Regular smoking of male ancestors in adolescence and fat and lean mass in young adult grandchildren and great-grandchildren

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Article

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Abstract

Our previous studies using the Avon Longitudinal Study of Parents and Children (ALSPAC) have shown that if men started smoking before the onset of puberty their sons, their granddaughters and great-granddaughters were more likely to have excess fat mass during childhood, adolescence and early adulthood. Here we assess associations between ancestral smoking during adolescence (ages 11-16years) with fat and lean mass of subsequent generations at ages 17 and 24. We found associations between adolescent smoking of the *paternal* grandfather and the adjusted fat mass of their grandchildren, but no associations with lean mass. Grandchildren at age 17 had an average excess fat mass of + 1.65 [95% CI + 0.04, + 3.26] Kg, and at age 24 an average excess of + 1.55 [95% CI -0.27, + 3.38] Kg. Adolescent smoking by the *maternal* grandfather showed similar, but weaker, associations: at 17 average excess fat mass of + 1.02 Kg [95% CI -0.20, + 2.25] Kg, and at 24 average excess of + 1.28 [95% CI -0.11, + 2.66] Kg. There were no significant differences between the sexes of the children. For the great-grandparents there were few convincing results. Confirmation of these associations is required, either in a further data set or by demonstrating the presence of supportive biomarkers.

Introduction

The major spur towards the initiation of recent studies of associations between human ancestral exposures and their successors concerned a detailed comparison of the survival of individuals born on the edge of the Arctic Circle between 1880 and 1915. Three cohorts of index individuals were identified, based on their years of birth in the village of Őverkalix. Their grandparents' exposures to famine and/or a harvest glut during their childhoods was identified and the details linked to their grandchildren's health indices. Detailed analyses highlighted the following: (i) there were strong relationships which were sex-specific, both in regard to the sex of the exposed grandparent and to the sex of the affected index grandchild, and (ii) the exposure effects were specific to particular ages of exposure – the most susceptible period being the ages prior to puberty [Pembrey et al 2006].

This study prompted a number of projects assessing associations between exposures during the pre-pubertal period and health and development of the grandchildren. For example, van den Berg and Pinger [2016] studied the children and grandchildren of individuals who were exposed to the Berlin famine at ages 8–12 years. They demonstrated that those whose *mothers* had been exposed during these ages had worse health outcomes, particularly if they were male. In the subsequent generation, those grandsons whose *paternal* grandfathers had been exposed pre-puberty had higher (better) mental health scores, and granddaughters had higher mental health scores if their *maternal* grandmothers had experienced the famine during the pre-puberty ages.

Among major cohort studies, information on environmental exposures during the childhood of parents has been collected only occasionally, and that of grandparents rarely. The Avon Longitudinal Study of Parents and Children (ALSPAC) was one pre-birth cohort which had collected information on the ages at which the parents of the study children had started smoking regularly. These data were used to determine whether children whose parents had a history of starting smoking pre-puberty were likely to have a different growth

pattern than children who started smoking later. We demonstrated that if the fathers had started smoking regularly prior to 11 years of age, their sons (but not their daughters) were more likely to have an increased body mass index (BMI), largely associated with excess fat mass at ages 13, 15 and 17 [Northstone et al 2014]. A subsequent detailed study of antecedents associated with fat mass at age 24 showed that the association remained with *paternal* smoking < 11, and actually increased in size on adjustment [Golding et al 2019]. However, this study also showed an adjusted association between fat mass of the offspring and *maternal* onset of smoking during adolescence (i.e. at ages 11–16).

We have subsequently determined whether the pre-pubertal ages at starting to smoke regularly of grandparents and/or great-grandparents was also associated with fat mass of the grandchildren and great-grandchildren. We compared the fat mass measurements of the different generations according to whether their ancestors had started smoking pre-puberty with those who started smoking in adolescence (11–16) [Golding et al 2022]. We hypothesised that any effects would differ according to the sex of both the ancestral smoker, and that of the grandchild and great-grandchild. In order to provide a comparison with the results for fat mass, we analysed the results for lean mass, and specifically looked at the outcomes of early onset smoking of the great-grandparents, grandparents and parents on the body composition of the index offspring in late adolescence and early adulthood (Fig. 1). The results showed that granddaughters, but not grandsons, whose paternal grandfather started smoking pre-puberty (< 13) were significantly fatter than those whose paternal grandfather started smoking between ages 13 and 16. There were similar associations with the great-granddaughters (but not great-grandsons) of fathers of maternal grandfathers who had started smoking pre-puberty [Golding et al 2022]. The analyses did not compare grandchildren and great-grandchildren of those ancestors who smoked during adolescence with those who did not.

Here we hypothesise: (i) that there are likely to be differences between the subsequent generations of children who started smoking before age 17 and those who either never smoked or who started smoking after age 16; (ii) that these are likely to vary with sex of the grandchildren and/or great-grandchildren, as well as with (iii) the mode of inheritance.

Results

Nomenclature used

The ways in which the ancestors are referred to are shown in Figure 1. In brief, the four individuals on the maternal side of generation F0 are referred to as MGMM (maternal grandmother's mother), MGMF (maternal grandmother's father), MGFM (maternal grandfather's mother) and MGFF (maternal grandfather's father). The paternal side of generation F0 are labelled PGMM, PGMF, PGFM and PGFF with similar meanings. For the F1 generation, the labels are MGM and MGF on the maternal side and PGM and MGF on the paternal side. F2 is represented by M (mother) and F (father). F3 is the proband who is referred to as the great-grandchild, grandchild, or child depending on the generation whose onset of smoking is being considered.

Grandparents' smoking in adolescence

Fat mass

The unadjusted associations between each of the grandparents who smoked regularly in adolescence and the fat mass of their grandchildren are shown in Table 1a. There were marked associations for increased fat mass for the grandchildren if a maternal or paternal grandparent had smoked regularly in adolescence; the associations at age 17 tended to be more likely to be significant at the P values we have used than those at age 24. There were no significant differences between the sexes.

The demographic variables associated with grandchild's fat mass are depicted for each grandparent in Supplementary Table 1. Those with R^2 >0.1% were included as covariates. The consequent adjusted associations are shown in Table 1b. The numbers involved in the adjusted analyses were only approximately half of the numbers in the unadjusted analyses due to missing data in the confounders. There were no adjusted associations with either of the grandmothers smoking in adolescence, but associations with the grandfather smoking in adolescence remained, especially for the paternal grandfather. There were no indications of differences in effect sizes between the sexes of the grandchildren (Table 1c).

Lean mass

In complete contrast with Table 1a: (i) whereas 23 of 24 associations were positive (i.e. greater fat mass if the grandparent had started smoking by 16 years of age), only 16 of the 24 associations with lean mass were positive; (ii) whereas 15 out of 24 unadjusted mean differences in fat mass were highlighted as reaching our defined P value cut-points, only 3 of the 24 unadjusted statistics for lean mass did so (Table 2a).

Interestingly, very few of the socioeconomic and demographic variables were associated with lean mass, compared with fat mass (Supplementary Table 1). For example, the social class and education levels of each of the grandparents contributed to the grandchild's fat mass, whereas this only occurred rarely for lean mass. Adjustment for these potential confounders showed little of interest (Table 2b) apart from an interaction with the sex of the grandchild if the PGM had smoked in adolescence (with increased effect size among 24-year-old grandsons compared to granddaughters) (Table 2c).

Great-grandparents' smoking in adolescence

Fat mass

The unadjusted associations between the great-grandparents' age <17 at smoking regularly and fat mass of the great-grandchildren is shown in Table 3a. When the maternal great-grandparents had smoked in adolescence, their great-grandchildren tended to have more fat mass on average, with the exception of the great-grandchildren of the MGFF's, where the association was negative. The only associations at P<0.10 concerned an excess of fat mass at age 17 if the MGMM had smoked regularly in adolescence; there was no such association at age 24. For paternal grandparents, there were four associations at P<0.20, each involving the 24-year-olds.

On adjustment (Table 3b), two of the 16 associations reached the P value stipulated in advance (P<0.10); both associations were negative and were related to the 24-year-olds (involving the MGFM and MGFF). This number of adjusted associations was no greater that would have been expected by chance. Similarly, examination of the 32 associations considering the sexes separately, only five were at the P value cut-off, and none exhibited consistency between the two age groups (Table 3c).

Lean mass

Of the 48 unadjusted associations, only five reached an appropriate P value – i.e. no more than would be expected by chance. On adjustment, two of the 16 comparisons reached a relevant P value, again no more than expected (Supplementary Tables 3a-3c).

Discussion

The aim of our research has been to test whether exposure to an environmental insult such as regular smoking in the adolescence of ancestors had any detectable consequences on the fat mass of the grandchildren and/or great-grandchildren. We have used lean mass effects as a contrast, to ensure that any effect of fat mass was not true of another anthropometric measure. Based on the Őverkalix studies, and our earlier findings of an association between pre-pubertal onset of paternal smoking and increased fat mass in sons, but not daughters, we showed in an earlier study [Golding et al 2022] that there were sex-specific effects on grandchildren and great-grandchildren if their ancestor had begun to smoke regularly pre-puberty. We found that, in spite of small numbers and wide confidence intervals, there was evidence of increased fat mass in the granddaughters and great-grand-daughters at the two ages analysed (17 and 24 years) associated with ancestors starting to smoke pre-puberty (< 13 years) compared with starting during adolescence (aged 13–16). We found no such associations with lean mass.

In this set of analyses, we have assessed whether there were associations between the amount of fat mass in the grandchildren and great-grandchildren of men and women who had smoked regularly in adolescence compared with the rest of their peers. We have shown here associations between the grandfathers smoking before age 17 and the fat mass of their grandchildren, and that this was apparent for the grandchildren in both their late teens and early adulthood (ages 17 and 24), and for both the maternal and paternal lines, contrary to our hypothesis. There were no such associations if either of the grandmothers had smoked in adolescence. There were no convincing associations between the great-grandparents smoking in adolescence and the fat or lean mass of their great-grandchildren.

Previous analyses have stressed the importance of the timing of exposures in regard to outcomes in succeeding generations. We have shown this in regard to exposures *in utero* as well as in the pre-puberty period, with apparent effects on outcomes as diverse as autistic traits, myopia, obesity and IQ [Golding et al 2021]. Here we have demonstrated an association with an exposure to cigarette smoking in the adolescent period and suggest that this period of time should also be considered in further multi-generational studies. However, it should be noted that, unlike the associations with pre-pubertal smoking, there was no indication

of any consistent associations in the great-grandchildren, indicating that the associations may indicate intergenerational rather than transgenerational inheritance.

This study has a number of weaknesses: (a) the data on age at onset of regular smoking of their ancestors was obtained retrospectively from their children and grandchildren. Although there is anecdotal evidence that ancestors who started smoking pre-adolescence are prone to remember and even boast about this, it is unclear as to whether those starting smoking at later ages (i.e. age 13–16) were as likely to recall such detail. (b) There was a large amount of information missing on age at onset of smoking; we did not try and impute these data since we were unsure concerning whether they were missing at random. Consequently, the adjusted analyses were carried out with complete data only, with obvious reduction in statistical power, particularly for the paternal line. To compensate for this, and to ensure that we did not ignore relevant associations, we considered P values < 0.10 for the maternal line and < 0.20 for the paternal line. (c) We were not able to evaluate replication of our findings as we are not aware of any other studies with relevant data.

The strengths of the study lie in: (i) its longitudinal nature; (ii) the fact that outcomes used the DXA measures of fat and lean mass which are considerably more accurate than indicators such as BMI (body mass index) which do not distinguish between fat, lean or bone mass, and (iii) the associations we demonstrated were apparent for the two ages tested.

In conclusion, our research question concerned whether exposures to cigarette smoking in the age group 13–16 years compared with not starting smoking until age 17 or later, or not at all, was associated with outcomes in the grandchildren or great-grandchildren. We have shown here that exposures to cigarette smoking at this age by the grandfather, but not the grandmother, were associated with fat, but not lean, body mass. The fact that no such effects were found among the great-grandchildren may indicate that the associations are intergenerational rather than transgenerational. Alternatively it may indicate weakening of effects across generations possibly obscured by a multitude of other factors. Clearly further longitudinal family studies are important in order to assess whether these results are generalisable.

Methods

The ALSPAC population

ALSPAC was designed to assess ways in which aspects of the environment and genes of individuals interact to result in benefits and disadvantages to health and development. It recruited pregnant women who were resident in a predefined area (that part of Avon that was within the South-West Regional Health Authority) and had an expected date of delivery between 1st April 1991 and 31st December 1992. Eligible women were contacted as early in pregnancy as feasible. The initial number of pregnancies enrolled is 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. These participants were followed throughout pregnancy and they, their partners and their offspring throughout subsequent years. The collection of

information continued with bolstering of the initial sample, with those who were eligible but who had not enrolled during pregnancy, taking place from the age of 7 years. The total sample size, therefore, for analyses using any data collected after the age 7 is 15,454 pregnancies, resulting in 15,589 fetuses, of which 14,901 were alive at 1 year of age [Northstone et al 2019]. Data were collected using a variety of methods including questionnaires completed by the mothers, their partners and their offspring; analyses of biological samples; linkage to standard data sets, and hands-on examinations including anthropometrical measures [Boyd et al 2013; Fraser et al 2013].

From the age of 22, study data were collected and managed using REDCap electronic data capture tools hosted at the University of Bristol. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies [Harris et al., 2009].

Please note that the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool: http://www.bristol.ac.uk/alspac/researchers/our-data/.

Ethical approval

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 the 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.

The Exposures

As part of the original data collected during pregnancy, the questionnaires sent to the study mother and her partner (usually the father of the study child) included details of their childhood and adolescence, including the age at which they had started smoking regularly, together with other information on their smoking habits, and those of their parents (i.e. the study child's grandparents (F1 – see Figure 1 for the generation F nomenclature). Unfortunately, the smoking habits of the F1s did not include details as to their ages when they had started smoking. Consequently, a recent endeavour has resulted in the sending of new questionnaires to those biological parents (F2) with whom the study was still in contact, to obtain further information on their parents (F1s) and grandparents (F0s), including whether they had started smoking regularly during childhood (defined as < 17 years). Questionnaires were mainly sent online, but for those who preferred paper alternatives, paper questionnaires were posted to them. Full details of the methodology and the questions asked can be found elsewhere [Golding et al 2020]. In brief, for each ancestor the question asked was: 'During his/her childhood, up to age 16, did he/she start smoking regularly?' If yes, the age at which the smoking was started (in years), with the option 'yes but don't know what age'. For the analyses presented here, we have included all who started smoking prior to age 17 and who were therefore smoking in adolescence.

The Outcomes

Total fat mass was estimated with the use of a Lunar Prodigy DXA scanner (GE Medical Systems Lunar, Madison, WI). For the present study we have concentrated on the measurements of fat mass at ages 17 (approximating to the end of puberty) and 24 years (early adulthood). Measurements of lean mass were measured at the same times using the same equipment. Both were measured on a continuous scale.

Confounders considered

For each ancestor studied, the following were considered as potential confounders: (i) their year of birth; (ii) ethnic group (white/non-white for F1s); (iii) social class based on occupation (manual/non-manual); (iv) no. of older siblings (0/1/2+); (v) no. of younger siblings; (vi) age of ancestor at the birth of the next generation; (vii) level of education (for F1s, not F0s and coded as equivalent to O-level+/ <O-level (examinations taken at the age of 16)); (viii) whether born in England (yes/no for F0s); (ix) trend in gross domestic product (GDP) of year of birth (F1s only); (x) business cycle of year of birth (F1s only).

Statistical analyses

Initial analyses determined the unadjusted associations between each of the 4 grandparents (F1s) and the eight great-grandparents (F0s) in regard to the grandchild's (F3) outcomes separately for (i) all grandchildren, (ii) grandsons only and (iii) granddaughters only.

For all outcomes, adjustments were made for potential confounders that contributed 0.1% or more to R^2 for the relevant outcome using multiple regression. The analyses were run for all grandchildren and great-grandchildren as appropriate. The analyses were then repeated with a term for the interaction between the sex of the F3 individual and whether or not the relevant ancestor had started smoking prior to 17 years of age.

The numbers of individual ancestors for whom relevant data were available for the unadjusted analyses are shown in Supplementary Table 4. This demonstrates that information was far more likely to be available for maternal great-grandparents (numbers ranged from 2224 to 3116) than for paternal great-grandparents (1128 to 1363). Similarly, for the grandparents, information was more likely to be returned in regard to maternal grandparents (3981 and 4112) than paternal grandparents (1900 and 2182). Given such discrepancies, it was decided to use as significant $P<0.10$ and $P<0.20$ for associations down the maternal and paternal lines respectively in order not to lose valid associations.

Declarations

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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*

Conflicts of Interest: The authors declare no competing interests.

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Tables

Table 1a. Unadjusted associations between regular smoking during the adolescence of the grandparents and **fat** mass in their grandchildren at ages 17 and 24. (In bold are results where the P value was ≤0.10 for maternal ancestors and ≤0.20 for paternal ancestors). The numbers involved in each analysis are shown in Supplementary Table 4.

Individual Ancestor	Age of F3	All grandchildren			Grandsons		Granddaughters	
		MD[95%CI]Kg	N	P	MD[95%CI]Kg	P	MD[95%CI]Kg	P
Maternal grandparents								
MGM	17	**1.62 [.56, 2.67]**	2462	**.003**	**1.94 [.38, 3.51]**	**.015**	1.01[-.24, 2.27]	.114
	24	**1.21 [.02, 2.40]**	2089	**.046**	1.16 [-.65, 2.97]	.209	.88 [-.64, 2.40]	.257
MGF	17	**1.67 [.82, 2.52]**	2148	**<.001**	**1.34 [.13, 2.55]**	**.030**	**1.50 [.46, 2.54]**	**.005**
	24	**1.54 [.59, 2.49]**	1819	**.001**	.77 [-.59, 2.13]	.267	**1.77[.52, 3.02]**	**.005**
Paternal grandparents								
PGM	17	**1.38[-.01, 2.76]**	1223	**.052**	**1.30 [-.67, 3.27]**	**.196**	**1.19 [-.48, 2.87]**	**.163**
	24	0.23[-1.37,1.82]	1065	.781	1.49 [-.90, 3.87]	.222	-.63 [-2.70, 1.45]	.552
PGF	17	**1.69 [.51, 2.87]**	1048	**.005**	**1.30 [-.67, 3.27]**	**.031**	**1.21[-.24, 2.67]**	**.102**
	24	**0.95 [-.37, 2.28]**	924	**.158**	.45 [-1.42, 2.32]	.636	1.16 [-.62, 2.93]	.203

CI = confidence interval; MD = mean difference in Kg fat mass; MGM = maternal grandmother; MGF = maternal grandfather; PGM = paternal grandmother; PGF = paternal grandfather

Table 1b. Adjusted associations between regular smoking during adolescence (<17) of grandparents and **fat** mass in their **grandchildren** (F3) at ages 17 and 24.

Ancestor F1	of F3	N	MD [95%CI]Kg	P	R^2	P_{int}
Maternal grandparents						
MGM	17	1340	+0.88 [-0.63, 2.38]	0.254	2.11	0.660
	24	1184	+1.03 [-0.57, 2.64]	0.208	2.94	0.984
MGF	17	1080	**+1.02 [-0.20, 2.25]**	**0.100**	2.31	0.814
	24	905	**+1.28 [-0.11, 2.66]**	**0.071**	3.01	0.718
Paternal grandparents						
PGM	17	509	-0.18 [-2.27, 1.90]	0.863	1.03	0.233
	24	449	-0.73 [-3.27, 1.82]	0.575	1.16	0.591
PGF	17	563	**+1.65 [+0.04, 3.26]**	**0.045**	1.93	0.793
	24	423	**+1.55 [-0.27, 3.38]**	**0.095**	1.85	0.483

CI = confidence interval; MD = mean difference in Kg fat mass; MGM = maternal grandmother; MGF = maternal grandfather; PGM = paternal grandmother; PGF = paternal grandfather

P_{int} = P value for interaction between the sexes

Table 1c. Adjusted associations between regular smoking during the adolescence of the grandparents and **fat** mass in their grandchildren at ages 17 and 24. (In bold are results where the P value was <0.10 for maternal ancestors and <0.20 for paternal ancestors).

Grandparent	MALE F3s			FEMALE F3s		
	n	MD [95%CI]	P	n	MD [95%CI]	P
Fat mass at 17						
MGM	590	0.94 [-1.29, 3.16]	0.408	750	0.45 [-1.33, 2.24]	0.620
MGF	468	0.48 [-1.32, 2.28]	0.601	612	0.85 [-0.60, 2.31]	0.249
PGM	222	1.12 [-1.58, 3.82]	0.414	287	-1.37 [-3.95, 1.20]	0.295
PGF	250	**1.44 [-0.55, 3.43]**	**0.155**	313	1.23 [-0.87, 3.34]	0.251
Fat mass at 24						
MGM	474	1.18 [-1.32, 3.68]	0.355	710	0.59 [-1.45, 2.64]	0.570
MGF	353	0.64 [-1.38, 2.66]	0.532	552	1.43 [-0.40, 3.27]	0.125
PGM	182	0.41 [-3.34, 4.16]	0.831	267	1.89 [-5.32, 1.55]	0.280
PGF	172	**2.07 [-0.55, 4.69]**	**0.120**	251	1.04 [-1.49, 3.57]	0.418

CI = confidence interval; MD = mean difference in Kg fat mass; MGM = maternal grandmother; MGF = maternal grandfather; PGM = paternal grandmother; PGF = paternal grandfather

Table 2a. Unadjusted associations between regular smoking in adolescence of grandparents and **lean** mass in their grandchildren at ages 17 and 24.

Individual ancestor	Age	All grandchildren MD [95%CI]Kg	P	Grandsons MD [95%CI]Kg	P	Granddaughters MD [95%CI]Kg	P
Maternal grandparents							
MGM	17	-0.01 [-1.05, 1.04]	.991	0.68 [-0.32, 1.69]	.182	0.32 [-0.26, 0.90]	.274
	24	-0.83 [-1.95, 0.29]	.147	0.13 [-1.26, 1.51]	.856	-0.08[-0.85, 0.69]	.835
MGF	17	-0.47 [-1.31, 0.37]	.274	0.40 [-0.38, 1.17]	.313	-0.17 [-0.65, 0.31]	.483
	24	-0.40 [-1.30, 0.49]	.380	0.59 [-0.45, 1.63]	.264	-0.13[-0.76, 0.50]	.677
Paternal grandparents							
PGM	17	0.43 [-1.04, 1.90]	.569	**1.13 [-0.31, 2.57]**	**.125**	0.47 [-0.33, 1.28]	.250
	24	0.49 [-1.06, 2.94]	.536	**1.55 [-0.31, 3.41]**	**.101**	0.05 [-1.01, 1.10]	.931
PGF	17	-0.23 [-1.43, 0.97]	.707	0.04 [-1.16, 1.23]	.953	**0.45 [-.21, 1.12]**	**.179**
	24	0.24 [-1.02, 1.49]	.712	0.79 [-0.71, 2.30]	.301	0.40 [-.47, 1.27]	.365

CI = confidence interval; MD = mean difference in Kg fat mass; MGM = maternal grandmother; MGF = maternal grandfather; PGM = paternal grandmother; PGF = paternal grandfather

Table 2b. **Adjusted** associations between regular smoking during adolescence (<17) of grandparents and **lean** mass in their **grandchildren** at ages 17 and 24.

Ancestor F1	Age of F3	N	MD [95%CI]Kg	P	R^2	P_{int}
Maternal grandparents						
MGM	17	1502	0.48 [-0.93, 1.88]	0.506	0.71	0.623
	24	1735	-0.60 [-1.84, 0.65]	0.346	0.11	0.476
MGF	17	1472	0.27 [-0.80, 1.34]	0.621	0.86	0.147
	24	1711	-0.26 [-1.19, 0.66]	0.574	0.06	0.142
Paternal grandparents						
PGM	17	502	0.79 [-1.60, 3.17]	0.518	1.64	0.384
	24	856	0.82 [-0.87, 2.50]	0.342	0.24	**0.090**
PGF	17	607	-0.20 [-2.04, 1.30]	0.662	0.31	0.223
	24	924	0.24 [-1.02, 1.49]	0.712	0.01	0.636

CI = confidence interval; MD = mean difference in Kg fat mass; MGM = maternal grandmother; MGF = maternal grandfather; PGM = paternal grandmother; PGF = paternal grandfather

P_{int} = P value for interaction between the sexes

Table 2c. Adjusted associations between regular smoking during adolescence of the grandparents (F1) and **lean** mass in their grandchildren at ages 17 and 24. (In bold are results where the P value was $\leq$0.10 for maternal ancestors and $\leq$0.20 for paternal ancestors).

Grandparent	GRANDSONS			GRANDDAUGHTERS		
	n	MD [95%CI]	P	N	MD [95%CI]	P
Fat mass at 17						
MGM	652	0.94 [-0.45, 2.33]	0.186	850	0.51 [-0.25, 1.27]	0.187
MGF	654	**0.96 [-0.05, 1.96]**	**0.063**	818	0.22 [-0.36, 0.79]	0.461
PGM	219	1.79 [-0.54, 4.12]	0.132	283	0.36 [-1.04, 1.77]	0.611
PGF	272	-0.38 [-1.88, 1.12]	0.619	335	0.51 [-0.45, 1.47]	0.298
Fat mass at 24						
MGM	695	0.48 [-1.03, 1.98]	0.533	1040	-0.24 [-1.10, 0.62]	0.584
MGF	661	0.69 [-0.39, 1.77]	0.210	1050	-0.19 [-0.83, 0.45]	0.561
PGM	331	**2.10 [0.07, 4.14]**	**0.043**	523	0.08 [-1.08, 1.25]	0.889
PGF	358	0.79 [-0.71, 2.30]	0.301	566	0.40 [-0.47, 1.27]	0.365

CI = confidence interval; MD = mean difference in Kg fat mass; MGM = maternal grandmother; MGF = maternal grandfather; PGM = paternal grandmother; PGF = paternal grandfather

Table 3a. Unadjusted associations between regular smoking in adolescence (<17) of great-grandparents and **fat** mass in their **great-grandchildren** at ages 17 and 24. Data shown comprise the mean differences (MD) between the fat mass of the great-grandchildren of those great-grandparents who smoked <17 compared with the rest of the population.

Individual	age	All		Males		Females	
		MD [95%CI]Kg	P	MD [95%CI]Kg	P	MD [95%CI]Kg	P
Maternal great- grandparents							
MGMM	17	**2.06 [0.60, 3.52]**	**.006**	**2.72 [0.70, 4.73]**	**.008**	**1.62 [-0.15, 3.39]**	**.073**
	24	1.16 [-0.48, 2.80]	.164	1.42 [-1.17, 4.00]	.281	0.63 [-1.44, 2.69]	.550
MGMF	17	0.57 [-0.72, 1.86]	.386	0.71 [-1.03, 2.46]	.423	0.53 [-1.05, 2.10]	.512
	24	1.08 [-0.32, 2.48]	.130	1.15 [-0.92, 3.21]	.276	1.04 [-0.80, 2.88]	.267
MGFM	17	0.57 [-1.13, 2.28]	.509	1.68 [-0.62, 3.98]	.151	-0.46 [-2.58, 1.65]	.667
	24	0.39 [-1.62, 2.40]	.700	0.12 [-2.65, 2.89]	.934	0.67 [-0.21, 3.38]	.630
MGFF	17	-0.17 [-1.69, 1.35]	.831	-0.16 [-2.38, 2.07]	.889	-0.30 [-2.15, 1.56]	.754
	24	-1.15 [-2.88, 0.58]	.192	-0.26 [-2.89, 2.37]	.845	-1.50 [-3.74, 0.73]	.186
Paternal great-grandparents							
PGMM	17	0.36 [-1.89, 2.60]	.754	1.33 [-1.94, 4.60]	.423	-0.33 [-3.00, 2.34]	.808
	24	-0.96 [-3.36, 1.44]	.432	0.81 [-3.07, 4.68]	.682	**-2.01 [-5.03, 1.02]**	**.192**
PGMF	17	1.86 [-0.96, 4.68]	.209	-0.01[-2.84, 2.82]	.994	1.21 [-0.87, 3.29]	.254
	24	0.33 [-1.60, 2.26]	.734	**-2.23 [-5.29, 0.84]**	**.153**	**1.74 [-0.71, 4.19]**	**.162**
PGFM	17	0.55 [-1.95, 3.04]	.608	2.18 [-1.49,5.84]	.243	-0.96 [-3.95, 2.03]	.529
	24	0.31 [-2.53, 3.16]	.828	2.42 [-2.83,7.66]	.363	-0.94 [-4.27, 2.39]	.578
PGFF	17	1.60 [-1.68,4.88]	.337	.85 [-3.76, 5.45]	.717	1.39 [-2.59, 5.38]	.491
	24	1.94 [-1.65,5.54]	.289	**5.47 [-.52, 1.15]**	**.073**	0.10 [-4.57, 4.37]	.966

CI = confidence interval; MD = mean difference in Kg fat mass MGMM = maternal grandmother's mother; MGMF = maternal grandmother's father. MGFM = maternal grandfather's mother; MGFF = maternal grandfather's father. PGMM = paternal grandmother's mother; PGMF = paternal grandmother's father. PGFM = paternal grandfather's mother; PGFF = paternal grandfather's father.

Table 3b. Adjusted associations between regular smoking in adolescence (<17) of great-grandparents and **fat** mass in their **great-grandchildren** at ages 17 and 24. Data shown comprise the mean differences (MD) between the **fat** mass of the great-grandchildren of those great-grandparents who smoked <17 compared with the rest of the population

Ancestor F0	Age of F3	N	MD [95%CI]Kg	P	R^2	P_{int}
Maternal great-grandparents						
MGMM	17	634	1.81 [-0.42, 4.04]	0.111	3.12	0.558
	24	563	0.66 [-1.88, 3.19]	0.611	3.41	0.927
MGMF	17	386	-0.41 [-2.36, 1.54]	0.679	3.71	0.763
	24	317	-0.88 [-3.05, 1.29]	0.425	4.89	0.439
MGFM	17	229	-0.54 [-3.86, 2.77]	0.748	6.56	0.509
	24	467	**-3.48 [-6.21, -0.74]**	**0.013**	2.49	0.547
MGFF	17	372	0.43 [-1.54, 2.40]	0.667	1.04	0.763
	24	282	**-2.00 [-4.26, 0.26]**	**0.082**	2.30	0.934
Paternal great-grandparents						
PGMM	17	186	-2.74 [-0.63,2.38]	0.254	2.11	0.660
	24	123	-2.45 [-8.96, 4.05]	0.457	3.45	0.853
PGMF	17	232	1.35 [-1.13, 3.84]	0.285	3.02	0.480
	24	139	0.17 [-3.30, 3.64]	0.924	4.91	**0.140**
PGFM	17	86	-1.23 [-6.60, 4.13]	0.649	1.93	0.823
	24	82	-2.38 [-8.83, 4.08]	0.466	2.90	0.596
PGFF	17	102	1.75 [-1.82, 5.32]	0.333	17.1	0.948
	24	102	0.05 [-3.60, 3.70]	0.980	13.4	0.886

MD = mean difference in Kg fat mass; MGMM = maternal grandmother's mother; MGMF = maternal grandmother's father. MGFM = maternal grandfather's mother; MGFF = maternal grandfather's father. PGMM = paternal grandmother's mother; PGMF = paternal grandmother's father. PGFM = paternal grandfather's mother; PGFF = paternal grandfather's father.

P_{int} = P value for interaction between the sexes

Table 3c. Adjusted associations between regular smoking during adolescence of the great-grandparents (F1) and **fat** mass in their great-grandchildren (F3) at ages 17 and 24. (In bold are results where the P value was <0.10 for maternal ancestors and <0.20 for paternal ancestors).

Great-Grandparent	MALE F3s			FEMALE F3s		
	n	MD[95%CI]	P	n	MD[95%CI]	P
Fat mass at 17						
MGMM	287	**3.43 [0.59, 6.27]**	**0.018**	347	1.68 [-1.15, 4.50]	0.244
MGMF	181	0.37 [-1.91, 2.64]	0.751	205	0.94 [-1.59, 3.46]	0.466
MGFM	110	0.64 [-3.86, 5.14]	0.779	119	-1.70 [-5.60, 2.20]	0.389
MGFF	174	1.07 [-1.70, 3.84]	0.447	198	0.03 [-2.38, 2.44]	0.980
PGMM	81	-0.94 [-7.02, 5.14]	0.760	105	**-4.23 [-8.64, 0.19]**	**0.060**
PGMF	58	1.76 [-1.93, 5.45]	0.343	86	0.43 [-1.33, 2.18]	0.629
PGFM	30	-0.05 [-6.22, 6.12]	0.986	56	**2.45 [-0.35, 5.25]**	**0.086**
PGFF	39	-0.96 [-5.47, 3.55]	0.668	62	-0.05 [-1.92, 1.81]	0.953
Fat mass at 24						
MGMM	232	0.11 [-3.71, 3.93]	0.956	331	0.71 [-2.56, 3.97]	0.671
MGMF	141	0.49 [-3.04, 4.02]	0.784	176	-1.30 [-3.98, 1.39]	0.342
MGFM	197	**-4.44 [-8.29, -0.59]**	**0.024**	270	-2.92 [-6.65, 0.80]	0.123
MGFF	126	-1.61 [-4.70, 1.49]	0.307	156	-2.51 [-5.62, 0.61]	0.114
PGMM	48	-3.14 [-14.8, 8.51]	0.589	75	-4.78[-12.9, 3.31]	0.242
PGMF	55	-2.36 [-7.57, 2.86]	0.369	84	1.65 [-3.21, 6.51]	0.501
PGFM	27	-3.16 [-31.3, 25.0]	0.817	55	**-4.17 [-9.53, 1.19]**	**0.125**
PGFF	38	0.78 [-6.55, 8.11]	0.829	64	-0.33 [-4.71, 4.05]	0.880

CI = confidence interval; MD = mean difference in Kg fat mass MGMM = maternal grandmother's mother; MGMF = maternal grandmother's father. MGFM = maternal grandfather's mother; MGFF = maternal grandfather's father. PGMM = paternal grandmother's mother; PGMF = paternal grandmother's father. PGFM = paternal grandfather's mother; PGFF = paternal grandfather's father.

Figures

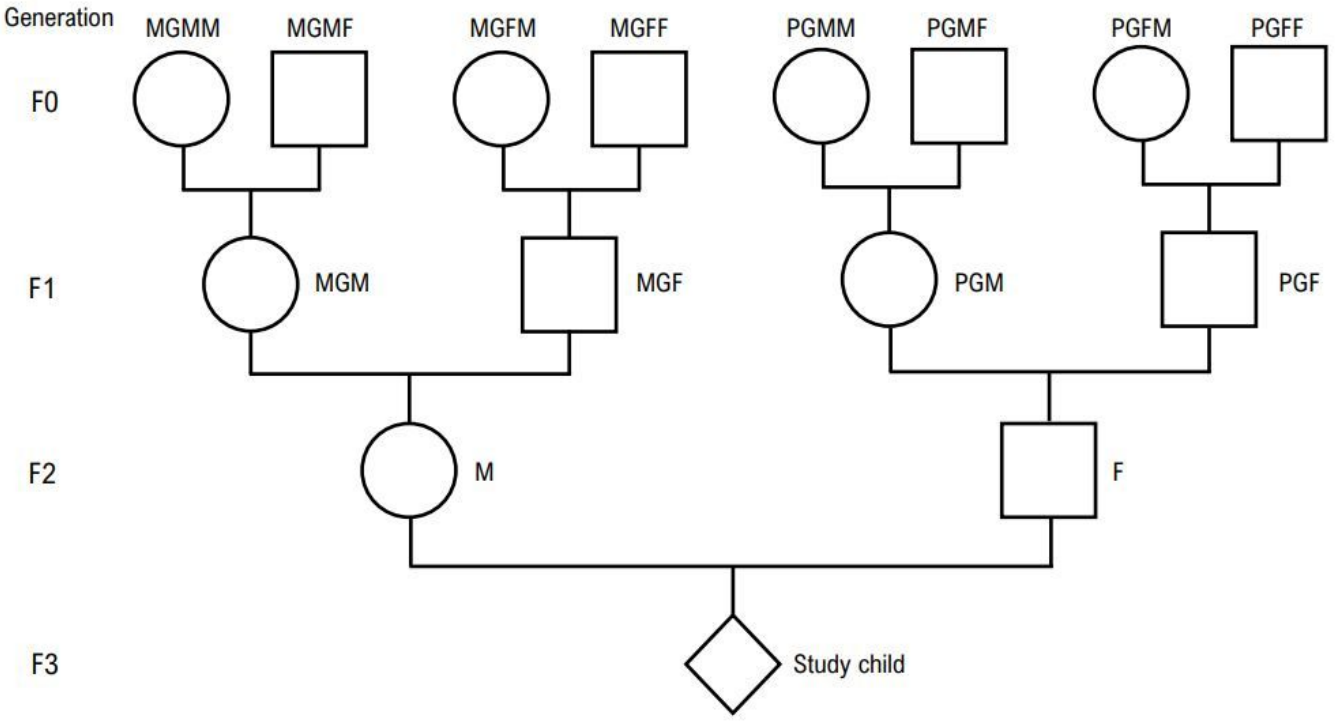

Figure 1

Family structure with nomenclature used (see text in Results section).

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