non-religious versus others, and (4) MDD cases versus controls. The dependent variable was permuted 1000 times. All regressions were corrected for PCs, city size, and CQC. The analyses for MDD also included religion (dichotomous) as a covariate.

Results

Geographic distribution and assortment on religion

Figure 2 shows that the North-South distribution of Protestants and Catholics in the Netherlands seen in previous generations is maintained today, but with an overall increase (especially in the larger cities displayed in Figure 1) of non-religious groups. When comparing the 26 cities in the Netherlands with a population size >100k with the rest of the Netherlands, the more densely populated cities show significantly fewer religious individuals ($\chi^2(1) = 289.05$, $p < .001$, Cramer's V = .261). The fact that the North-South distribution of Protestants and Catholics is still visible today may have been influenced by strong religious assortment. Spouse pairs (3,042 pairs who had a measure for religious affiliation) showed highly significant religious assortment ($\chi^2(4) = 3226.67$, $p < .001$, Cramer's V = .728; see Table 1). Of the 3,042 spouse pairs, 2,486 (81.72%) reported the same religious affiliation (Protestant, Catholic, or no affiliation).

These results confirm the stability of the religious distribution of Catholics and Protestants. The PC explaining most variation, thus showing the greatest ancestry differences in the Dutch population, shows a high correlation with the North-South gradient based on current living address ($r=.603$), and mainly separates the Catholic and Protestant regions of the Netherlands (see Figure 2d). The association between geographic proximity and shared ancestry within the Catholic and Protestant regions is in line with the stable geographic distribution of religions during the last centuries, and indicates that current religious affiliation (Figures 2b and 2c) is likely to correspond to those of a person's ancestors (Figure 2a) for Catholics and Protestants.

Religion and autozygosity

Data on religious affiliation were available for 4,022 unrelated individuals with Dutch ancestry in three categories: Catholic (N=1,069; mean F_{roh} = .0016), Protestant (N=839; mean F_{roh} = .0019), and non-religious (N=2,114; mean F_{roh} = .0013). Linear regressions with F_{roh} as the dependent variable were performed to test whether the F_{roh} differences between these three groups were significant. Regressions included: ancestry-informative PCs (PC1=North-South PC, PC2=East-West PC, PC3=middle-band PC), city size, and CQC. To account for the non-normal distribution of F_{roh} (see Figure 3), F_{roh} was permuted 100,000 times.

Without religion as a predictor, only the North-South PC, East-West PC, and city size contributed significantly to F_{roh} variation (see Appendix 1), with the most significant contribution coming from the North-South PC, where the southern part has fewer/shorter ROHs. Including religion in the regression analysis led to a significant increase in explained variance of F_{roh}. Post-hoc analyses showed that this was due to the significant difference between the non-religious group and the two religious groups, since there was no significant difference between the two religious groups (see Table 2, Appendix 1, and Figure 3). To further investigate whether this effect is due to closer inbreeding, we removed subjects with more extreme F_{roh} values and repeated the analyses. When removing the 2.5% tail ($Z_{Froh} >$ 1.96, N=103, of which 66% is religious, compared to 47% of the remaining individuals), the effect remains the same, and significant. Removing outliers with an approximate equivalent of half-cousin inbreeding ($F_{roh} > .03125$) or even distant inbreeding ($F_{roh} > .005$) also results in a significant effect (Table 2 and Appendix 1). This suggests that these effects are not due to recent or close inbreeding. The highest observed F_{roh} value was .0583, so there were no individuals with values higher than or equivalent to cousin-cousin inbreeding ($F_{roh} > .0625$).

MDD and autozygosity

Being religious was protective for MDD, while living in a larger city increased the chance for MDD: a logistic regression with MDD case/control status as the dependent variable, religion as a dichotomous (religious versus non-religious) predictor ($= -.84$, $p < 10^{-16}$), and city size as a dichotomous predictor ($= .62$, $p = 5.71 \times 10^{-15}$) showed a significant negative relation between MDD and religion and a significant positive relation between MDD and city size.

MDD controls showed higher F_{roh} values than MDD cases for each F_{roh} cutoff (see Table 4). Linear regressions with F_{roh} as the dependent variable were performed that included the three ancestry PCs, city size, and CQC as predictors. We did the analysis for the entire dataset (2,916 unrelated individuals with MDD and religion measured), and repeated it for the datasets excluding positive outliers (i.e., excluding closer inbreeding). MDD showed a nominally significant association with F_{roh} for the dataset excluding the 2,5% F_{roh} tail (significantly more variance is explained with than without MDD as an independent variable: empirical $p = .042$). When repeating this analysis with religion added as a predictor, the effect disappears (empirical $p = .236$), while religion remains highly significant ($p = 2.09 \times 10^{-5}$; see Table 5 and Appendix 2).

ROH mapping analysis

In order to test whether specific regions of the genome were overrepresented in one of the religious groups, or in MDD cases/controls, genome-wide analyses were conducted on ROHs. Analyses were done on each ROH segment that showed overlap between individuals. Each segment was tested in genome-wide analyses with the following dichotomous dependent variables: (1) Catholics versus others, (2) Protestants versus others, (3) non-religious versus others, and (4) MDD cases versus controls (see Tables 3–4 for sample sizes). Association analyses were run on (1) the overall burden of ROHs at each location throughout the genome, and (2) on each allelically distinct ROH. All regressions included the three ancestry PCs, city size, and CQC as covariates, and the analyses for MDD also included religion as a covariate. There were no significant results (all empirical p's > .05), indicating no association between specific genomic regions and religion or MDD.

Discussion

We observed significant autozygosity differences between religious and non-religious groups, due to by greater levels of background parental relatedness in the religious groups and a more outbred non-religious group. Individuals with the same religious affiliation are more likely to share ancestry due to a combination of geographic proximity and the strong assortment and segregation of religious groups throughout the past centuries. The non-religious group showed a significantly lower ROH burden, which could not be explained by the fact that these groups are more prevalent in urban areas that attract people with a greater variation in genetic background. The decrease of ROHs in non-religious groups is likely caused by increasing variation in the gene pool of possible mates, induced by the relatively recent absence of denominational restrictions on mate selection.

In the non-religious groups there were significantly more MDD cases. Religion has often been reported to be an important coping mechanism with a positive influence on mental health (Koenig 2009). Numerous studies have been conducted on the relation between religion and depressive disorder, of which the majority reported religious people to have significantly lower rates of depressive disorder or fewer depressive symptoms (Koenig 2009). The association between religion and MDD led to a nominally significant negative association between autozygosity and MDD. Without knowledge of the relationship between religion and autozygosity, and between religion and MDD, the significant association may have led to the hypothesis that inbreeding protects against MDD (i.e., that recessive alleles unmasked by ROH are protective and an important part of the genetic architecture of MDD). This could be interpreted as a result of selection against the inability to develop MDD. This would be in line with the analytical rumination hypothesis which poses that MDD is an evolved response to complex problems, which functions by focusing the limited cognitive processing recourses on the analysis of the problems in the individuals life (Andrews and Thomson Jr 2009). However, after including religion as a covariate, MDD did not show a significant association with ROHs, while religion still contributed significantly to F_{roh} variation. This suggests that the relation between autozygosity and MDD is a consequence of a stratification artifact.

The North-South PC contributed most significantly to F_{roh} variation in all regressions. This was no surprise, as the Dutch North-South cline significantly correlates with genome-wide homozygosity (F), making the stratification captured by the North-South PC also well detectable by F_{roh}. This correlation is likely due to a serial founder effect that is also visible in the European North-South cline that correlates highly ($r = .66$) with the Dutch North-South cline (Abdellaoui et al. 2013). Unlike F_{roh}, F does not require a minimum amount of consecutive homozygous SNPs, and thus is able to capture much shorter (hence older) ROHs. Given that the serial founder effect captured by the North-South PC is expected to have occurred in more ancient times (during the European South-North expansions), we would expect a higher correlation between PC1 and F than between PC1 and F_{roh}, which is indeed what we observe ($r_{PC1,F} = .245$, $p < .001$; $r_{PC1,Froh} = .082$, $p < .001$).

Genome-wide ROH mapping analyses did not reveal any genomic regions that were significantly more homozygous in any religious group, or in MDD cases or controls. This was not unexpected, as there was no reason to assume that past religious assortment was based on specific genomic regions, and there was no association between F_{roh} and MDD.

A larger meta-analysis also found no significant relationship between autozygosity (F_{roh}/ROHs) and MDD (9,238 MDD cases and 9,521 controls, including a subsample of the current study genotyped on a different microarray), and observed inconsistent directions of effect between the datasets (Power et al. 2013). The study included nine datasets from 5

countries (1 UK, 2 Australian, 3 German, 2 US, and 1 Dutch dataset), and the direction of the effects were consistent across countries (higher F_{roh} protective for MDD: Australia, Netherlands, and US; lower F_{roh} protective for MDD: UK and Germany). This suggests that there may be similar demographic/social factors associated with both autozygosity and MDD in other populations as well. In-depth analyses similar to those in the present study are needed to further explore this hypothesis in other populations.

Differences in autozygosity between social groups can unveil additional dimensions of stratification within populations. It shows in the Netherlands how recent secularization can have genetic consequences in a relatively small country. This can confound studies on traits associated with the stratifying factor, even when correcting for PCs reflecting ancestry and geographic factors. To detect effect sizes seen from known inbreeding studies in outbred populations, larger sample sizes than that of the current dataset are needed (~12,000–65,000) (Keller et al. 2012; Keller et al. 2011). The effects of within population differences as observed in the current study however are well detectable with the current sample size. It is crucial to the investigation of the effects of inbreeding on the well-being of offspring to take social factors into account that are predictive for both parental relatedness and the trait under investigation. Considering the sensitivity of F_{roh} for social stratification not detected by PCAs, we also encourage to consider accounting for these effects in GWASs.

Acknowledgments

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Figure 1.
Map of the Netherlands, its major rivers, and its 26 largest municipalities (population size >100k as of April 2012) (CBS 2012).
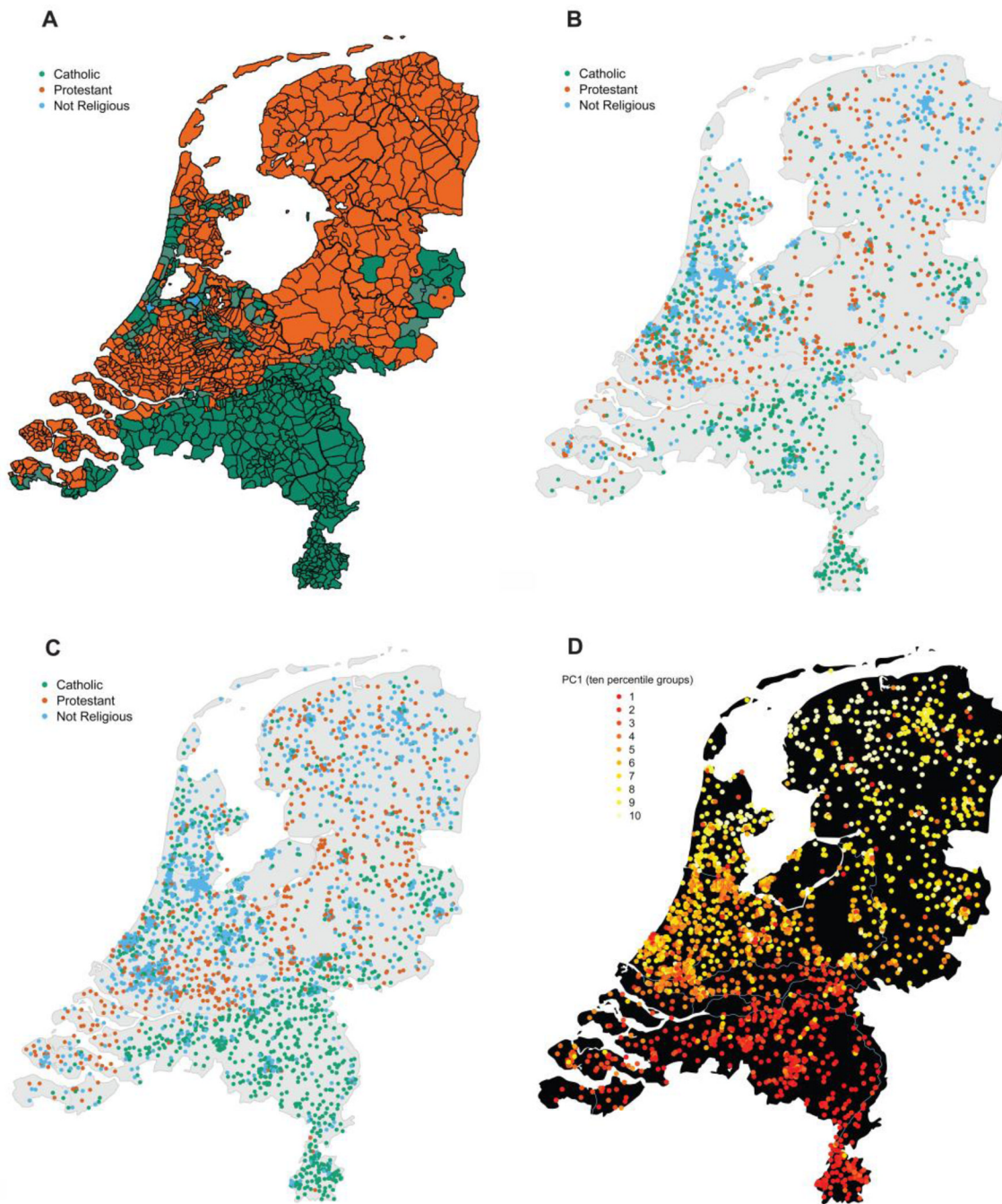
Figure 2. Geographic distribution of religious groups in the Netherlands
A shows the geographical distribution of Catholic, Protestant and not religious groups in the Netherlands in 1849 (Dimitri 2007; Knippenberg 1992). **B** and **C** show the distribution of the current genotyped dataset (including related individuals: N=6,464) and the full dataset with a measurement for religious affiliation respectively (N=25,450). Each postal code was given the color of its most prevalent religious group. **D** shows the geographic distribution of the North-South PC, where the mean PC value per postal code (current living address) was computed, divided into 10 percentiles, and plotted.
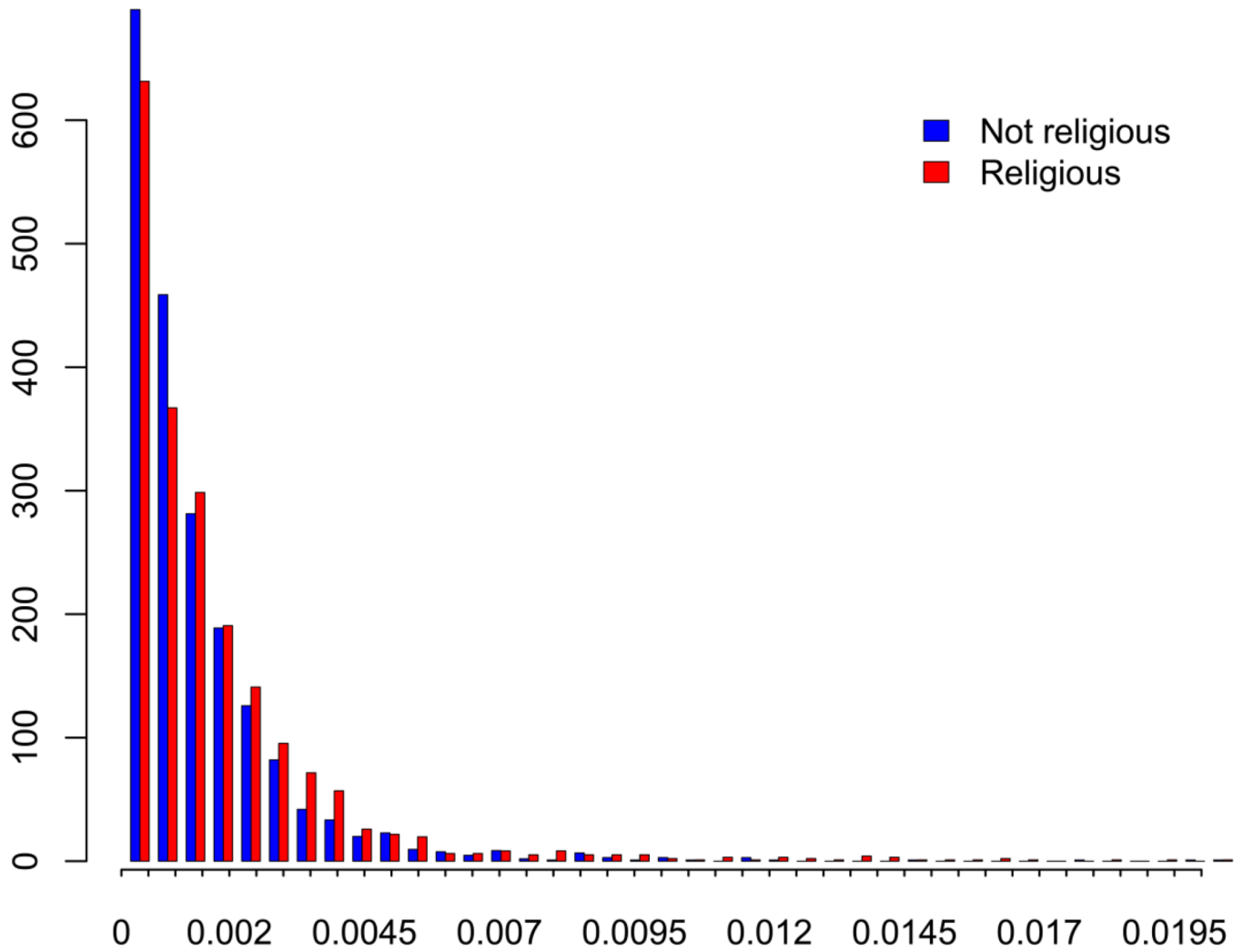
Figure 3. Density plot of the F_{roh} distribution for religious and non-religious individuals
The bars have been scaled to have a total density of 1 for both groups in order to allow a comparison between the groups.

Table 1

Distribution of religious affiliation in 3,042 spouse pairs, including ^{2} test for non-random assortment between spouses and the spouse correlation (Cramer's V).

^{2} (4) = 3226.67, *p* < .001, Cramer's V = .728		Wife		
		Protestant	**Catholic**	**Not Religious**
	Protestant	**646 (202.5)**	52 (324.8)	69 (239.8)
Husband	Catholic	42 (312)	**1049 (500.5)**	91 (369.5)
	Not Religious	115 (288.5)	187 (462.8)	**791 (341.7)**

The numbers between brackets is the expected number of spouse pairs in that cell under the null hypothesis of no religious assortment. Observed values higher than the expected values are in bold.

Table 2

Results of 100k Permutations of multiple linear regression on F_{roh}, including the three Dutch PCs, whether the subject lives in a city with >100k residents and CQC as predictors.

Included in model		F (df)	R² change	p-value	Empirical p
All available individuals (N=4,022)					
Main test	Two religious groups and non religious group (as two dummy variables)	9.78 (2 4014)	.005	5.80×10^{-5}	7×10^{-5}
Post-hoc tests	Not religious vs. Protestant	10.27 (1 2946)	.003	1.36×10^{-3}	1×10^{-3}
	Not religious vs. Catholic	17.08 (1 3176)	.005	3.67×10^{-5}	8×10^{-5}
	Catholic vs. Protestant	.088 (1 1901)	4.57×10^{-5}	.766	.770
Individuals with $Z_{Froh} < 1.96$ (i.e., excluding the 2.5% tail; N=3,923)					
Main test	Two religious groups and non religious group (as two dummy variables)	9.99 (2 3915)	.005	4.70×10^{-5}	3×10^{-5}
Post-hoc tests	Not religious vs. Protestant	8.62 (1 2880)	.003	3.35×10^{-3}	3.18×10^{-3}
	Not religious vs. Catholic	14.38 (1 3110)	.004	1.52×10^{-4}	1.8×10^{-4}
	Catholic vs. Protestant	.001 (1 1835)	5.75×10^{-7}	.973	.973
Individuals with $F_{roh} < .03125$ (i.e., excluding half-cousin inbreeding; N=4,017)					
Main test	Two religious groups and non religious group (as two dummy variables)	13.08 (2 4009)	.006	2.17×10^{-6}	$<10^{-5}$
Post-hoc tests	Not religious vs. Protestant	14.49 (1 2943)	.005	1.44×10^{-4}	1.1×10^{-4}
	Not religious vs. Catholic	20.23 (1 3172)	.006	7.12×10^{-6}	$<10^{-5}$
	Catholic vs. Protestant	.065 (1 1898)	3.38×10^{-5}	.798	.797
Individuals with $F_{roh} < .005$ (i.e., excluding elevated levels of distant inbreeding; N=3,855)					
Main test	Two religious groups and non religious group (as two dummy variables)	12.04 (2 3847)	.006	6.11×10^{-6}	$<10^{-5}$
Post-hoc tests	Not religious vs. Protestant	13.59 (1 2835)	.005	2.31×10^{-4}	2.4×10^{-4}
	Not religious vs. Catholic	14.94 (1 3056)	.005	1.13×10^{-4}	1.3×10^{-4}
	Catholic vs. Protestant	.580 (1 1798)	3.08×10^{-4}	.446	.445

Significance threshold for post-hoc tests = .05/3 = 0.0167. Bold = significant.

Table 3

Sample size and mean F_{roh} for each religious group per additional F_{roh} cutoff

Religious group	All subjects		$Z_{Froh} < 1.96$		$F_{roh} < .03125$		$F_{roh} < .005$	
	N	Mean F_{roh} (SD)	N	Mean F_{roh} (SD)	N	Mean F_{roh} (SD)	N	Mean F_{roh} (SD)
Catholic	1,069	.00159 (.0031)	1,036	.00119 (.0013)	1,067	.00151 (.0024)	1,013	.00109 (.0011)
Protestant	839	.00186 (.0031)	806	.00138 (.0013)	838	.00180 (.0026)	792	.00131 (.0012)
Not Religious	2,114	.00133 (.0024)	2,081	.00112 (.0012)	2,112	.00128 (.0020)	2,050	.00105 (.0011)

Table 4

Sample size and mean F_{roh} for MDD cases and controls per additional F_{roh} cutoff

	Cases		Controls	
	N	**Mean F_{roh} (SD)**	**N**	**Mean F_{roh} (SD)**
All subjects (N = 2,916)	1,608	.00143 (.0025)	1,308	.00156 (.0024)
$Z_{Froh} < 1.96$ (N = 2,835)	1,570	.00116 (.0012)	1,265	.00124 (.0013)
Froh < .03125 (N = 2,914)	1,607	.00140 (.0022)	1,307	.00154 (.0023)
Froh < .005 (N = 2,787)	1,549	.00110 (.0011)	1,238	.00114 (.0011)

Table 5

R^2 change, and its significance, between multiple regressions on F_{roh} with and without MDD as independent variable (once with, and once without religion as a covariate). F_{roh} was permuted 100,000 ×.

		F (df)	R² change	p	Empirical p
All individuals	No religion included	1.65 (1, 2909)	.0006	.199	.200
N = 2,916	Religion included	.07 (1 2908)	2.4×10^{-5}	.787	.788
$Z_{Froh} < 1.96$	No religion included	4.14 (1, 2828)	.0014	**.042**	**.042**
N = 2,835	Religion included	1.41 (1 2827)	.0005	.235	.236
$F_{\text{roh}} < .03125$	No religion included	2.84 (1, 2907)	.0010	.092	.093
N = 2,914	Religion included	.46 (1 2906)	.0002	.497	.496
$F_{\text{roh}} < .005$	No religion included	2.51 (1, 2780)	.0009	.113	.113
N = 2,787	Religion included	.71 (1 2779)	.0002	.400	.399

Appendix 1

Regression coefficients (*p*-values between brackets) for each of the predictors included in the linear regressions.

	Religious Affiliation	Religious Affiliation	PC1 (North-South)	PC2 (East-West)	PC3 (Middle-Band)	City Size	CQC
All available individuals (N=4,022)							
No religion included	NA	NA	.0211 (3.96×10^{-11})	.0067(.037)	.0060(.060)	−.0003 (1.77×10^{-4})	.0001 (.102)
Two dummy variables	.0004 (.002)	.0004 (1.09×10^{-4})	.0230 (1.09×10^{-11})	.0071 (.025)	.0063 (.052)	−.0002 (.014)	.0001 (.093)
Not religious vs. Protestant	NA	.0004 (.001)	.0261(8.56×10^{-11})	−.0010 (.790)	.0107 (.005)	−.0002(.106)	8.15×10^{-5} (.372)
Not religious vs. Catholic	NA	.0004 (3.68×10^{-5})	.0235 (3.79×10^{-11})	.0114 (.001)	.0016 (.657)	−.0002 (.055)	.0002 (.019)
Catholic vs. Protestant	NA	-4.76×10^{-5} (.766)	.0207 (1.57×10^{-4})	.0120 (.014)	.0081 (.109)	−.0004 (.011)	.0001 (.366)
Individuals with $Z_{Froh} < 1.96$ (i.e., excluding the 2.5 % tail; N=3,923)							
No religion included	NA	NA	.0178 $(<10^{-15})$	.0007 (.644)	−.0030 (.039)	−.0002 (7.65×10^{-5})	.0001 (.001)
Two dummy variables	.0002 (.002)	.0002 (7.63×10^{-5})	.0187 $(<10^{-15})$	.0009 (.523)	−.0029 (.052)	−.0001 (.008)	.0001 (.0009)
Not religious vs. Protestant	NA	.0002 (.003)	.0228 $(<10^{-15})$	−.0005 (.756)	.0009 (.604)	-4.44×10^{-5} (.341)	7.93×10^{-5} (.067)
Not religious vs. Catholic	NA	.0002 (1.52×10^{-4})	.0175 $(<10^{-15})$	.0023 (.175)	−.0033 (.046)	−.0001 (.010)	.0002 (7.39×10^{-5})
Catholic vs. Protestant	NA	2.25×10^{-6} (.974)	.0168 (6.999e-13)	.0010 (.646)	−.0059 (.0059)	−.0002 (.0017)	.0001 (.019)
Individuals with $F_{roh} < .03125$ (i.e., excluding half-cousin inbreeding; N=4,017)							
No religion included	NA	NA	.0205 (2.89×10^{-15})	.0018 (.480)	.0039 (.134)	−.0003 (2.50×10^{-4})	.0001 (.065)
Two dummy variables	.0004 (7.49×10^{-5})	.0004 (2.28×10^{-5})	.0219 (1.78×10^{-15})	.0023 (.382)	.0040 (.127)	−.0002 (.029)	.0001 (.056)
Not religious vs. Protestant	NA	.0004 (1.44×10^{-4})	.0261 (6.00×10^{-15})	.0005 (.875)	.0090 (.004)	−.0001 (.123)	8.95×10^{-5} (.238)
Not religious vs. Catholic	NA	.0004 (7.12×10^{-6})	.0218 (2.75×10^{-14})	.0046 (.110)	-9.74×10^{-5} (.973)	−.0001 (.109)	.0002 (.011)
Catholic vs. Protestant	NA	3.30×10^{-5} (.798)	.01855 (2.54×10^{-5})	.0014 (.722)	.0048 (.240)	−.0003 (.026)	9.85×10^{-5} (.365)
Individuals with $F_{roh} < .005$ (i.e., excluding elevated levels of distant inbreeding; N=3,855)							
No religion included	NA	NA	.0161 $(<10^{-15})$	.0018 (.182)	−.0024 (.069)	-8.03×10^{-5} (.022)	9.22×10^{-5} (.006)
Two dummy variables	.0002 (7.81×10^{-5})	.0002 (9.61×10^{-5})	.0167 $(<10^{-15})$	.0020 (.136)	−.0024 (.075)	-3.32×10^{-5} (.361)	9.32×10^{-5} (.005)
Not religious vs. Protestant	NA	.0002 (2.31×10^{-4})	.0202 $(<10^{-15})$	.0011 (.492)	.0006 (.708)	5.85×10^{-6} (.889)	5.75×10^{-5} (.137)
Not religious vs. Catholic	NA	.0002 (1.13×10^{-4})	.0156 $(<10^{-15})$	.0029 (.051)	−.0021 (.159)	-2.41×10^{-5} (.542)	.0001 (.002)
Catholic vs. Protestant	NA	4.60×10^{-5} (.446)	.0149 (9.54×10^{-13})	.0018 (.353)	−.0056 (.004)	−.0001 (.048)	.0001 (.025)

Appendix 2

Regression coefficients (*p*-values between brackets) for each of the predictors included in the linear regressions of the MDD analyses. These results are from analyses done on individuals with $Z_{Froh} < 1.96$.

	Religious Affiliation (dichotomous)	MDD	PC1 (North-South)	PC2 (East-West)	PC3 (Middle-Band)	City Size	CQC
No Religion & No MDD	NA	NA	.0156 ($<10^{-15}$)	.0038 (.030)	−.0053 (.002)	−.0001 (.003)	.0001 (.006)
No Religion & MDD	NA	−.0001 (.042)	.0159 ($<10^{-15}$)	.0038 (.031)	−.0052 (.003)	−.0001 (.013)	.0001 (.004)
Religion & No MDD	.0002 (4.99×10^{-6})	NA	.0162 ($<10^{-15}$)	.0042 (.017)	−.0051 (.003)	-7.81×10^{-5} (.097)	.0001 (.005)
Religion & MDD	.0002 (2.09×10^{-5})	-5.66×10^{-5} (.235)	.0163 ($<10^{-15}$)	.0041 (.018)	−.0050 (.003)	-7.00×10^{-5} (.141)	.0001 (.004)