Possible transgenerational associations between grandparents' childhood exposures and religious belief in their granddaughters

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Abstract

Research in epigenetics indicates that grandparents' smoking habits and their childhood experiences of trauma can influence the physical and psychological attributes of their grandchildren. We explored whether such grandparental exposures were associated with the religious and/or spiritual beliefs (RSBs) of their grandchildren. In line with other inter/trans-generational human studies we hypothesised that: (H1) Childhood exposures to cigarette smoking and/or traumatic events of grandparents will be associated with their grandchild's RSB; (H2) associations will differ between maternal and paternal inheritance; (H3) relationships will vary with age at grandparental exposure, and (H4) associations will differ between grandsons and granddaughters.

Information from the Avon Longitudinal Study of Parents and Children (ALSPAC) was used. We found significant associations between both grandparents' smoking and their childhood trauma histories with their grandchild's RSB (H1 supported); associations were mainly found in the maternal line (H2 possibly supported); results varied with age of exposure of the grandparents (H3 supported); and granddaughters were more affected than grandsons (H4 supported). We hope that these results will motivate collection of similar data to further evaluate these questions in other populations, including a possible role for epigenetic mechanisms.

Keywords: ALSPAC; religion; spirituality; intergenerational; transgenerational; grandparent; childhood traumas; grandparental smoking.

Introduction

Definitions of religious or spiritual belief (RSB) are not clear, but the general population has an implicit understanding of the terms, and such beliefs have permeated civilisations worldwide. Perhaps because of the difficulty of definition, there have been few detailed epidemiological studies of the antecedents of such beliefs in individuals. Neurobiological studies have shown associations between RSBs and activity in different regions of the brain, particularly the inferior parietal lobe.[1] A review by Rim and colleagues in 2019[2] concluded that the experience of RSB has specific neurobiological correlates that differ from 'the neurobiological correlates for comparable non-RS phenomena'. Others have studied the structures of the brains of those with positive RSBs and shown associations with a thicker cortex,[3] and a specific neural circuit in the brain stem.[4]

Thus, there is evidence of neurobiological differences between those with positive and negative RSBs (i.e. between individuals who believe in a divine being (hereinafter named as

having ‘positive’ RSBs) and non-believers (those with negative RSBs)), but few studies as yet have attempted to identify what the causes of these differences might be. There is some evidence, using twin and adoption studies, that religious beliefs are heritable, but that genes do not play a major role in whether or not an individual is affiliated to a particular religion .[5] Genes that have been associated with RSBs include those involved with serotonin (e.g. 5-HTTLPR) and dopamine (e.g. DRD4)[6] but, to our knowledge, no GWAS type studies have been undertaken with RSB as the outcome.

Heredity does not necessarily involve genes, however. Epigenetics is an example of non-genetic heritability. This has been shown in experiments in rodents whereby a parent who has been exposed as a fetus to an environmental challenge to its mother (either chemical or stressful) and subsequently mated, has grandchildren and great-grandchildren (but not children) with particular physical or neurobiological changes. The mechanism is assumed to concern the effect of the original challenge on the stem cells of the gonads of the developing fetus. A different mechanism is involved when the non-pregnant parent (male or female) is exposed to the challenge before conception. This time the offspring, as well as subsequent generations, may be affected.[7] Biomarkers for both types of effects are found in specific DNA methylation sites in observational human studies as well as in experimental animal studies.[7]

To our knowledge, there have been no observational studies to date to determine whether such ancestral exposures are associated with RSBs. Ancestral exposures that have been shown to have effects on later generations in humans involve cigarette smoking[8-10] and starvation and exposure to major traumas such as war .[11-13] The associations of these challenges on subsequent generations (e.g. grandchildren) have been shown biologically with changes in DNA methylation in the grandchild when the grandmother smoked in the pregnancy resulting in the mother[14] and in grandchildren whose grandparents were Holocaust survivors.[15]

In this paper we investigate whether there is any evidence indicating possible epigenetic associations involving either ancestral exposures to cigarette smoking or traumatic events in regard to RSBs. Since other studies of inter/trans-generational influences have shown the pre-pubertal age to be of particular importance.[13,16], as well as the sex of both the grandparent and the grandchild,[9,10,17] we hypothesised that:

> (H1) Childhood (or fetal) exposures to cigarette smoking and/or trauma in the grandparent generation are associated with RSBs in their grandchildren.
>
> (H2) The associations will vary according to whether inheritance is down the paternal or maternal line.
>
> (H3) The relationships will vary according to the ages at exposure of the grandparents.
> (H4) The results will vary with the sex of the grandchild.

Results

Nomenclature for Ancestors

The nomenclature used is shown in Figure 1, with MGM, MGF, PGM and PGF indicating maternal grandmother, maternal grandfather, paternal grandmother, and paternal

grandfather respectively. It should also be noted that the granddaughters and grandsons studied here are not biologically related to one another – they are the parents of the subsequent generation (not studied in this paper).

Validity of exposure

Although it would be prohibitively costly to validate participant reports of grandparental smoking, there are multiple lines of evidence supporting the accuracy of the reports used here. In line with national statistics at the time, the proportion of men (our grandfathers) reported as smoking was twice that of the women (our grandmothers), and the proportion of grandfathers reported as smoking increased as his year of birth decreased. Consistent with previous studies reporting lower birthweight in pregnancies exposed to cigarette smoke, the children of the grandmothers who were reported as smoking during pregnancy in our study have lower mean birthweight.[18] Furthermore, the grandchildren of these grandmothers have DNA methylation differences compared to those who reported that their grandmothers did not smoke during pregnancy.[14]

Unadjusted Associations with the Granddaughters' Religious Belief

Of the 12,351 pregnant women who answered the question on religious belief, 50% reported positively.[19] The ways in which the prevalence of such belief varied with demographic features of each of her four grandparents is shown in Table 1. Associated at $P<0.05$ were: (a) the year of birth of the MGM which showed a linear trend, the more recently her MGM had been born the less likely was her granddaughter to believe ($P<0.001$); (b) the age of the MGM at the birth of the granddaughter's mother (the older the MGM was the more likely her granddaughter to believe ($P = 0.023$); (c) the more sisters the MGF had, the more likely his granddaughter to believe ($P = 0.014$). There was an additional association with the year of birth of the PGF, but this was not associated with a linear trend and is likely to be a chance finding. Thus, of the seven demographic features (28 tests), only three were associated at $P<0.05$ (expected 1.4).

As a control assessment to determine whether these associations were likely to be cultural rather than specific to the granddaughter's religious beliefs, we compared the same 28 features of *her partners'* grandparents with her belief (Supplementary Table 1). The years of birth of her partner's maternal grandparents were associated with her belief at $P<0.05$, but there was no sign of a significant trend. We concluded that, since the results were not similar, the demographic associations found with the granddaughters' grandmothers' years of birth were unlikely to be culturally determined.

Compared with the demographic features in Table 1, the 18 relationships with the grandparents' childhood environmental exposures show many more associations at $P<0.05$ (10 compared with <1 expected; $P<0.01$; Table 2). The results showed positive trends with the number of traumatic events occurring between the ages of 6 and 11 years of the MGM, the MGF and the PGM, such that the more events that had occurred the more likely the granddaughters to have reported having a religious belief. Similarly, there were positive associations with the number of traumatic events between ages 12 and 16 for MGM, MGF and PGF. There was also evidence of an association between starting to smoke regularly during adolescence (by age 16) of the MGF and the PGM; each was associated with a reduced likelihood of belief in their granddaughter. In addition, if the paternal grandmother

had smoked during the pregnancy resulting in the birth of her son, then her granddaughter had a lower likelihood of religious belief.

As with the demographic characteristics of the grandparents, we computed the proportion of granddaughters with a religious belief according to the environmental exposures of *her partners'* grandparents during childhood. In contrast with Table 2 (where there were 10 associations at P<0.05), there was only one association at P<0.05 and that was between her partner's maternal grandmother's number of traumatic events that had occurred before she was aged 6 and the granddaughter's belief (Supplementary Table 2). We therefore concluded that the associations between the granddaughters' beliefs and their grandparents' childhood exposures are unlikely to be the result of cultural associations.

Unadjusted Associations with the Grandsons' Religious Belief

The proportion of grandsons with a religious belief (37% of 9789 fathers) at the time of pregnancy in 1991-2 was considerably lower than that of their female partners (50%). The only notable unadjusted associations with the demographic features of their grandparents concerned trends in associations with the age of their grandfathers (both MGF and PGF) at the time of birth of their grandson's parents: the older the grandfather was the more likely his grandson to have a religious belief (Table 3).

For the environmental features in the grandparents' childhoods, there was evidence of associations of early childhood traumas (< 6 years) in the MGM, the MGF and the PGF being associated with a lower rate of religious belief in their grandsons, but no associations with traumatic events occurring later in childhood. There were no associations with smoking at the P<0.05 level, nor was there any indication of differences in RSB if the grandmothers had smoked during pregnancy (Table 4).

Patterns of association with traumatic events in childhood

In Tables 5a and 5b the associations with the actual traumatic events are outlined for the grandchildren of the MGMs and MGFs. It is of interest that the odds ratios (ORs) for the beliefs of the grandchildren of the MGMs were almost all less than 1.00 (17/20 traumatic events for both grandsons and granddaughters). In contrast the ORs of the grandchildren of the MGFs were predominantly greater than 1.00 (16/18 of the grandsons and 15/18 of the granddaughters). The number of associations with P<0.05 did not differ from those expected by chance for the grandchildren of the MGMs (3 observed; 2 expected) or those of the MGFs (4 observed, 1.8 expected). The numbers available were too small for further analyses by particular types of childhood trauma; however, for interest we present the associations found when splitting the traumatic events by the age at occurrence in Supplementary Tables 3 and 4. For the grandchildren of the PGMs and PGFs, numbers were too small for useful comparison.

Adjusted associations with the granddaughters' beliefs (Figure 2)

Statistical analyses to identify the independent associations with RSBs of the granddaughters used stepwise regression on the demographic and environmental features of each of the grandparents for whom there was sufficient data available. We found:

(i) *The maternal grandmother (MGM):* The features of the MGM that showed unadjusted associations with religious belief in the granddaughters at P<0.05 were

(a) her year of birth; (b) her age when the mother was born; (c) no. of traumatic events aged <6; (d) no. of traumatic events at ages 6-11; (e) no. of traumatic events aged 12-16. Of these, the age variable was accounted for by the year of birth, and the traumatic events at ages <6 and >11 were secondary to the traumas at ages 6-11. Thus, the final model comprised just the two variables: traumas pre-puberty and year of birth of the MGM (Figure 2).

(ii) *The maternal grandfather (MGF).* The unadjusted associations at P<0.05 were (a) the number of sisters he had; (b) no. of traumas aged 6-11 years; (c) no. of traumas aged 12-16; (d) started smoking regularly before age 17. Of these, the no. of traumatic events aged 6-11 predominated over those occurring at ages 12-16 but smoking regularly aged <17 was independently associated. These two variables comprised the final model for the MGF.

(iii) *The paternal grandmother (PGM).* There were no demographic variables related to the PGM which had an unadjusted association at P<0.05 with her granddaughters' religious belief. However, there were three environmental associations: (a) no. traumatic events aged 6-11; (b) started smoking regularly before age 17; (c) smoked in the pregnancy resulting in the birth of her granddaughter's father. Of these, backward stepwise analysis resulted in just the number of traumatic events aged 6-11 remaining in the model. Conversely, forward stepwise analysis resulted in the pregnancy smoking variable entering but not the traumas; however, the numbers in this final model were considerably smaller than the previous one (n = 1030 v. 1925).

(iv) *The paternal grandfather (PGF).* Only one variable concerning the PGF was associated with RSB in the granddaughter – and that concerned the number of traumatic events he had been exposed to aged 12-16. When offered to the factors associated with his wife (PGM), his exposure did not contribute independently to his granddaughter's religious belief.

Discussion

This is the first study (to our knowledge) to have assessed whether there is any intra- or trans-generational evidence for religious beliefs being associated with ancestral environmental features. We have taken grandparent smoking and traumatic events in childhood as our exemplars of possible environmental factors that might have such an effect, since there is evidence that these factors in ancestors have been associated with different outcomes in their grandchildren. For example, grandmothers smoking during pregnancy have been demonstrated to be associated with a number of different outcomes in the grandchildren;[10] onset of regular smoking of the grandfather prior to puberty is associated with obesity in the grandchild,[9,17] and a grandchild's exposure in mid-childhood to famine is associated with long-term survival and improved mental health.[13,16] We hypothesised that if there was a relationship between religious belief in the grandchildren and exposures of grandparents to these factors, the associations would be likely to be associated differently according to the sex of the grandchild as well as of the type of grandparent. That is indeed what we have demonstrated.

We have shown that the probability of a *positive* religious belief of the granddaughters (but not the grandsons) was associated with the number of traumatic circumstances in the childhoods of three of their grandparents (MGM, MGF and PGM) at ages 6-11; conversely the number of traumatic experiences the grandparents (MGM, MGF and PGF) had experienced aged <6 years was associated with *reduced* likelihood of religious belief in the granddaughters (but not the grandsons). Of these, the association with MGF trauma at ages 6-11 survived adjustment for multiple tests ($p < 0.001$).

It is notable that there were few demographic features of the grandparents (including social class) that were associated with religious belief in their grandchildren. The one exception concerned the years of birth of the maternal grandmother which was strongly associated with RSB of her granddaughter such that the more recently the MGM had been born, the less likely the granddaughter to believe (Figure 2). This was not explained by social characteristics or by the age of the MGM when her daughter was born. Using a control experiment associating the characteristics of the grandparents of the index woman's partner with her own beliefs, we showed that there were associations with the years of birth of his MGM and MGF, but they were in the opposite direction – the more recently they had been born, the greater the likelihood that the index woman would have a religious belief. This suggests that the association of the year of birth of the MGM of the granddaughter with her own religious belief was not a statistical artifact due to cultural similarities.

Cigarette smoking of the MGF did demonstrate some associations with the religious belief of his granddaughter such that if he had started smoking in childhood (<17 years), she (but not his grandson) would be at reduced likelihood of believing. There was also a possible association with PGM smoking in pregnancy with the granddaughter which remained in the analysis when forward, but not backward, stepwise analyses were carried out (see Figure 2).

Putting the results in context

The first study to demonstrate a traumatic incident to grandparents in childhood being associated with outcomes to the grandchildren involved the occupants of the rural town of Överkalix on the edge of the Arctic Circle in Sweden. It showed that exposure of the occupants to famine during what the authors called the slow growth period, but which we would more accurately call pre-puberty, was associated with mortality and survival of their adult grandchildren.[17] The results were related to the harvest records and showed associations with very poor and excessively good harvests which were specific to the sex of the grandparent and to that of the grandchild. These results were validated by a larger study in Uppsala.[20] Moreover, van den Berg and Pinger analysed data on the grandchildren whose grandparents had been exposed to a major famine in Berlin, and demonstrated that if the paternal grandfather had been exposed pre-puberty, then his grandsons (but not granddaughters) had better mental health than expected, but if the maternal grandmother was exposed pre-puberty, then her granddaughters (but not grandsons) had better mental health.[13] These results raise the question as to whether the association is with poor nutrition, or with the stress that will have been associated with famine, especially during a war which was the case in Berlin. In the present study, there were few grandparents who were reported as having insufficient food during childhood.[21] However, we have shown that exposure of the maternal grandparents to one or more traumas in mid or late-childhood was associated with increased likelihood of religious belief in the granddaughters (but not the grandsons). Conversely there

was a suggestion that exposures in early childhood were associated with reduced likelihood of religious belief in their grandsons (but not granddaughters).

Several different cohorts have shown that onset of regular cigarette smoking in childhood is associated with increased obesity and asthma in the next generation.[9,22] In addition, several studies have considered the possible effects of a grandmother smoking in pregnancy on the grandchild. The results, summarised for ALSPAC publications to date in Table 6, indicates that when the MGM smokes prenatally, the grandson is more likely to be associated with discernible effects, whereas if the PGM smokes during pregnancy, the associations are with effects to the granddaughter. This study shows a similar association with the PGM smoking by demonstrating a reduced chance of the granddaughter having a religious belief. This accumulating evidence of smoking by a grandmother in pregnancy being associated with a sex-specific outcome is now complemented by results of DNA methylation showing results in the maturing grandchild (age 15-17) that indicate different associations depending on the type of grandmother, and the sex of the grandchild.[14]

Strengths and Limitations

The major strengths of the study concern the fact that it is based on a geographically defined population. Data on religious belief was collected in 1990-2 during the relevant pregnancy of the study mothers. There was no bias in selection (especially regarding characteristics of the grandparents) other than that the women had to be pregnant, have an expected date of delivery between April 1991 and December 1992 and live in the defined area. A further advantage to this study lies in the fact that information was available on the ancestors of the women's partners, and we were able to use the relationships between the exposures to the woman's partners' grandparents on the religious belief of the woman as a control.

There are, however, several limitations. (a) The population of pregnant mothers was asked to include their partners if they were happy to do so – but the ALSPAC study team had no way of contacting them other than via the mothers for many years after the study had started. Consequently, the response rate of the index men in these analyses is much lower than that of the index women, with some loss of statistical power. (b) The details collected on the traumatic events of the grandparents during their childhoods were not collected from the study parents until 2018, by which time their response rate had dropped substantially due to mortality and loss to follow up. (c) The accuracy of response may be questionable, especially as many of the grandparents had died, and details of their childhoods with them. (d) The statistical power of the study was low for the grandparents of the grandsons and precluded more detailed statistical analysis. In spite of this, the association with MGF's trauma at age 6-11y did survive adjustment for multiple tests. (e) The traumatic events during each time period were summed for individuals; although this assumed the results were similar regardless of the type of trauma, this may not have been appropriate. (f) It is not clear how relevant the results might be to other populations.

Biomarkers supporting the epigenetic consequences of smoking and traumatic exposures

We have shown that the changes with potential epigenetic environmental exposures, such as grandmother smoking in pregnancy, are associated with changes in DNA methylation levels at specific CpG sites, including on the X chromosome of the grandchild.[14] There is also

abundant evidence from experimental studies that traumatic events can have effects on the DNA methylome for several generations.[e.g.7] Evidence in humans is less clear, although there are reports of changes in DNA methylation among offspring of Holocaust survivors,[11,15] those exposed to famine[23] and to domestic violence.[12]

Conclusions

The results of this study may point to environmental reasons for changes in individual RSBs over the past 100 years. We suggest that further research should be carried out with other population-based studies to fill the gaps regarding biological consequences of ancestral exposures to both cigarette smoking and traumatic events during childhood.

Material and Methods

The study population

The Avon Longitudinal Study of Parents and Children (ALSPAC) started to collect data from pregnant women in 1990. The eligible sample was defined as all women resident in a defined geographic area centred on the city of Bristol, whose expected date of delivery was between the beginning of April 1991 and the end of December 1992. The aim of the study was to determine the environmental factors that might interact with the genes to influence the health and development of their offspring.[24] Information concerning the backgrounds of the women and their partners was collected during pregnancy, and thereafter throughout the childhood, adolescence, and early adulthood of the offspring. This paper is focused on the beliefs of these prospective parents, who we refer to as the granddaughters and grandsons. Their offspring are not considered here.

The initial number of pregnancies enrolled was 14,541 (for these at least one questionnaire has been returned or a “Children in Focus” clinic had been attended by 19/07/99). Of these initial pregnancies, there was a total of 14,676 fetuses, resulting in 14,062 live births and 13,988 children who were alive at 1 year of age..[25-27]

Recently collected data were managed using REDCap electronic data capture tools hosted at the University of Bristol. REDCap (Research Electronic Data Capture). This is a secure, web-based software platform designed to support data capture for research studies.[28] Please note that the study website contains details of all the data available through a fully searchable data dictionary and variable search tool: [https://www.bristol.ac.uk/alspac/researchers/our-data/]. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee (ALEC; IRB00003312) and the Local Research Ethics Committees. Detailed information on the ways in which confidentiality of the cohort is maintained may be found on the study website: http://www.bristol.ac.uk/alspac/researchers/research-ethics/
All methods were performed in accordance with the relevant guidelines and regulations. Informed consent for use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.[29]

Asked within the initial questionnaires completed by the grandchildren were details of their four grandparents.[21] The nomenclature used is as shown in Figure 1. Data collected included demographic details of them and their families as well as potential epigenetic associations (their smoking history and traumatic events that occurred during their childhoods).

The outcome measure: religious belief

During the study pregnancies, each grandchild (i.e. the pregnant woman and her partner) was asked to complete a questionnaire which included a variety of questions on religion, spirituality, belief, and religious behaviour.[19] For this study we use the positive response to the question: 'Do you believe in God or some divine power?' as our definition of RSB.

Data concerning potential epigenetic exposures

Information on smoking of their grandparents (as well as their parents) was collected during the initial contacts. The study grandchildren were asked (i) whether their grandparents had started smoking before the age of 17, and (ii) whether their grandmother had smoked when pregnant with the individual who was to become their parent.

Subsequently these grandchildren were contacted for further information about their grandparents. For each, questions were asked concerning whether the grandparent had, during their childhood: (a) suffered from a serious illness; (b) attended boarding school; (c) been taken into care by family or (d) by others; (e) had been in a war situation; (f) became a refugee; (g) had been subjected to violence directly or (h) whether there was violence in their home; (i) not enough to eat at times or (j) had an unhappy childhood. In addition, during their childhood whether any of the following had occurred to each of their parents including (k, l) whether either had died; (m, n) been seriously ill; (o, p) been in a war situation or (q, r) become a refugee. Finally, they were asked (s) to describe any other major events or additional comments concerning their ancestor's childhood. For each of these 19 questions, multiple responses were allowed: yes aged 0-5; yes aged 6-11; yes age 12-16; yes, but age not known; no.[21] We have used the number of different events in each age period as the exposure of interest. An additional question concerned whether the grandparent had moved home, but this was not considered to be traumatic enough to warrant inclusion in the totals of traumatic events.

Potential confounders

For each of the four grandparents the following data had been collected: whether they were born in England; their years of birth; their ages at the birth of the parents of the study grandchildren, their social classes based on their occupations; the numbers of brothers and sisters they had and whether they were the firstborn.

Statistical analyses

This is an exploratory analysis to determine whether there are any indications that there is an epigenetic aetiology to RSB. In particular we examine whether there is evidence that grandparents' exposure to smoking or to traumatic events are related to the RSB of their grandchildren. Because of its exploratory nature we take pains to reduce the chance of Type II errors. To do this we assess the numbers of associations with P< a predetermined value,

based approximately on the statistical power, given the available numbers. We then compare the numbers of associations at P<0.05 that would be expected to occur by chance with those observed. When the statistic $[(O-E)^2/E]$ is more than 3.96 we consider the associations as possibly indicative of a true association. It is important to recognise that such evidence is exploratory and requires other confirmatory data before assuming the associations to be robust. We consider Type I errors for individual statistical tests by also adjusting p-values for multiple tests, i.e. $p < 0.05$/number of tests. In particular, Tables 2 and 4 each present findings for 20 tests (4 grandparents x 5 exposures). Hence, the adjusted p-value threshold for each is $p = 0.00125 = 0.05/40$.

Numbers of individuals available for analysis are shown in Table 7. The numbers of granddaughters with data available were greater than the numbers of grandsons; the numbers relating to the maternal grandparents were greater than those relating to the paternal grandparents and, apart from social class, the numbers available for other features were greater for grandmothers than grandfathers. Consequently, the statistical power is greatest for the maternal grandparents of the granddaughters.

Separate sets of analyses were undertaken for the granddaughters and grandsons. The initial analyses first assessed the associations between the potential confounders (the demographic variables) and the outcomes, and secondly the associations with the possible epigenetic exposures. In order to determine whether any significant results between grandparent exposures and grandchild's beliefs were due to cultural associations we compared them with those found using their spouse's grandparents' exposures. If there was a true significant epigenetic relationship, there should *not* be a significant association with the exposures to the spouse's ancestors. If the original results were due to cultural similarities rather than epigenetic effects, one would expect similar results for the spouse's grandparents.

Factors that were significant at the P<0.05 level for any one type of grandparent were selected for further analysis. Because there was considerable multi-collinearity between some of the variables, backwards stepwise logistic regression was used to determine those factors that were most likely to be independently associated with each index individual. This involved starting with a model containing all factors, then removing the variable with the least significant contribution to the model (i.e. the highest P value >0.05), repeating until the contribution of each remaining variable in the model was P <0.05. As a check on these results, the analyses were repeated using forwards stepwise analyses, and differences were documented. Forward stepwise analysis involved starting with an empty model, then adding the variable with the most significant (i.e. lowest P value) contribution to the model, repeating until no remaining variable was associated with a contribution at $P < 0.05$. Analyses then proceeded to adjust for the demographic factors that had been identified as potential confounders, using logistic regression. The latter analyses were carried out using STATAv.9.

Because we did not believe that the data were missing at random, we have not imputed missing information. Consequently, the small numbers (particularly on the paternal side) indicate that the power to show significant results is low. Partly for reasons of statistical power, but also so that we avoid type I errors, we do not correct for multiple testing. Rather, we concentrate on the answers to our original research hypotheses but take account of the numbers that should have P<0.05 based on chance.

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Author contributions: This publication is the work of the authors; JG and SG will serve as guarantors for the contents of this paper. SG carried out the statistical analyses; JG, MP and MS derived the concept and design of the study, and all authors contributed to writing and rewriting of several versions of the paper.

Data availability: ALSPAC data is available to researchers for particular projects, provided no attempt is made to reveal the identities of the subjects. Guidelines for access are found on the ALSPAC website: *www.bristol.ac.uk/alspac/researchers*

Legends for Figures

Figure 1. Family structure with nomenclature used.

Figure 2. The final models: features of the grandparents independently associated with the index granddaughter's religious belief

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