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A study of relative maternal protection against type 1 diabetes in offspring: Statistical analysis plan

Version History

Version:	Version Date:	Changes:
1.0	19/01/2023	1 st version
1.1	24/07/2023	<p>Decision made to change cohorts included: <i>Exclusion of DARE cohort due to insufficient data regarding family history</i> <i>Exclusion of EXE-T1D/MODY cohorts as inclusion criteria meant genetic data skewed towards individuals with higher GRS</i> <i>Inclusion of BDD as additional cohort</i></p> <p>Decision to remove exploratory analyses around HbA1c, DKA and autoantibody profile at diagnosis due to lack of data across cohorts</p>

Researchers:

Lowri Allen,
Peter Taylor,
Annelie Carlsson,
Diane Fraser,
William Hagopian,
Emma Hedlund,
Anita Hill,
Angus Jones,
Jonny Ludvigsson,
Georgina Mortimer,
Suna Onengut-Gumuscu,
Maria Redondo,
Stephen Rich,
Claire Williams,
Kathleen Gillespie,
Colin Dayan,
Richard Oram.

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1. Abbreviations

Acronym	Meaning
BDD	Better Diabetes Diagnosis
BOX	Barts Oxford Family Study
T1D	Type 1 diabetes
T1DGC	Type 1 diabetes Genetic Consortium
T1D-GRS	Type 1 diabetes genetic risk score
GRS2	Most recent iteration of the T1D-GRS
TrialNet PTP	TrialNet Pathway to Prevention

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2. Statistical analysis plan authorship

The author of the statistical analysis plan is the principal researcher (Dr Lowri Allen). The protocol will be finalised and agreed by researchers prior to starting statistical analysis (target spring-summer 2023).

3. Background

Type 1 diabetes (T1D) is a multifactorial disease, representing the end result of a combination of genetic susceptibility and environmental exposures. The risk of T1D is significantly greater amongst first-degree relatives of individuals with T1D (8-15 times higher than in the background population)^{1,2,3,4}. However, studies have consistently shown the risk to be higher (around twice as high) if the affected relative is the father rather than the mother⁵⁻¹³. Comparison with data from siblings has demonstrated this to be due to a relatively lower than expected risk of T1D amongst the offspring of affected mothers. Though maternal protection is only relative, as the risk remains higher than in the background population, it remains clinically significant.

Published studies describing the risk of T1D in the offspring of mothers compared with fathers, have focused specifically on the risk of T1D developing during the childhood and early adult life of offspring (maximum follow up to age 30 years⁶⁻¹³.) It is therefore not known whether maternal T1D offers short-term relative protection against T1D in offspring, effectively delaying disease development as compared with paternal T1D. Alternatively, maternal protection could result in long-term or even lifelong relative protection against T1D as compared with paternal disease.

Limited progress has been made in identifying the mechanism responsible for relative maternal protection against T1D in offspring. Broadly, plausible mechanisms can be divided into genetic and environmental mechanisms. The genetic architecture of diabetes risk is increasingly understood^{2,14,15}. Genetic hypotheses including selective loss of fetuses with high-risk genes in mothers, preferential transmission of susceptibility genes from fathers and/or parent of origin effects influencing gene expression have been proposed as possible explanations for the observed relative maternal protection^{5,16}. However, no study has definitively demonstrated whether genetic mechanisms account for the lower rate of T1D amongst the offspring of affected mothers compared with fathers. Genome wide association studies have identified common genetic variants (single nucleotide polymorphisms) contributing to the genetic predisposition to T1D. The type 1 diabetes genetic risk score (T1D-GRS) describes an individual's polygenic susceptibility to T1D^{17,18}. Comparison of T1D-GRS between individuals with T1D according to whether they have an affected father versus mother is likely to provide important insights into the relative importance of genetics in determining relative maternal protection against T1D in offspring.

An alternative way of trying to elucidate the relative importance of genetic and environmental factors in underpinning maternal protection against T1D in offspring, is to determine whether maternal protection is only observed amongst offspring whose mothers were diagnosed prior to their birth.

A better understanding of mechanisms which offer relative protection against T1D in children of mothers with T1D may offer the opportunity to identify interventions that could reduce the risk of T1D in high-risk individuals, such as children of men with T1D.

3.1 Study aims

Overall aim: To determine whether maternal T1D offers relative protection against T1D in offspring beyond childhood and early life. To further our understanding of the likely underlying mechanism.

The specific objectives are:

- To describe the number and proportion of individuals within each cohort and overall, with affected fathers compared with mothers.
- To determine the proportion of individuals within each cohort and overall, with affected fathers compared with mothers, after subdividing individuals into two subgroups based on age at diagnosis (≤ 18 years and >18 years.)
- To compare age at diagnosis of individuals with T1D with affected fathers versus mothers.
- To determine whether the proportion of individuals with an affected father versus mother is significantly different depending on whether parental diagnosis is made before or after the birth of the individual in question.
- To describe the distribution of GRS2, as a measure of inherited genetic susceptibility to T1D, and determine whether T1D-GRS is significantly different depending on whether an individual with T1D has an affected father versus mother.

4. Study population

As there are a relatively small number of studies with accurate family history and details of progression to T1D, we are applying to multiple datasets with accurate family history and availability of genetic data to generate the GRS2. These datasets include BOX¹⁹, Type 1 Diabetes Genetic Consortium (T1DGC) families²⁰, StartRight²¹, TrialNet Pathway to Prevention (PTP)²², Better Diabetes Diagnosis (BDD)²³.

We plan to perform analyses with each cohort separately but aim to write the project up as one paper, with a similar question asked in multiple datasets as a way to strengthen findings. We are not planning to merge all data to analyse due to different recruitment criteria for each study and the potential for collider bias. Instead, we will use random effects meta-analysis methodology to derive overall estimates of effects across cohorts.

4.1 Cohorts

Dataset	Study population	Recruitment period	Number of individuals eligible for inclusion
BOX ¹⁹	An observational cohort study that recruits individuals diagnosed with type 1 diabetes prior to their 21 st birthday alongside their families in the Oxford Regional health authority area.	Since 1985	3040
Type 1 Diabetes Genetics Consortium (T1DGC) Families Dataset ³	An international, multicenter program as part of which a ‘families dataset’ comprised of affected sibling-pairs and parent-child trios with type 1 diabetes was generated. Participants were diagnosed with type 1 diabetes between ages 0-32 years.	2004-2009	2662
StartRight ²¹	An observational cohort study that recruited approximately 1800 individuals diagnosed with diabetes after the age of 18 years, from 55 UK sites.	2015-2020	561
TrialNet Pathway to Prevention ⁸	An international multi-centre cohort of	2000	1316

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	individuals aged 2.5-45 years who have a first or second degree relative with type 1 diabetes or who have tested positive for at least one type 1 diabetes associated autoantibody outside of the study, and who have not yet been diagnosed with diabetes themselves. Progression to T1D is recorded.		
BDD ²³	A nationwide study in Sweden which has recruited individuals with newly diagnosed type 1 diabetes under the age of 18 years since 2005.	2011	3647

4.2 Inclusion criteria

- *. Confirmed diagnosis of T1D
- *. Family history data available

4.3 Exclusion criteria

- * Not diagnosed with T1D (i.e. diagnosed with a different form of diabetes e.g. type 2 diabetes, or did not progress from autoimmunity to clinical T1D)
- *. Uncertainty around the nature of the relationship with a relative with T1D e.g. documented to have an affected parent but unclear whether the affected parent is the mother or father

4.4 Power calculation

Previously published studies suggest that around 2-3% of individuals with T1D have an affected mother, and 4-6% of individuals with T1D have an affected father. Using, the lower limits of these estimates, and assuming power 80% and 2-sided alpha=0,05, to be adequately powered to detect a significant difference in the proportion of individuals with affected mothers and fathers within our cohort we require n=317.

In pilot data from a T1D cohort but which is not entirely representative of the population in question, the T1D-GRS mean and variance were 16.05 and 2.19. Assuming these values, power 80% and 2- sided alpha=0.05, to detect a 0.5 standard deviation difference in T1D-

GRS we require n=62 in each group. To detect a 0.25 standard deviation difference, we require n=242 in each group.

5. Data

5.1 Baseline characteristics of interest (exposures)

For all cases we will collect the following information where available:

<u>Baseline Characteristic/Exposure</u>	<u>Type of variable</u>
Date of birth	Continuous (dates)
Diabetes date of diagnosis	Continuous (dates)
Age at diagnosis	Continuous (years)
Family history: First degree relative with diabetes Classification Age at diagnosis Insulin treatment Date of diagnosis	Categorical (Yes/No) Categorical (Mother/Father/Sibling) Continuous (years) Categorical (Yes/No) Continuous (dates)

5.1.1 Family history

In all cohorts, family history data has been collected through direct questioning of participants and/or family members. In some cohorts the type of diabetes a relative has may be unknown. Our approach to dealing with this is outlined in subsection 7 (missing data.)

5.2 Outcomes of interest

- i) Proportion of individuals with affected fathers versus mothers
- ii) Age at diagnosis of T1D of individuals with affected fathers versus mothers
- iii) GRS2 of individuals with affected fathers versus mothers

<u>Outcome</u>	<u>Type of variable</u>
Family history	Father affected (Binary – Yes/No) Mother affected (Binary – Yes/No)
Age at diagnosis	Continuous (years)
GRS2*	Continuous

*The GRS2 is based on 67 genetic variants (HLA and non-HLA) associated with increased T1D risk. It is calculated based on the sum of the number of alleles associated with increased T1D risk across single nucleotide polymorphisms multiplied by the natural logarithm of the odds ratio for each allele, alongside HLA-DQ interaction terms. Genotyping was undertaken as part of the original studies.

6. Statistical analyses

6.1. Principal analyses

We will describe the number and proportion of individuals within each individual cohort with an affected father compared with mother. We will calculate an odds ratio based on the odds of an individual having an affected father compared with the odds of having an affected mother. We will use a random-effects meta-analysis to derive an overall estimate of effects across cohorts.

We will subsequently utilise this same approach to compare the results between the following subgroups:

- Individuals diagnosed with T1D ≤ 18 years versus >18 years of age
- Individuals with parents diagnosed before versus after their birth

We will use summary statistics to describe the age at diagnosis of T1D, overall and by subgroup. We will use random effects meta-analyses to derive an overall estimate of the difference in age (in years) between those with affected mothers versus fathers.

We will use summary statistics to describe the GRS2 distribution, overall and by subgroup. We will use random effects meta-analyses to derive an overall estimate of the difference in GRS2 between those with affected fathers versus mothers.

6.2 Potential confounders

We have not identified any potential confounders that would need to be accounted for in our analysis of the proportion of individuals with affected fathers versus mothers.

Parents diagnosed at a younger age are likely to have a longer duration of T1D prior to the birth of offspring. It is therefore possible that if we demonstrate that maternal protection is dependent on maternal diagnosis prior to offspring birth, this could be driven by either a younger age of the mother at diagnosis or a longer duration of maternal disease prior to birth. We do not anticipate having sufficient data/power to facilitate a detailed analysis of the interaction between maternal age, timing of diagnosis and duration of maternal diabetes prior to the child's birth. However, we will aim to address this issue broadly by undertaking the analyses outlined in the exploratory analyses subsection outlined below.

Genetics are likely to be the most powerful determinant of age at diagnosis of T1D. Since this is on the causal pathway between parental T1D and age at diagnosis in the offspring, we will not adjust for this when comparing age at diagnosis between those with affected fathers versus mothers.

The GRS2 is determined at conception and is independent of environmental exposures. We will therefore not adjust for any environmental exposures in our analyses of the GRS2.

6.3 Exploratory analyses

We will use summary statistics to describe the age at diagnosis of T1D of mothers diagnosed before and after the birth of offspring. We will subsequently subdivide mothers diagnosed

prior to birth into two subgroups, using the median age at maternal diagnosis for this group. To determine whether maternal protection is observed in both subgroups, the proportion of individuals with affected mothers will be compared against the proportion with affected fathers diagnosed prior to birth and with a comparable age of diagnosis for both subgroups. Similarly, we will describe the proportion of individuals with an affected father compared with mother for individuals whose parents had a duration of diabetes above and below the median duration of maternal diabetes prior to birth.

7. Missing data

7.1 Missing family history data

Cohorts have been selected for inclusion in this study specifically because they collected family history data. Though all cohorts recorded family history of diabetes, not all cohorts recorded the type of diabetes in relatives. Where it is unclear whether a relative has T1D or another form of diabetes (type 2/gestational etc) we will use the following criteria as a surrogate marker of T1D: relative with insulin (only) treated diabetes. We will compare the results obtained in this way with those obtained when using increasingly restrictive definition of diabetes (treatment with insulin only and age at diagnosis ≤ 25 years, ≤ 30 years, ≤ 35 years, ≤ 40 years.) We would anticipate that this approach will ensure that we minimise the inclusion of relatives with type 2 diabetes or other forms of diabetes. When reporting our findings we will acknowledge the limitations of this approach.

7.2 Missing outcome data

We will describe the proportion of missing outcome data and examine whether the population with missing data are different to those with complete data with respect to demographic and baseline characteristics. We will undertake analysis only in those with outcome data available. We will not use multiple imputation to account for missing data.

8. Software

Statistical analyses will be carried out using Stata version 17.

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