Study Title: One In Seven Gestational Diabetes Study
Sponsor: Prenome
Protocol Number: PRE001/OM001
IRB Submission Number: Pro00049373
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One in Seven

GESTATIONAL DIABETES GENETIC AND SOCIOECONOMIC RISK PROTOCOL

VERSION DATE: 05/05/2021

STUDY SUMMARY

Investigational Agent(s) (Drugs or Devices)	N/A
IND / IDE / HDE #	No
Special Population(s)	Pregnant Women
Sample Size	10,000 Participants
Funding Source	Prenome (Industry)
Indicate the type of consent to be obtained	Electronic Consent Written Consent
Site	500 Lincoln Centre Drive, Foster City, CA 94404
Research Related Radiation Exposure	Yes No ✔
DSMB / DMC / IDMC	Yes ✔ No

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OBJECTIVES

The objective of the **Gestational Diabetes Genetic Socioeconomic Risk Study** is to generate genome wide association study data (GWAS) to calculate polygenic risk scores (PRS) for the development of gestational diabetes in pregnant women. Prenome's GWAS study will be conducted by collecting DNA samples alongside medical and socioeconomic data and applying data science methodology to generate a polygenic risk score algorithm for gestational diabetes. Our hypothesis is that key genetic variants linked to gestational diabetes will be identified, and sociodemographic characteristics may impact epigenetic factors which further contribute to this risk of gestational diabetes. The PRS generated through our study will be combined with an analysis of epigenetic factors to produce a new method for predicting risk of developing gestational diabetes during pregnancy.

BACKGROUND

Gestational diabetes is a condition where high blood glucose levels occur during pregnancy and the mother was not diabetic before pregnancy. Between 6-9% of pregnancies are affected by this disorder. Gestational diabetes is usually diagnosed with a glucose challenge test where a blood glucose level of 190mg/dL or above would be indicative of gestational diabetes. This test usually occurs around 24-28 weeks into pregnancy. If a patient has other risk factors such as obesity or family history of diabetes, the assessment may be performed earlier.

Gestational diabetes increases risk of complications in both the mother and the baby. For the child, it may result in excessive weight at birth, a preterm birth, hypoglycemia, Type II diabetes later in life, and potential stillbirth. For the mother, it drastically increases the risk of high blood pressure, preeclampsia, and developing diabetes later in life. Gestational diabetes can also lead to further pregnancy related complications and increased risk of maternal morbidity and mortality events[1].

Very few genome-wide association studies have been carried out to correlate genetic variants to gestational diabetes, and have identified a small non-exhaustive number of genes linked to the condition. The list comprises genes encoding transcription factors such as TCF7L2 and genes required for glucose processing

such as GCK and GCKR[2]. Many of these genes are also associated with the risk of Type II diabetes. A large number of GWAS have identified genes tightly associated with Type II diabetes including CDKALI 1, FOXO1, GCKR and FTO, and the risk of gestational diabetes and type II is strongly correlated[3]. These variants, along with demonstrated non-genetic risk factors such as obesity and high blood pressure, have demonstrated a clear ability to assess risk of different types of diabetes including gestational diabetes . However, there are currently no PRS for gestational diabetes available as a market product in any country. Additionally, the studies mentioned above do not wholly combine genome data with sociodemographic and lifestyle risks. Very few large scale GWAS studies have focused solely on female participants or pregnancy related complications. Further, existing literature and publications lack diversity among female participants and often focus on specific sub-populations. Prenome intends to conduct a large-scale GWAS in conjunction with the additional socioeconomic qualifications to generate a novel PRS to assess the risk of gestational diabetes with focus on inclusion of diverse populations in our samples. With these added quantifications of qualitative data, Prenome's PRS will be more representative of a patient's personal health and risk of developing gestational diabetes.

STUDY ENDPOINTS

The end point of this study is the generation of a polygenic risk score for gestational diabetes from whole genome sequencing of 10,000 DNA samples and sociodemographic data from currently pregnant or recently (< 1 year since birth) pregnant participants.

STUDY INTERVENTION

This study does not require any intervention procedures. Participants will be recruited for saliva samples for DNA isolation and data collection only.

PROCEDURES INVOLVED

In order to generate a polygenic risk score for gestational diabetes, Prenome will conduct a GWAS by gathering DNA samples and information from 10,000

participants who satisfy one of the following qualifications: women who are currently > 24 weeks pregnant, women who have been pregnant within the last year, women with or without a history of gestational diabetes, and women who currently have gestational diabetes.

Participants will be recruited through OB/GYN clinics, patient recruitment organizations or directly by Prenome through an established waitlist and an online advertising campaign. Interested persons will receive materials fully explaining the study and consent processes in detail through a series of pre-prepared email templates. This will include a video, a detailed description in the consent form, and information displayed on the company or study websites (www.prenome.com or www.gestationaldiabetesstudy.com). All interested persons will have the option to consult Prenome staff about this study by contacting the clinical@prenome.com email. This information will be presented online and in communication emails. Prenome will be accessing participant's personal information through medical records to collect information on impactful risk factors and pregnancy outcome data. Samples will be collected from two participant cohorts: a control group containing 5000 participants with normoglycemic pregnancies and a second group containing 5000 diagnosed with gestational diabetes.

Registered participants may request collection kits to be sent directly to a desired address (personal or clinical recruitment site) and may consent to participate electronically; this is to address safety concerns due to COVID-19. Once recruited into the study, participants will be mailed a DNA sample collection kit (provided by Prenome) containing either an Oragene OGD-510 or OCD-100 saliva tube or Zymo R1210-E saliva tube. These sample collection devices have received FDA 510(k) clearance, and contain chemistry which lyses enveloped viruses such as SARS-CoV2, ensuring subject safety. Participants will register the identifier barcode on the collection device, which will be used to link medical data to their sample. The participant will spit directly into the saliva collection tube for DNA sample collection, and subjects will be asked to complete a questionnaire about socioeconomic demographics and medical history. Access to this questionnaire will

be granted online at the point of sample collection kit registration. This questionnaire will take no more than 30 minutes to complete.

Regardless of the recruitment site, samples will be returned to the Prenome study lead site for processing, DNA sequencing, and data analysis. Research scientists handling participant samples will have access to the unique identifier number on the collection device ONLY. This is to ensure participant privacy and protection of PHI, while also allowing processing to remain blind to participants' diabetic status.

The samples will undergo DNA isolation and library preparation following the DNA/RNA Prep Work Flow with Exome Enrichment using Illumina preparation chemistry. Whole genome and whole exome sequencing will be carried out, sequencing all samples to a minimum coverage of 1X. The sequencing data will be used to identify linkage of associated alleles and variants that determine risk of gestational diabetes, and then tied to medical or socioeconomic data which increases or decreases risk.

This clinical study will result in a novel PRS which can be used to assess risk of gestational diabetes. Any remaining isolated DNA or saliva will be stored in the Prenome BioBank using the de-identified barcode assigned at collection.

DATA AND SPECIMEN BANKING

An anonymised participant ID database with assigned storage space will be used to monitor storage of participant DNA. This database will include information on anonymised ID, DNA concentration, date isolated, estimated destruction date and sequencing status. All isolated DNA will be stored at -80°C with duplicates of each sample held in two separate freezers. DNA samples will be stored for 10 years after collection and then will be destroyed. Final DNA libraries generated from library preparation of participant samples will be stored for 6 months after preparation and sequencing. These samples will also be catalogued by anonymised IDs, along with quality control data and sequencing status. If participants remove themselves from the study, all of their samples will be destroyed upon the exit request.

As a secondary storage method DNA Genotek Oragene or Zymo saliva collection allows for long term saliva sample storage at -20°C. Once appropriate sample volumes have been taken for DNA isolation and library preparation, the remaining sample will be aliquoted into 1ml cryogenic storage tubes as per DNAGenotek guidelines. The samples will be stored and catalogued using the ID assigned at kit registration, and will be stored for 10 years after collection. As with DNA samples, if participants withdraw from the study and request for their samples to be removed, they will be thawed and destroyed.

SHARING RESULTS WITH PARTICIPANTS

As this study is collecting samples retrospectively, the polygenic risk scores calculated in this study will not be returned to participants as their pregnancy outcomes are already defined. However, the results of this study will be published to an open access journal, and updates on the study status will be available on the Prenome Website. No information published will contain personal information or personal PRS outcomes.

STUDY TIMELINES

The One in Seven Gestational Diabetes Risk Study will be collecting samples from participants for a 1 year period, with data analysis and PRS calculation continuing for 1 year after the final sample collection.

INCLUSION AND EXCLUSION CRITERIA

The control cohort will include 5000 adult women who are over 24 weeks pregnant or have given birth to a child within 1 year, who have not received a diagnosis of gestational diabetes and were not diabetic during or before their pregnancy. The diabetic cohort will include 5000 adult women who are over 24 weeks pregnant or have given birth to a child within 1 year, who received any diagnosis of gestational diabetes during their pregnancy. There is no adult age, race or ethnicity exclusion criteria for this study, as our intention is to generate PRS based on diverse populations in line with percentage diversity of the US population. However, minors are not eligible to participate in this study. Male participation is excluded from this

study. Non-english speakers are excluded from recruitment, this is due to the consent process being available in English only.

VULNERABLE POPULATIONS

Vulnerable populations include pregnant women, visually impaired participants, and women with a history of gestational diabetes. No conversations or documentation given to participants will discuss pregnancy termination. information produced in this study will be returned to participants, and will be not used to influence decisions related to termination of pregnancy. No inducements, monetary or otherwise will be offered to influence pregnancy termination. In order to ensure the safety and comfort of these participants, the collection kit aims to be as minimally invasive as possible and available to participants in their homes to reduce Covid-19 exposure in clinical environments. Visually impaired persons will have access to this study, and the instructions will be available in a verbal format to increase understanding.

RECRUITMENT METHODS

Participants will be recruited through two channels. The first is through partnered clinical sites referred to Prenome by healthcare providers, where participants receive obstetric care. Clinicians at these sites will be able to identify qualifying participants and register them through the company or study websites (www.prenome.com or www.gestationaldiabetesstudy.com) which are connected to a HIPAA compliant CRM portal, and initiate their study involvement.

Participants will also be able to directly sign up to this study through a clinical study sign up form featured on the Prenome or study websites (www.prenome.com or www.gestationaldiabetesstudy.com). Any person currently over 24 weeks pregnant or previously pregnant within the last year may register interest in participation on the Prenome website. Prenome will use a series of standardized email templates to communicate with participants at every stage of the study including sign up, consent and kit collection. Interested persons will have access to a video and written materials explaining the study

and consent processes in detail. They will then be able to complete the sign-up and consent process online. Participants may request a consultation with Prenome to discuss the study if they require more information. Information about study participation through this route will be advertised online including social media and pregnancy related blogs. External sites will be given access to the same advertising materials for recruitment.

COMPENSATION FOR PARTICIPATION IN RESEARCH ACTIVITIES

All participants will receive a $20 Uber Eats or Amazon gift card, upon delivery of their saliva sample to the Prenome lead site, for contributing to this research study. The gift card will be emailed to participants by default, but participants may request a physical gift card be mailed to them.

WITHDRAWAL OF PARTICIPANTS

Participants may withdraw from the study at any stage of the process. In order to formally withdraw, participants can request a withdrawal form through the Prenome contact email (clinical@prenome.com) supplied in their communication emails, consent form, collection kit or on the Prenome website. Participants can physically or digitally sign the withdrawal form, and return this to the contact email address or Prenomes mailing address. Upon receiving the withdrawal request, all sequencing, medical and sociodemographic data tied to the participant ID will be removed from the Prenome database and CRM. All saliva, DNA and library samples tied to that participant will be removed from storage and destroyed immediately.

RISKS TO PARTICIPANTS

The risk to participants from study involvement is minimal. Participants will be required to refrain from eating or drinking for 30 minutes before sample collection. Participants will be asked to provide 1ml of saliva into the collection device, this can be collected over 5-10 minutes. Both of these requirements may cause some participant discomfort, but do not pose high risk of participant harm or distress. Participants will be able to collect their saliva samples at home, at a time of their choice to help negate this discomfort.

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ETHICAL CONSIDERATION

This study poses a very low risk to participants, as sample collection is non-invasive and can be done at home reducing risk to unnecessary COVID-19 exposure for a high risk group. Further, no individual study outcomes will be reported to participants, as the pregnancy outcomes will already be determined, reducing the chances of mental stress or harm to participants. The outcomes of this study will allow for the creation of a predictive test for gestational diabetes. This can be used by women to mitigate and reduce the potentially dangerous pregnancy outcomes associated with gestational diabetes such as preterm birth, diabetes later in life and stillbirth, improving the lives of new mothers and their children.

DATA ANALYSIS

Data generated from sequencing will under-go quality control analysis using the basespace software platform produced by Illumina. This will be completed before any other analysis is performed, to ensure high quality DNA sequencing was successful by assessing the QC30 score, clusters passing filter and error rate calculated according to the PhiX spike in from illumina platform protocols.

Once data has passed quality control, the reads generated will be aligned to reference genomes, using illumina's basespace platform to produce BAM files containing aligned reads which can be linked to the index barcodes for each participant. The participant data will be linked to the sequencing data using the individual barcode assigned at sample collection. This data will not contain names or full addresses of participants but will contain medical information and sociodemographic survey information. We will combine the study sequencing data with public genetic datasets (UKBioBank, BioBank Japan) which contain information on pregnant women. This information will be analysed for specific alleles and gene variants that can be linked to gestational diabetes using Python to identify GWAS data. The GWAS data will then be run through a variety of PRS algorithms using Python for Prenome's preprietory algorithm and Allelica for publicly available PRS algorithms.

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DATA MANAGEMENT AND CONFIDENTIALITY

All sequencing data from participant samples will be generated on the NovaSeq 6000. Sequenced data will be transferred from sequencing equipment to password protected cloud storage. This information will be anonymised and catalogued using unique identifiers to protect confidentiality. Genome sequencing data will be checked for quality control management, and then reads will be filtered and aligned to a reference genome.

These aligned reads will be matched to participant medical records, used to generate GWAS data and identify linkage of associated alleles or gene variants which can be correlated with gestational diabetes. This data may be combined with publicly available whole genome sequencing data such as the UKBiobank and Biobank Japan. Once variants have been detected, polygenic risk scores will be generated using publically available algorithms and Prenome's internal algorithms. These scores will be tied to sociodemographic risk factors, generated from the study questionnaire, to calculate a complete risk score composed of genetic and epigenetic factors. All data outcomes and statistical analyses produced by this study will be reviewed by a data monitoring committee (DMC).

Throughout the data analysis process, only assigned data scientists will have access to participant data. All analysis will be performed by Prenome employees, and must be conducted on company hardware which is encrypted and password protected. None of the data generated in this study will be shared or sold to third parties. Any data published to academic or clinical journals will be de-identified and will not include individual participant information.

ECONOMIC BURDEN TO PARTICIPANTS

There is no cost for participation in this study, all costs related to sample collection, data generation and analysis will be covered by Prenome.

CONSENT PROCESS

Research study participants will be able to provide consent via an electronic consent form unless a physical form is requested. This request can be emailed to

clinical@prenome.com. All patients recruited will provide electronic consent using an encrypted online module through Prenome's websites (www.prenome.com or www.gestationaldiabetesstudy.com) and stored within a HIPPA certified CRM. Prenome will have a phone number that visually impaired patients can call to listen to consent documents prior to signing.

The purpose of participant consent forms and contact forms is to ensure that participants are fully aware of the study's methods, goals and risks. The consent form will be written in clear and understandable language, outlining all steps involved in the study as well as information on how participants can exit the study. At any point during the consent process or study, participants can contact Prenome directly (clinical@prenome.com) to set up a call for information or to ask questions about this study.

PROTECTED HEALTH INFORMATION (PHI AND HIPAA)

Prenome's **One in Seven Gestational Diabetes Risk Study** will involve the collection of PHI and HIPAA protected information. Specifically, Prenome will be collecting the participant's age, geographic location in the form of a zip code, and medical record numbers used in this study. Further, pregnancy related outcomes and health data will be extracted from participant medical records or gathered through the sociodemographic questionnaire. No participant names, detailed addresses or unauthorized information will be used for data analysis in this study. Participants will sign a HIPAA and PHI authorization form as part of the consent for this study. No unauthorized personnel will have access to this information, and this information will be accessible only on Prenome devices.

QUALIFICATIONS TO CONDUCT RESEARCH AND RESOURCES AVAILABLE

10,000 participants will be recruited across partner sites, online advertising and waitlist registrations. Currently, Prenome has over a thousand women who have expressed an interest in participating in this study and will undergo recruitment upon IRB approval. Prenome's established waitlist has been accrued from direct recruitment and women expressing interest through our website sign up portal.

The study will have a dedicated team of researchers for a minimum of 1 year. This will include the Principal Investigator, NGS Scientist, Research Associate, Data Scientist and a Recruitment Coordinator. Prenome has access to the appropriate laboratory space, sequencing equipment, reagents and storage facilities to complete the research study. All employees involved will be required to follow SOPs to conduct research protocols.

MULTI-SITE RESEARCH

Participants will be recruited at multiple external sites for this study. All sites will be required to sign a IRB authorization agreement, and must follow the protocols for recruitment outlined by Prenome. Sites will identify and register qualifying participants, and register them using a secured CRM. All external sites will be provided with the advertising materials created by Prenome for social media based advertising.

The materials for sample collection will be provided by the lead site, and shipped to the participant or shipped to external collection sites. All communications with external sites will be carried out via the secured CRM or through a secure and confidential Clinical Prenome email address (clinical@prenome.com). This will be used to notify the lead site of newly recruited participants, reporting any problems with recruitment, or requests to withdraw from the study.

All samples collected will be processed, stored and analysed at Prenome's lead research site. Samples collected in oragene devices which contain stabilizing reagents, as well as reagents which lyse enveloped viruses. Samples collected in OCD-100 or OGD-510 saliva tubes will contain a bacteriostatic reagent, which inhibits the growth of bacteria from time of sample collection to processing. These stabilizing and securing reagents allow for the sample to be shipped securely to the lead site using biohazard-cleared shipping and protects against any risk of SARS-CoV2 transmission. Prenome staff will be responsible for sample accessioning and tracking throughout the study, external sites will not have access to this information.

CONFLICT OF INTEREST

Personnel	Role	Declaration of COI
Barbara Levy	Principal Investigator	Consultant for Flo, AbbVie, Dorsata and Clinical Professor at UCSD.
Sarah Brozio	Head of Research	None
Stevie Cline	Cofounder	Consultant for Natera
Brittany Dismuke	Cofounder	None

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