Inherited susceptibility to miscarriage: a nested case-control study of 31565 women from an intergenerational cohort

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Disclosure of interests

This study forms part of the thesis of AW for her PhD at the University of Aberdeen. There are no conflicts of interest to disclose.

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Condensation:

There may be an inherited susceptibility to miscarriage transmitted from mothers to daughters.

Short title

Inherited susceptibility to miscarriage

AJOG at a Glance

A –This study investigated if daughters were at higher risk of miscarriage if their mother had a history of miscarriage.

B – There may be an inherited risk of miscarriage transmitted from mothers to daughters.

C – This intergenerational study is the largest population-based study investigating familial predisposition to miscarriage between mother-daughter pairs.

Keywords

Familial, family history, inheritance, inherited predisposition, intergenerational, miscarriage, mother-daughter pairs, recurrent miscarriage, hereditary

Abstract

Background

Miscarriage can be a devastating outcome for couples and most miscarriages are unexplained. Many adverse obstetric outcomes are thought to be inherited such as pre-eclampsia, preterm birth and growth restriction. It is possible these conditions could share similar pathophysiological mechanisms with miscarriage such as endothelial dysfunction. Therefore, it was hypothesised that there could be a susceptibility to miscarriage transmitted from mother to daughter.

Objective

This study aimed to investigate the association between a maternal history of miscarriage and the risk of miscarriage in daughters.

Study design

A case-control study nested within an intergenerational cohort was conducted. Mother-daughter pairs were identified from the intergenerational cohort within the Aberdeen Maternity and Neonatal Databank (AMND), United Kingdom. A mother's history of miscarriage was the exposure. The primary outcome was miscarriage in daughters. There were 31, 565 mother-daughter pairs eligible for inclusion. A population average model using Generalised Estimating Equations (GEE) with robust standard errors was used to estimate odds of a mother's history of miscarriage in daughters with a miscarriage compared to daughters with only livebirths. This method accounted for clustering of daughters within mothers and multi-adjusted analyses were performed to include confounders at the daughter's pregnancy level.

Results

Daughters who miscarried had 11% greater odds of being born to mothers with a history of miscarriage (adjusted Odds Ratio (aOR) 1.11; 95% Confidence Intervals (95% CI) 1.01 to 1.22). Daughters with recurrent miscarriage (two or more) were also more likely to be born to a mother with a history of miscarriage (aOR 1.25; 95%CI 1.04 to 1.49).

Conclusions

There may be an inherited predisposition to miscarriage transmitted from mothers to daughters. Future research should investigate genetic or familial environmental factors which may predispose women to miscarriage.

Introduction

Miscarriage is the commonest complication of pregnancy, but it is often unexpected, can be devastating and unexplained. In the United States of America, 13.5% of pregnancies end before 12 weeks due to spontaneous complications including miscarriage.[1] Miscarriage is defined as a spontaneous pregnancy loss occurring at less than 24 weeks' gestation in the United Kingdom.[2] Miscarriage could be an isolated event or can occur repeatedly. It is generally accepted that 1 in 5-6 pregnancies end in miscarriage but recurrent miscarriage affects 1-2% of couples.[3,4] One in four women experience miscarriage before the age of 39.[5] Miscarriage is associated with greater obstetric and perinatal risk in subsequent pregnancies.[6,7] Therefore it is pertinent that we try to understand the aetiology and determine women who may be at increased risk of miscarriage.

The pathophysiology of miscarriage may be different depending on the number of miscarriages experienced, the gestational age when the loss occurs, the fetal genotype and the mother's health at the time of an individual pregnancy. Equally it is possible that parents – mothers and fathers – may possess characteristics, such as an inherited genetic predisposition or tendency to particular environmental or lifestyle factors, which increases the risk of miscarriage or recurrent miscarriage. Approximately half of miscarriages are thought to be secondary to fetal chromosomal abnormalities[8] however most of the remainder are unexplained. Several genetic mutations have been associated with a parental predisposition to unexplained miscarriage including genes involved in immunity, coagulation, metabolism and angiogenesis.[9-11] Serum markers of endothelial dysfunction are increased in non-

pregnant women with a history of recurrent miscarriage and women with a history of severe pre-eclampsia.[12] This suggests that there could be similar pathophysiological mechanisms involved in miscarriage and disorders such as pre-eclampsia. Polymorphisms in vascular endothelial growth factor (VEGF) gene have been associated with recurrent miscarriage.[13] Women with recurrent miscarriage have been found to have a greater risk of preterm birth in subsequent pregnancies.[14,15] This could reflect shared pathophysiology between miscarriage and preterm birth. Pre-eclampsia and preterm birth have been shown to be at least partly hereditary.[16-21] Therefore, it is possible that inherited factors could have a role in miscarriage or recurrent miscarriage. It is also possible that some couples may be predisposed to repeated losses due to a predisposition to pregnancies with fetal chromosomal aberrations.

The aim of this study was to investigate if a maternal history of miscarriage was associated with an increased risk of miscarriage in daughters.

Materials and Methods

Study design and conduct

A nested case-control study within an intergenerational cohort of mother-daughter pairs from the Aberdeen Maternity and Neonatal Databank (AMND)[22] was conducted. The study design and statistical methods described in this paper are similar to our previously published work.[23] The study is reported in accordance with the STROBE statement for observational studies.[24] The population consisted of all mother-daughter

pairs who each had pregnancies delivered (livebirths or miscarriages) from 1949 until 2016 at Aberdeen Maternity Hospital, Scotland, UK. Mothers who had pregnancies between 1949 and 2000, and daughters who had pregnancies between 1965 and 2016 were included. Mother-daughter pairs were identified by deterministic matching using unique Scottish Community Health Index (CHI) numbers where available or probabilistic matching on surname (daughters' maiden name), post code and dates of delivery by the AMND data management team at the University of Aberdeen, UK. An anonymised dataset was given to researchers for analysis. Only singleton births in both mothers and daughters were included. Mothers who gave birth to live born sons but not daughters were excluded. Miscarriage was defined as a spontaneous pregnancy loss at a gestation of less than 24 weeks. The AMND holds routinely collected information recorded within the medical records of hospital treated miscarriages only.

Cases were defined as daughters with a history of at least one miscarriage in any of their pregnancies. Controls were defined as daughters who only ever had a history of live births. The exposure was a mother's history of miscarriage, and secondly, a mother's history of pregnancy loss at any gestational age (miscarriage or stillbirth). A subgroup analysis was carried out to investigate the risk of recurrent miscarriage (daughters with history of 2 or more miscarriages) compared to controls. This included daughters with two miscarriages or more in any pregnancy whether consecutive or non-consecutive. Individual daughters were included and the first pregnancy characteristics were used for both cases (first miscarriage) and controls (first livebirth) in the main analyses as well as in subgroup analyses.

All eligible women recorded within the AMND were included. Assuming a 20% prevalence of miscarriage in the unexposed group a power calculation using nQuery advisor software {(nQuery (2017) Statistical Solutions Ltd, 4500 Avenue 4000, Cork Airport Business Park, Cork, T12 NX7D, Ireland)} showed that there was 99% power to detect a difference in prevalence of 5% in 4284 daughters of mothers with at least one miscarriage compared to 26212 daughters with a mother with all livebirths, with p=0.05 in a two-sided test. After taking account of the clustered data structure, with large numbers of mothers, small numbers of daughters per mother, and assuming very small Intraclass correlation (ICC)), the power of the study was expected to be over 90%.

Statistical analysis

All data were stored and analysed using SPSS software (*IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp*.). The analyses were carried out under a multilevel framework, using a population average model[29-31] with Generalised Estimating Equations (GEE) to account for the clustering of multiple daughters (level 1) nested within the same mother (level 2). Specifically, the robust standard errors of the regression co-efficients were estimated by specifying a working exchangeable correlation structure which assumes that the risk of miscarriage is the same in any daughter if the mother had history of miscarriage. Unadjusted and adjusted analyses were carried out to determine associations between maternal history of miscarriage and a daughter's history of miscarriage after adjusting for socio-demographic characteristics. Odds Ratios (OR) and 95%

confidence intervals (95%CI) were calculated. This method was used for the main analysis and sub group analysis. A p-value of less than 0.05 was considered to be statistically significant.

Potential confounders included at the daughter's level were adjusted for in a multivariable model were: daughter's age at delivery or miscarriage, smoking status (non-, ex- and current smoker), deprivation category[25] (most deprived (4-6) and least deprived (1-3)) and year of delivery. Deprivation category[25] is a Scottish measure of socioeconomic deprivation using national information on factors such as income, employment, health, education and housing, whereby 1 represents the least deprived and 6 represents the most deprived. Further information on the collection and coding of variables included within in the AMND is available in a similar study published previously.[23]

Missing values

Where >5% of covariate data were missing, values were aggregated from complete data in another of the same daughter's pregnancies. There were no missing data for outcome or exposure status. Aggregated missing data were used for daughter's smoking status and deprivation category only. Complete case analysis was then carried out using the aggregated covariate data. Where there was more than one pregnancy record available for the same daughter from which to aggregate data:

i. maximum recorded deprivation category score was used (highest value representing most deprived)

ii. ‘smoker’ was accepted over ‘ex-smoker’ and ‘non-smoker’;

Results

Daughters with a history of one or more miscarriages (n=5161) were compared to daughters with a history of only live births (n=26404). The flowchart of the selection process is shown in Figure 1. Demographic characteristics of daughters with a history of miscarriage were compared to those who only had live births (Table 1). Daughters with a history of miscarriage were significantly more likely to be older, smokers and socioeconomically deprived at the time of first miscarriage compared with daughters with a history of only live births. Daughters were then compared according to their mother’s reproductive history (Table 2).

Daughters with a history of miscarriage were significantly more likely to be born to mothers with a history of miscarriage (aOR 1.11; 95%CI 1.01 to 1.22)). A maternal history of a pregnancy loss at any gestation was also significantly associated with daughters who had a history of miscarriage (aOR 1.12; 95%CI 1.02 to 1.22). A mother’s history of 2 or more miscarriages was significantly associated with miscarriage in their daughters in the unadjusted analyses however this was not statistically significant in adjusted analyses. Mother’s history of stillbirth was not associated with daughter’s history of miscarriage. Table 3 demonstrates the results of the subgroup analysis where daughters with 2 or more miscarriages were compared to controls. Daughters with a history of two or more miscarriages (n=1017) had 25% greater odds of having a mother with a history of miscarriage (aOR 1.25; 95%CI 1.04 to 1.49) compared to daughters with only livebirths (controls, n = 26404).

Comment

Principal findings

This large population-based intergenerational study suggests there could be an inherited susceptibility to miscarriage transmitted from mothers to daughters.

Strengths and weaknesses of the study

A major strength of the intergenerational study is the use of registry-based data thereby significantly reducing the risk of recall bias. The AMND is recognised as a robust data source with regular quality checks[22] which contains over 30,000 mother-daughter pairs. It is one of the rare intergenerational databases which holds information on early pregnancy complications. A low outmigration rate in this population (3.8% of the population who have a birth record in AMND later subsequently move out of the area)[22,26] suggests that the results are valid for the population of the North of Scotland and are likely to be generalizable to the wider UK population as well as other high-resource countries with similar access to health care. The nature of this cohort means there is minimal risk of selection bias as the majority of pregnancies for women in the region covered are included. There remains a risk that a small number of mothers and daughters pregnancies may not be recorded within the AMND. The AMND only records clinically recognised miscarriages where women have presented to hospital. There is no record of whether these were biochemical miscarriages or ultrasound confirmed pregnancies. This potentially leads to a bias in the results as different pathological processes could be involved in miscarriages at earlier or later gestations. Given this inevitable exclusion of many women with conservatively managed or very early spontaneous miscarriages who would not have

attended hospital, it is also possible that this has led to an underestimate of the true effect size.

This is the first intergenerational study in this subject area to employ a population average model to adjust for individual daughters nested within mothers ensuring the associations presented are as robust as possible. This statistical method is also discussed in a previously published paper by our group investigating inherited predisposition to stillbirth.[23]

We were able to adjust for confounding factors such as age, smoking, socioeconomic deprivation and year of delivery. BMI was available within the dataset however as less than 2% of BMI measurements were recorded at the time of miscarriage (which was significantly less than that recorded for controls) and as BMI was very likely to change over the reproductive life course, the authors felt it was inappropriate to substitute using either aggregation or multiple imputation. Therefore BMI was not included in the adjusted analyses. By using aggregated data this meant that missing data for covariates including in the adjusted models were <10%. This approach has been published previously by our group.[23] It was deemed an appropriate method for accounting for missing data, as many sociodemographic characteristics will remain the same over the woman's reproductive life course. However, there is a risk if a woman has a single pregnancy recorded and it remains incomplete then there are no other records from which to aggregate data. Aggregated data was used for smoking (original missing data, 27%; after aggregation, 8.7%) and deprivation category (original missing data, 20.6%; after aggregation, 4.7%). As the missing was <10% we

did not proceed to multiple Imputation. We were not able to include all potential confounding factors such as maternal autoimmune disease, stress, caffeine intake, maternal medication or illicit drug use, alcohol intake or body mass index (BMI) as these factors were not available within the dataset which is a significant limitation to the study.

Defining daughters as cases and controls was felt to be clinically relevant as this is how family history would be elicited in a clinical setting, whereby women would reflect retrospectively on their mother's obstetric history. A potential limitation of this study is that confounding factors were not considered at the mother's level. Mother's history of miscarriage could be affected by age, BMI, deprivation and smoking. However, as cases and controls were defined as daughters, confounders were not included at the exposure level (mothers) in this study.

Analysing the first miscarriage (cases) versus the first live born pregnancy (controls) could lead to bias in the results as the first miscarriage may not have been the first pregnancy. We used this method ensuring that cases and controls were only included in the analysis once, with no effect of clustering of multiple pregnancies within daughters. A population average model using GEE was then used to account for clustering of multiple daughters within mothers. With assumption that any inherited risk would be present throughout a woman's reproductive life, the risk of bias was thought to be small. We felt the most appropriate control group was women with a history of only livebirths. Using pregnancy level data or selecting a particular pregnancy such as the first pregnancy is however controversial in obstetric

observational studies.[27] It could be argued that by including only one pregnancy (such as the first pregnancy loss or first livebirth) there is a risk of losing valuable information from the remaining pregnancy records. This is because the first pregnancy may not be reflective of the overall woman's reproductive history and it reduces statistical power.[27] This is a potential limitation to this study.

Another potential caveat to any association seen in this study relates to the study design as it is not possible to prove causation from these results. Furthermore, there were only 236 daughters with three or more miscarriages in our intergenerational study therefore it was likely it was underpowered to detect a difference when considering three of more miscarriages in the daughter. Therefore, daughters with a history of two or more miscarriages were categorised as "recurrent miscarriages" in this study. Consecutive and non-consecutive miscarriage were included in the analysis of 2 or more miscarriages in daughters. Current guidelines on whether recurrent miscarriage should only include consecutive miscarriages varies[3,28,29] and we were unable to determine consecutive miscarriages within this dataset therefore this is a potential limitation. We were unable to ascertain cause of miscarriage for individual daughters within the dataset. As most miscarriages are not investigated in the U.K. this is not routinely collected information for the majority of miscarriages.

Results in the context of what is known

There are very few epidemiological studies which have investigated familial predisposition to miscarriage.[30-41] Compared to the published literature, the results of our intergenerational study results show a more conservative association between a

mother's history of miscarriage and a similar outcome in a daughter than other studies which investigated any family history of miscarriage.[30-41] This likely reflects the robust methods used to account for clustering of multiple daughters within mothers, as well as adjustment for confounding factors such as age and smoking in this study. Of two previous similar studies which specifically investigated maternal history of miscarriage as did our study,[31,37] one [37] found no association – a result which contrasts with our study and another cohort study conducted in China.[31] However, studies varied in quality with few adjusting for confounding factors and all used self-obtained exposure and/or outcome data.

Obstetric conditions other than miscarriage, such as pre-eclampsia, preterm birth and growth restriction[16-21,42] are thought to be inherited through families. A Danish cohort study also found that daughters were at higher risk of an ectopic pregnancy if their mother had experienced an ectopic (Rate Ratio 1.50, 95%CI 1.19-1.88).[43] This suggests the possibility that underlying pathophysiological mechanisms could be genetically inherited. Over fifty maternal genetic polymorphisms have been found to be associated with recurrent miscarriage.[10] It is possible that many genes are involved, or indeed differences in gene expression or signalling may contribute to the risk of miscarriage. Studies investigating human leucocyte antigen (HLA) haplotypes found that sisters who shared the same HLA haplotype had higher rates of miscarriage.[44,45] Couples known to carry a structural chromosomal abnormality have been shown to report a history of two or more miscarriages in their siblings[41]. Women with a history of miscarriage are at increased risk of ischaemic heart disease in later life.[46-48] Endothelial dysfunction has been associated with recurrent miscarriage,[12] pre-clampsia,[12] and is recognised as a pathophysiological mechanism in

cardiovascular disease.[49] Thus women who miscarry may have an inherited predisposition to endothelial dysfunction which could affect the vasculature of the placenta and the process of implantation.

It is also possible that exposure to a detrimental intrauterine environment could negatively affect the offspring's own future reproduction by altering the unborn fetus' DNA predisposing them to reproductive failure. An intergenerational study found that being exposed to cigarette smoke *in utero* increased the risk of miscarriage in the offspring.[50] Thus, intrauterine exposures may be responsible for any intergenerational association seen between mothers and daughters.

Any familial predisposition found may relate to shared environmental exposures or common lifestyle choices among family members. We were able to account for some of these potential factors in our adjusted analyses such as smoking and socioeconomic deprivation, however other factors[51-54] such as medications or illicit drugs, alcohol, caffeine intake or stress would have been preferable. Occupational exposures may also be common between family members who work in similar industries. Exposures such have night shift work have been associated with an increased risk of miscarriage.[55]

Clinical implications

Miscarriage is likely to be multifactorial, but this paper highlights an association with maternal history which has not been widely recognised previously. Our results

highlight the importance of understanding maternal genetic factors as well as other familial factors such as shared environments or lifestyle tendencies that may be associated with miscarriage in order to identify new avenues for research to inform individualised prevention or treatment plans according to the risk status of each woman.

Research implications

There does not appear to be a dose response with increasing number of miscarriages of mother's history of recurrent miscarriage (OR of 1.21 for 2 or more; OR of 1.08 for 3 or more) in our results. This is most likely due to the very small number of daughters with a mother who had 3 or more miscarriages – which may relate to the reduced reproductive success of mothers with 3 or miscarriages i.e. they may be less likely to have live born daughters recorded within the AMND. However, given this finding further epidemiological research using large intergenerational datasets is warranted to confirm or refute our findings which suggest an inherited predisposition to miscarriage transmitted from mother to daughter.

Future research should explore the genetic basis for the inherited predisposition to miscarriage through studies involving candidate genes or genome-wide association studies. Knowledge of appropriate genetic variants could lead to Mendelian randomisation studies to determine the impact of various modifiable risk factors for miscarriage.

Conclusions

There may be an inherited predisposition to miscarriage and recurrent miscarriage transmitted from mothers to daughters. Research now needs to focus on the potential genetic factors or shared environmental factors among family members that could be involved in the pathophysiology of miscarriage.

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Table 1 Comparison of demographic characteristics for daughters with and without a history of miscarriage (N = 31565)

Daughter's characteristic Daughter's characteristic	Daughters – ever had a miscarriage (N=5161) n (%)	Daughters – never had a miscarriage (N=26404) n (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	P-value
Age at delivery in years					<0.001*
<20	1239 (24.0)	7461 (28.3)	1.03 (0.95 – 1.12)	0.82 (0.74 – 0.90)	
21-25	1407 (27.3)	8726 (33.0)	1.00	1.00	
26-30	1343 (26.0)	6678 (25.3)	1.25 (1.15 – 1.35)	1.55 (1.41 – 1.70)	
31-35	737 (14.3)	2900 (11.0)	1.58 (1.43 – 1.74)	2.04 (1.82 – 2.28)	
36-40	342 (6.6)	598 (2.3)	3.55 (3.07 – 4.10)	4.19 (3.57 – 4.91)	
>40	92 (1.8)	41 (0.2)	13.92 (9.59 – 20.19)	15.30 (10.42 – 22.47)	
Missing*	1	0			
Smoking status					
Non smoker	2224 (50.2)	13154 (54.0)	1.00	1.00	
Current Smoker	1768 (39.9)	9059 (37.2)	1.15 (1.08 – 1.23)	1.33 (1.18 – 1.49)	<0.001*
Ex-smoker	439 (9.9)	2152 (8.8)	1.21 (1.08 – 1.35)	1.21 (1.11 – 1.31)	
Missing*	730 (14.1)	2039 (7.7)			
Deprivation category					
Least deprived (1-3)	2050 (45.0)	13364 (52.4)	1.00	1.00	<0.001*
Most deprived (4-6)	2509 (55.0)	12161 (47.6)	1.34 (1.26 – 1.43)	1.56 (1.45 – 1.67)	
Missing*	602 (11.7)	879 (3.3)			

*denotes statistically significant.

**Missing data was not included when calculating proportions.

Multi-adjusted models adjusted for age at delivery, smoking, deprivation, year of delivery and exposure of Mother's history of miscarriage.

Missing covariates where possible aggregated from other pregnancy records from same daughter for smoking and deprivation; thereafter complete case analysis carried out with aggregated values for covariates included.

Table 2 Comparison of mother's reproductive history between daughters with and without a history of miscarriage (N = 31565)

Mother's reproductive history	Daughters – ever had a miscarriage (N=5161) n (%)	Daughters – never had a miscarriage (N=26404) n (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	P-value
Mother's history of miscarriage					
No	4403 (85.3)	22878 (86.6)	1.00	1.00	0.024*
Yes	758 (14.7)	3526 (13.4)	1.12 (1.03 – 1.22)	1.11 (1.01 – 1.22)	
Mother's history of recurrent miscarriage					
None or 1	5012 (97.1)	25782 (97.6)	1.00	1.00	
2 or more	149 (2.9)	622 (2.4)	1.23 (1.03 – 1.48)	1.21 (0.99 – 1.47)	0.064
Mother's history of recurrent miscarriage					
None or up to 2	5129 (99.4)	26255 (99.4)	1.00	1.00	
3 or more	32 (0.6)	149 (0.6)	1.10 (0.75 – 1.62)	1.08 (0.71 – 1.66)	0.718
Mother's history of stillbirth					
No	5042 (97.7)	25834 (97.8)	1.00	1.00	0.489
Yes	119 (2.3)	570 (2.2)	1.07 (0.88 – 1.31)	1.08 (0.86 – 1.36)	
Mother's history any pregnancy loss					
No	4304 (83.4)	22421 (84.9)	1.00	1.00	0.015*
Yes	857 (16.6)	3983 (15.1)	1.12 (1.03 – 1.22)	1.12 (1.02 - 1.22)	

*denotes statistically significant.
Multi-adjusted models adjusted for age at delivery, smoking, deprivation, year of delivery and mother's reproductive history as shown in Table.
Missing covariates where possible aggregated from other pregnancy records from same daughter for smoking and deprivation; thereafter complete case analysis carried out with aggregated values for covariates included.

Table 3 Comparison of mother's reproductive history between daughters with and without a history of recurrent miscarriage (2 or more) (N = 27421)

Mother's reproductive history	Daughters – ever had a miscarriage (N=1017) n (%)	Daughters – never had a miscarriage (N=26404) n (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	P-value
Mother's history of miscarriage					
No	845 (83.1)	22878 (86.6)	1.00	1.00	0.016*
Yes	172 (16.9)	3526 (13.4)	1.32 (1.11 – 1.56)	1.25 (1.04 – 1.49)	

Contribution to authorship

AW wrote the first and subsequent drafts, each of which were reviewed by SohB, SB, PD and EAR. The final draft of the paper was reviewed and edited by all authors. AW, SohB, SB conceived the idea for the intergenerational study and designed the study. PD was involved in initial planning and provided clinical input. AW and EAR performed statistical analyses for the intergenerational study.

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Ethical considerations

Approval to conduct this study was obtained from the AMND steering committee. The AMND has an overall Research Ethics Committee approval (Reference No.:1/0/58-13-NS-0050 North of Scotland Research Ethics committee) which allows data recorded within AMND to be used for steering committee approved research projects. The researchers only had access to an anonymised dataset.