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Association between pregnancy losses in women and risk of atherosclerotic disease in their relatives: a nationwide cohort study†

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Aims A common underlying mechanism with a genetic component could link pregnancy losses with vascular disease. We examined whether pregnancy losses (miscarriages and stillbirths) and atherosclerotic outcomes co-aggregated in families.

Methods and results Using Danish registers, we identified women with pregnancies in 1977–2008, and their parents (>1 million) and brothers (>435 000). We followed parents for incident ischaemic heart disease (IHD), myocardial infarction (MI), and cerebrovascular infarction (CVI), and brothers for a broader combined atherosclerotic endpoint. Using Cox regression, we estimated hazard ratios (HRs) for each outcome by history of pregnancy loss in daughters/sisters. Overall, parents whose daughters had 1, 2, and ≥3 miscarriages had 1.01 [95% confidence interval (CI) 0.99–1.04], 1.07 (95% CI 1.02–1.11), and 1.10 (95% CI 1.02–1.19) times the rate of MI, respectively, as parents whose daughters had no miscarriages. For parents with ≥3 daughters, the HRs were 1.12 (95% CI 1.02–1.24), 1.29 (95% CI 1.13–1.48), and 1.33 (95% CI 1.12–1.57). Effect magnitudes did not differ for fathers and mothers. We observed similar patterns for IHD and CVI (parents) and the atherosclerotic endpoint (brothers). Parents whose daughters had stillbirths had 1.14 (95% CI 1.05–1.24) and 1.07 (95% CI 0.96–1.18) times the rates of MI and CVI, respectively, as parents whose daughters had no stillbirths.

Conclusion Certain pregnancy losses and atherosclerotic diseases in both heart and brain may have a common aetiologic mechanism. Women in families with atherosclerotic disease may be predisposed to pregnancy loss; conversely, pregnancy losses in first-degree relatives may have implications for atherosclerotic disease risk.

Keywords Atherosclerosis • Epidemiology • Familial aggregation • Ischaemic disease • Pregnancy loss

Clinical summary

Pregnancy losses (miscarriage and stillbirth) are associated with ischaemic disease in both heart and brain, and probably also with the wider range of atherosclerotic diseases. These associations are seen not only at the level of the individual woman but also within families. This suggests that some types of pregnancy loss may share an underlying aetiologic mechanism (which potentially has a genetic or epigenetic component) with atherosclerotic disease, and that in families with ischaemic and/or atherosclerotic disease, women may be predisposed to miscarriage and stillbirth. Careful phenotypic characterization of both the affected pregnancies and the atherosclerotic diseases in families exhibiting both traits, and genetic studies in families with several affected relatives, are needed to identify the likely link between the two types of events, which could potentially lead to new treatment options. The impact of adding pregnancy loss to algorithms evaluating risk of atherosclerotic diseases should also be tested.

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Introduction

Recently, we showed that pregnancy losses are associated with increased risks of later atherosclerotic outcomes [myocardial infarction (MI), cerebrovascular infarction (CVI), and renovascular hypertension], with increasing numbers of losses leading to ever greater increases in risk.[1] These findings, and those of other groups,[2–5] suggest a link between pregnancy losses and later atherosclerotic disease in multiple organ systems. A common mechanism could predispose to both repeated pregnancy loss and atherosclerosis; alternatively, multiple pregnancy losses might induce physiologic changes that then increase the risk of later atherosclerotic events.

A familial aggregation study is useful in determining which explanation is most likely, since any link between a woman's pregnancy losses and atherosclerotic outcomes in family members could not be due to any direct physiologic burden conferred by the losses themselves, leaving shared genetic or behavioural mechanisms as the most plausible explanations for any observed associations. A recent study reported an association between recurrent miscarriage before first live birth in a daughter and parental ischaemic heart disease (IHD).[6] The authors concluded that recurrent miscarriage and IHD might share pathophysiology and genetic predispositions. However, an unfortunate methodologic limitation cast uncertainty on the study's findings, and insufficient statistical power precluded firm conclusions about other outcomes.

We conducted a large register-based familial aggregation study to examine associations between pregnancy losses (miscarriages and stillbirths) in women and (i) their parents' risks of IHD, MI, and CVI, and (ii) their brothers' risk of any atherosclerotic outcome. Rather than limiting ourselves to losses in individual women prior to first live birth, we looked at total losses among the group of daughters/sisters, while accounting for family size.

Methods

Data sources

Established in 1968, the Danish Civil Registration System is a population register that contains demographic and kinship information on all Danish residents.[7] Updated daily, the system allows for virtually complete follow-up of study subjects and linkage to information from Denmark's population-based registers via a unique personal identification number assigned to each person. The Medical Birth Register contains information on all live and stillbirths in Denmark since 1973.[8] The Hospital Discharge Register, which has registered all inpatient diagnoses since 1977 and all outpatient diagnoses since 1995, also includes information on live births, stillbirths, and miscarriages.[9,10]

Cohort definitions and pregnancy loss variables (exposure)

Using information from the Medical Birth and Hospital Discharge Registers, we identified women with ≥1 pregnancy between 1977 and 2008 that ended in a miscarriage, stillbirth, or singleton live birth. We then identified parents and siblings for these women using the Danish Civil Registration System. The parents constituted our parent cohort; the brother cohort included all male siblings.

Our definition of miscarriage included missed abortions and spontaneous abortions. Miscarriages registered within 8 weeks of molar pregnancies, induced abortions or extrauterine pregnancies were ignored, however. Stillbirths were defined as foetal deaths ≥28 weeks from 1977 to 2003 and ≥22 weeks from 2004 onwards (the definition of stillbirth evolved over time); losses at earlier gestational ages were considered miscarriages. For consistency with our previous study,[1] we did not consider pregnancies ending in multiple live births, although the concern over the link between multiple pregnancies and cardiovascular outcomes was less relevant to a familial aggregation study where pregnancy loss and atherosclerotic events were not occurring in the same person.

For the parent cohort, we counted the number of pregnancy losses and live singleton births occurring over time among a couple's daughters from the first time a daughter's pregnancy ended in one of these outcomes. Since the number of miscarriages, stillbirths, and live births was tracked over time, exposure to miscarriage or stillbirth in ≥1 daughters was considered as a time-dependent variable. Parents whose daughter(s) first had live births were considered unexposed until such time as a daughter experienced a pregnancy loss (if ever), at which time they were considered exposed to the relevant type of loss. If the first pregnancy in a daughter resulted in a loss, the parents were considered exposed to that type of loss from the start of follow-up. Once exposed, parents could not become unexposed; they could, however, become 'more' exposed with increasing numbers of miscarriages and stillbirths to daughters.

Similarly, for the brother cohort, we counted the number of miscarriages, stillbirths, and live singleton births occurring among a man's sisters over time, again considering number of pregnancy losses as a time-dependent variable.

Follow-up and outcomes

We followed each person in the parent cohort from the end of the first pregnancy in a daughter until the first of the following events in the parent: (i) ischaemic outcome; (ii) death; (iii) emigration; (iv) registration as 'missing' in the Civil Registration System; or (v) 31 December 2008 (end of follow-up). Persons with incident ischaemic outcomes during follow-up were identified using the Hospital Discharge Register. We considered the following ischaemic outcomes: MI [International Classification of Diseases (ICD) 8th revision code 410, ICD 10th revision code I21]; IHD (ICD-8 codes 410–414, ICD-10 codes I20-25); and CVI (ICD-8 codes 433, 436.01, 436.09, 436.90, 436.99, ICD-10 code I63).

Because the brother cohort was too young to experience sufficient ischaemic outcomes during follow-up, we followed the brothers for a broad range of atherosclerotic events. Each man was followed from his 18th birthday or the end of the first pregnancy in a sister, whichever came later, until the first of (i) combined atherosclerotic outcome; (ii) death; (iii) emigration; (iv) registration as 'missing' in the Civil Registration System; or (v) 31 December 2008 (end of follow-up). The combined atherosclerotic outcome was defined as registration of any of the following in the Hospital Discharge Register: ICD-8 codes 400.09–414.99, 432.00–436.90, 437.00–438.09, 440.09–442.99, 444.00–445.09; ICD-10 codes I10–24.1, 24.8–25.9, 63–66.9, 67.2, 67.2A, 67.4, 67.8–67.8B, 69.3, 69.4, 70–70.2A, 70.8–71.9A, 73.9–74.9.

For both cohorts, persons who experienced the outcome of interest before the start of follow-up were excluded from the relevant analysis. Persons who died or emigrated before the start of follow-up were excluded from the study cohort.

Statistical analyses

We used Cox regression with age as the underlying time scale to estimate hazard ratios (HRs) comparing outcome rates in persons with

and without daughters (parent cohort) or sisters (brother cohort) with pregnancy losses. Using age as the underlying time ensured that we compared rates in persons of the same age. In addition, all comparisons were conducted within 3-year strata of birth year, so that outcome rates were compared for persons from similar birth cohorts. Parent cohort analyses included sex as an additional internal stratifying variable, such that fathers were compared with fathers and mothers with mothers.

Since a tendency to pregnancy loss might be heritable, family size could reflect a family's predisposition to pregnancy loss. Therefore, in one set of analyses, we adjusted for total number of children/siblings by estimating HRs within strata of family size—e.g. among parents with two children—and then combining the stratum-specific estimates into a single adjusted estimate. In separate analyses, we stratified by number of daughters/sisters contributing pregnancies.

Because miscarriages in older women are more likely than in younger women to be due to fertility problems, congenital abnormalities, and other age-related issues unrelated to any mechanism that might link miscarriage and ischaemic/atherosclerotic outcomes, we conducted sub-analyses where we considered only miscarriages occurring at <40 years of age as contributing to parental exposure to miscarriages among daughters.

Information on cardiovascular risk factors often shared by family members (alcohol consumption, physical inactivity, smoking, high BMI, hypertension, diabetes, and dyslipidaemia) was either unavailable in Danish registers or incomplete. However, such factors could only have meaningfully confounded our results if they are also (i) shared to a significant extent by family members and (ii) risk factors for pregnancy loss. Smoking best fulfilled these conditions: smoking is strongly associated with cardiovascular disease (relative risks estimated to be 1.8 and 2.1 for men and women, respectively[11]) and also modestly associated with pregnancy loss (odds ratio 1.3[12]); furthermore, smoking behaviours are often shared within families.[13] We therefore conducted sensitivity analyses to determine the degree to which unmeasured confounding by smoking—as the most likely potential confounder of our associations—might have biased the unadjusted HR estimates, using the method outlined by Greenland and Lash.[14] We used relative risk estimates from Refs 11,12, applied the extreme assumption that all offspring of smokers themselves smoked, and varied the population prevalence of smoking between 30 and 50%.

Results

For the miscarriage analyses, our parent cohort included >1 million persons (MI analyses: 1 016 755; IHD analyses: 1 002 734; CVI analyses: 1 025 814) followed for >9.7 million person-years. For the stillbirth analyses, our parent cohort included >985 500 persons (MI analyses: 999 647; IHD analyses: 985 522; CVI analyses 1 008 448) followed for >12 million person-years. Our brother cohort included 435 284 men followed for almost 5 million person-years.

We identified >52 000 MIs (miscarriage analyses: 53 363; stillbirth analyses: 52 360), >111 600 instances of IHD (113 744 and 111 640, respectively), and >33 100 CVIs (33 828 and 33 109, respectively) in the parent cohort during follow-up. There were 15 625 incident atherosclerotic outcomes in the brother cohort during follow-up.

Associations between miscarriages in daughters and ischaemic outcomes in their parents

When we adjusted for number of offspring, persons whose daughters had miscarriages had modest but statistically significant increases in the rates of all three ischaemic outcomes, compared with persons whose daughters had no miscarriages (*Figure 1A–C* and Supplementary material online, *Table S1*). Rates tended to increase as the total number of miscarriages among daughters increased. This pattern was even more evident when we combined the three ischaemic outcomes (IHD, MI, and CVI) into a single endpoint (*Figure 1D* and Supplementary material online, *Table S1*); of particular interest, persons with ≥3 miscarriages among their daughters had a 15% increased rate of an ischaemic outcome, compared with persons with the same number of children whose daughters had never miscarried. When we subdivided the '≥3 miscarriages' group, we found the following associations with parental ischaemic outcomes (combined): 3 miscarriages among daughters, HR 1.15, 95% confidence interval (CI) 1.09–1.21; 4 miscarriages among daughters, HR 1.13, 95% CI 1.02–1.24; ≥5 miscarriages among daughters, HR 1.24, 95% CI 1.09–1.42. Hazard ratio magnitudes did not differ meaningfully by parental sex for any of the outcomes (see Supplementary material online, *Table S2*).

When we stratified by number of daughters contributing pregnancies, we saw the same pattern (*Figure 1A–D* and Supplementary material online, *Table S1*). For MI and IHD, the associations with increasing numbers of miscarriages were strongest among persons with ≥3 daughters, whereas for CVI, association magnitudes were similar regardless of number of daughters contributing pregnancies to the analyses. However, the CVI analyses were underpowered relative to the MI and IHD analyses (as reflected by the comparatively wider CVI confidence intervals); the CVI estimates are consistent with the pattern we observed for MI and IHD.

Restricting our analyses to miscarriages occurring before 40 years of age resulted in only minimal changes to HR magnitudes and had no effect on our conclusions (see Supplementary material online, *Table S3*). When we evaluated the effect of unmeasured confounding by smoking on our estimates, we found that our highest estimates (those for associations with miscarriages in families with many daughters contributing pregnancies) were reduced by ≤10%, even when we assumed a population prevalence of smoking of 50% (see Supplementary material online, *Table S4*).

Since the number of affected daughters might also be informative, we further looked at associations between the number of daughters with miscarriages and parental rates of the combined ischaemic outcome. Although there were relatively few families with >1 affected daughter, our results suggested that having ≥2 daughters with miscarriages was associated with increased parental rates of an ischaemic outcome, particularly among persons with many daughters (*Table 1*).

Rates of ischaemic outcomes associated with stillbirths in daughters

Although there were far fewer stillbirths than miscarriages among daughters, stillbirths among daughters were also associated with parental rates of ischaemic outcomes (*Table 2*). Analyses adjusted for number of children showed that stillbirths among daughters

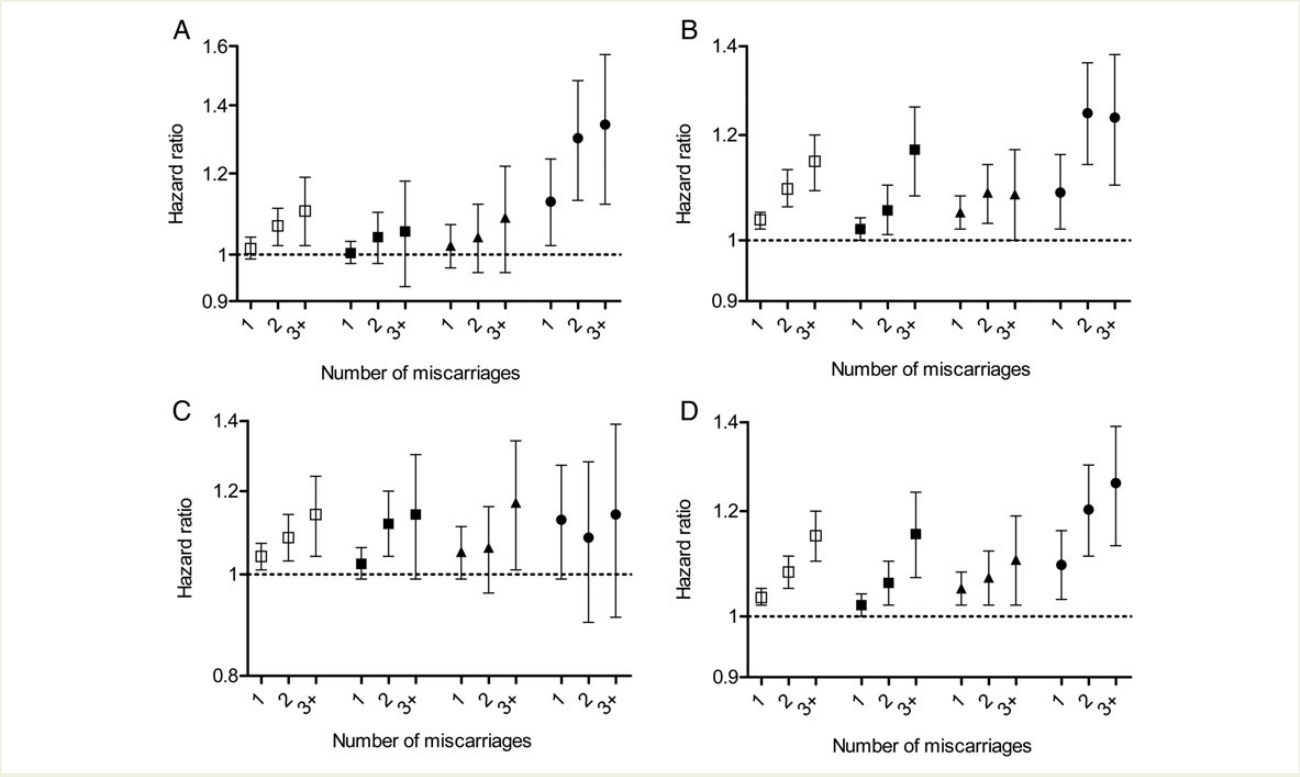

Figure 1 Hazard ratios with 95% confidence intervals for myocardial infarction (*A*), ischaemic heart disease, excluding myocardial infarction (*B*), cerebrovascular infarction (*C*), and the combined ischaemic outcome (*D*) in parents by total number of miscarriages among daughters. The overall hazard ratios (open squares) combine stratum-specific estimates for parents with different numbers of daughters with miscarriages, adjusted for total number of offspring. Separate hazard ratios then estimate the association between number of miscarriages and the outcome for persons with the specified number of daughters contributing pregnancies to the analyses (closed squares, 1 daughter contributing pregnancies to the analyses; triangles, 2 daughters contributing; circles, 3 daughters contributing). The combined ischaemic outcome was defined as ischaemic heart disease, myocardial infarction, or cerebrovascular infarction, whichever came first during follow-up.

Table 1 Hazard ratios for ischaemic outcome (ischaemic heart disease, myocardial infarction, or cerebrovascular infarction) in parents by number of daughters experiencing miscarriages, stratified by number of daughters contributing pregnancies

Number of daughters contributing pregnancies	Number of daughters with miscarriages	Person-years ($\times 10^3$)	Number of events	Hazard ratio	95% confidence interval
1	0	7918	83 729	1	(ref)
	1	1690	19 523	1.03	1.01, 1.05
2	0	1484	17 161	1	(ref)
	1	678	8626	1.05	1.03, 1.08
	2	85	1133	1.07	1.01, 1.14
≥3	0	212	2660	1	(ref)
	1	147	2105	1.10	1.04, 1.16
	2	40	689	1.28	1.18, 1.40
	≥3	5.6	90	1.16	0.94, 1.43

All hazard ratios were adjusted for parental sex and birth year.

were associated with modest (up to 15%) increases in parental rates of MI, IHD, and CVI (*Table 2*). As with miscarriages, when we stratified by number of daughters contributing pregnancies to the analysis, the association between daughters' stillbirths and parental rates of ischaemic outcomes appeared to be strongest in persons with ≥3 daughters, at least for IHD and CVI (*Table 2*).

Table 2 Hazard ratios for ischaemic outcomes in parents by total number of stillbirths among daughters

Number of stillbirths among daughters contributing pregnancies[a]	Myocardial infarction				Ischaemic heart disease				Cerebrovascular infarction			
	Person-years ($\times 10^3$)	Number of events	HR	95% CI	Person-years ($\times 10^3$)	Number of events	HR	95% CI	Person-years ($\times 10^3$)	Number of events	HR	95% CI
Adjusted for number of children[b]												
0 stillbirths	12 485	51 794	1	(ref)	12 022	110 411	1	(ref)	12 699	32 762	1	(ref)
≥1 stillbirths	117	566	1.14	1.05, 1.24	111	1229	1.15	1.08, 1.21	120	347	1.07	0.96, 1.18
Stratified by number of daughters[c]												
1 daughter												
0 stillbirths	9810	40 162	1	(ref)	9468	84 300	1	(ref)	9972	25 265	1	(ref)
≥1 stillbirths	71	344	1.17	1.05, 1.30	68	707	1.14	1.06, 1.22	72	220	1.15	1.00, 1.31
2 daughters												
0 stillbirths	2269	9773	1	(ref)	2169	21 704	1	(ref)	2313	6262	1	(ref)
≥1 stillbirths	37	172	1.08	0.93, 1.26	35	391	1.10	0.99, 1.21	38	87	0.84	0.68, 1.03
≥3 daughters												
0 stillbirths	406	1859	1	(ref)	385	4407	1	(ref)	415	1235	1	(ref)
≥1 stillbirths	9	50	1.17	0.89, 1.55	9	131	1.32	1.11, 1.57	10	40	1.37	1.00, 1.87

All hazard ratios were adjusted for parental sex and birth year.
HR, hazard ratio; CI, confidence interval.
[a]Total stillbirths among all daughters contributing pregnancies.
[b]Total number of children the parent has.
[c]Number of daughters contributing pregnancies to the analyses.

Rates of atherosclerotic outcomes associated with miscarriage in sisters

Despite our use of a combined atherosclerotic outcome, there were relatively few events in the brother cohort. Nevertheless, we observed the same pattern in the brother cohort as in the parent cohort: as the number of miscarriages among sisters increased, so did brothers' rates of atherosclerotic outcomes (*Table 3*). An analysis adjusted for number of siblings showed that men whose sisters had ≥2 miscarriages among them had a 10% increase (HR 1.10, 95% CI 1.03–1.17) in rate of atherosclerotic outcomes, compared with men with the same number of sisters among whom there were no miscarriages. When we stratified by number of sisters contributing pregnancies to the analysis, the association between increasing numbers of miscarriages and rate of atherosclerotic outcomes appeared to be strongest among men with ≥3 sisters, although we had insufficient power to state this definitively (*Table 3*). Restricting our analyses to the oldest brother in each family (to eliminate any influence of correlated outcomes among brothers) did not produce meaningful changes in our results (see Supplementary material online, *Table S5*).

Discussion

Our study suggests that miscarriages and stillbirths among daughters are associated with parental risk of ischaemic outcomes (IHD, MI, and CVI). The greater the combined number of miscarriages among daughters, the greater the parental risk. More importantly, the greater the number of daughters with miscarriages, the greater the parental risk of ischaemic outcomes. There was a similar tendency among men whose sisters had had miscarriages, with the risk of atherosclerotic outcomes increasing as the total number of miscarriages among sisters increased, particularly for men with many sisters.

A previous study suggested that before a first live birth, repeated miscarriages in individual daughters were associated with increased parental rates of IHD.[6] Its many strengths aside, the study had two unfortunate limitations. First, although the study cohort included the parents of almost 75 000 women, power to examine associations with outcomes other than IHD was limited by insufficient numbers of parental outcomes, making it difficult to establish whether only IHD is mechanistically linked with miscarriage or whether the link extends to other ischaemic/atherosclerotic diseases. Secondly, by including parental outcomes that occurred before a daughter had a miscarriage, the study conditioned on future events in daughters, which could have produced unreliable results.

With our cohort of >1 million parents, we had enough power to exclude parents with outcomes preceding the first pregnancy in a daughter and conduct analyses stratified by the number of daughters contributing pregnancies (to account for any familial tendency to miscarry and allow us to compare the experiences of similarly sized families). We also had sufficient power to look at a man's overall risk of atherosclerotic outcomes associated with miscarriages in his sisters. We expanded the exposure definition used by Smith *et al.*[6]—miscarriages before first live birth—to include non-consecutive miscarriages and examined family patterns of miscarriage, rather than

Table 3 Hazard ratios for atherosclerotic outcomes in brothers by total number of miscarriages among sisters

Number of miscarriages among sisters contributing pregnancies[a]	Person-years (×10^3)	Number of events	Hazard ratio	95% confidence interval
Adjusted for number of siblings[b]				
0 miscarriages	4840	11 822	1	(ref)
1 miscarriage	1046	2833	0.99	0.95, 1.03
≥2 miscarriages	278	970	1.10	1.03, 1.17
Stratified by number of sisters[c]				
1 sister				
0 miscarriages	4125	9843	1	(ref)
1 miscarriage	758	1973	0.99	0.94, 1.04
≥2 miscarriages	154	509	1.06	0.97, 1.16
2 sisters				
0 miscarriages	617	1690	1	(ref)
1 miscarriage	237	702	1.00	0.91, 1.09
≥2 miscarriages	91	327	1.12	0.99, 1.26
≥3 sisters				
0 miscarriages	96	289	1	(ref)
1 miscarriage	52	158	0.95	0.78, 1.15
≥2 miscarriages	33	134	1.17	0.95, 1.44

All hazard ratios were adjusted for birth year.
[a]Total number of miscarriages among all sisters contributing pregnancies.
[b]Total number of siblings the man has.
[c]Number of sisters contributing pregnancies to the analyses.

focusing on number of miscarriages in individual daughters. Despite somewhat thin data for CVI in families with many daughters and for atherosclerotic events among brothers, our analyses produced very suggestive findings.

Multiple miscarriages in a single daughter were associated with an up to 15% increase in parental risk of ischaemic outcomes, supporting the common underlying aetiology hypothesized for at least some miscarriages and atherosclerotic conditions not only in the heart but also in the brain. The finding that persons with many daughters with miscarriages had even greater increases in risk of ischaemic outcomes is even more indicative of a joint family predisposition to both types of events. Association magnitudes of similar strength for fathers and mothers, and similar patterns of association for atherosclerotic outcomes in brothers, further support this contention. Associations for mothers, but not fathers, might suggest a link operating via a familial tendency towards pregnancy loss, whereby mothers' own losses predisposed them to atherosclerotic outcomes due to physiologic changes associated with pregnancy loss. However, this mechanism cannot explain associations in fathers and brothers.

Corresponding analyses involving stillbirth were limited by the infrequency of late pregnancy losses. However, we observed similar patterns for stillbirth and parental risk of ischaemic outcomes, hinting that there is likely also an aetiologic link between some types of stillbirth and atherosclerotic conditions.

Our findings are especially noteworthy in light of the fact that we observed consistent associations despite the 'noise' from pregnancy losses due to causes unrelated to atherosclerosis risk (e.g. congenital abnormalities, uterine structural problems, infection). Our findings were robust to the exclusion of miscarriages in women $\geq$40 years of age, which are more likely than miscarriages in younger women to be due to age-related issues and less likely to be linked to atherosclerosis.

The sensitivity and specificity of registered diagnoses are crucial to the validity of register-based study results. Registration of MI, CVI, and miscarriages is fairly complete and the validity of registered diagnoses is excellent,[15–17] minimizing the likelihood that these conditions were misclassified. However, the sensitivity for the less acute outcomes included in our combined atherosclerotic outcome is probably low, as general practitioners, whose diagnoses are not registered in the Hospital Discharge Register, typically diagnose, e.g. primary hypertension. Consequently, registered diagnoses probably represent serious cases requiring hospital contact or serendipitously noted co-morbidities. On the other hand, the specificity of these diagnoses is probably excellent, given that they are not registered lightly. Therefore, bias due to misclassification in the combined atherosclerotic outcome—predominantly due to mislabelling of persons with non-acute atherosclerotic outcomes as healthy—was most likely negligible.

Mothers and daughters may share a tendency to miscarry, suggesting that the observed associations between miscarriages in daughters and ischaemic outcomes in mothers may have been confounded by the mothers' own history of miscarriage (for which information was lacking). To reduce potential confounding by familial clustering of miscarriage, we estimated effects within strata of family size, such that parents were only compared with other parents whose families were of the same size. Furthermore, our observation that the strength of association was the same for mothers and fathers argues against such confounding.

Information on some behaviours and conditions often shared by family members and potentially associated with both pregnancy loss and cardiovascular disease was unavailable or incomplete. However, the results of our sensitivity analyses suggested that while adjustment for smoking might have reduced our estimates slightly, family smoking habits were insufficient to explain the observed associations, particularly in families with many daughters and many miscarriages, even with the extreme assumption that all offspring of smoking parents themselves smoked (i.e. 100% heritability). Since other behavioural cardiovascular risk factors (e.g. obesity, alcohol consumption) are less strongly associated with cardiovascular disease or pregnancy loss than smoking, and/or are less likely to cluster in families, these factors were also unlikely to explain away our findings. Although confounding by smoking (or other cardiovascular risk factors) cannot absolutely be ruled out, we further note that when Smith *et al.*[6] adjusted for social and behavioural variables (including smoking), their results changed very little. Consequently, shared familial behaviours, including smoking, are probably not to blame for the observed co-aggregation of miscarriage and IHD in families.

Our results add to the growing body of evidence indicating that pregnancy losses are associated with ischaemic disease in multiple organ systems and probably even with a broader range of atherosclerotic diseases, implying that at least some pregnancy losses share underlying aetiologic mechanisms with atherosclerotic diseases. Evidence of familial co-aggregation of these conditions (i.e. evidence that an association exists not only within individual affected women but also across their first-degree relatives) further suggests that this mechanism may have a genetic or epigenetic component, although a shared behavioural mechanism cannot be definitively ruled out. Our findings also indicate that in families with atherosclerotic disease, women may be predisposed to pregnancy loss. Conversely, pregnancy losses in first-degree relatives may have implications for an individual's atherosclerotic disease risk.

Identification of pathways common to pregnancy loss and atherosclerotic diseases will improve our understanding of these conditions and may lead to improved treatment. Genetic variants or epigenetic modulation causing changes in endothelial function,[18] vasoreactivity,[18] inflammatory or immune responses,[19–21] or coagulation profiles,[19,22,23] are likely culprits, but hitherto unknown mechanisms could also be responsible. Specific candidates include genetic variants that change concentrations of, or receptor affinities for, nitric oxide, vascular endothelial growth factor, complement system components, and factors involved in the platelet activation cascade or the metabolic syndrome. Phenotypic characterization of both pregnancy losses and atherosclerotic diseases in families exhibiting both traits, and genotyping/sequencing studies in families with several affected relatives, are logical next steps toward identifying the underlying link between pregnancy loss and atherosclerotic disease. With further refinements, our findings could also inform tests of the impact of pregnancy losses, to oneself and to a first-degree relative, in algorithms to evaluate personal risk of atherosclerotic disease.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Authors' contributions

L.J.D. and J.S.: performed statistical analysis; H.A.B., H.B., and M.M.: handled funding and supervision; M.F.R. and I.B.: acquired the data; M.F.R., H.B., M.M., and H.A.B.: conceived and designed the research; M.F.R. and H.A.B.: drafted the manuscript; M.F.R., L.J.D., I.B., H.B., J.S., M.M., and H.A.B.: made critical revision of the manuscript for key intellectual content.

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Conflict of interest: M.F.R. worked at Statens Serum Institut from 2010 until 2014, but is currently employed by Novo Nordisk A/S. H.B. has received lecture fees from MSD, AstraZeneca, Shire, Novartis, and Pfizer. None of the above-mentioned firms played any role in any aspect of the study. None of the remaining authors have any potential conflicts of interest to declare.

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