COVER PAGE

Recall by Genotype: Neuropeptide Stimulation

Clinical Trials ID: NCT05901467

Date: 10/31/2025

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BROAD GOALS

The overall goals of this protocol are to 1) investigate the neuroendocrine control of reproduction, 2) investigate the physiology of kisspeptin administration in the human.

I. BACKGROUND & SIGNIFICANCE

A Shifting Paradigm in Reproductive Endocrinology: Discovery of a GPR54 as a Key Modulator of GnRH Secretion

The hypothalamic hormone GnRH sits atop the reproductive cascade of all mammals as a central driver of pituitary gonadotropin secretion. Its commanding role in this biological hierarchy allows it to control pulsatile gonadotropin secretion, modulate gonadal steroid feedback, and bring about full fertility in the adult. Yet, what turns on GnRH secretion at the time of puberty? And once it is turned on, what sustains the physiological amplitude and frequency so essential for normal reproductive function?

To find modulators of GnRH secretion, we utilized genetic tools in patients with GnRH deficiency (i.e., idiopathic hypogonadotropic hypogonadism [IHH]), a clinical syndrome defined by low sex steroids in the setting of low gonadotropins that results from the failure of a normal pattern of pulsatile GnRH secretion [1,2]. In 2003, we discovered that, **in both the human and the mouse**, mutations in *GPR54* cause hypogonadotropic hypogonadism which can be corrected by the administration of exogenous GnRH [3]. More recently, we identified an activating mutation in *GPR54* in a girl with central precocious puberty [4]. Taken together, these loss- and gain-of-function mutations demonstrate that the kisspeptin/GPR54 pathway is a genetic determinant and important gatekeeper of pubertal function in the human.

Kisspeptin: the Ligand for GPR54

The ligand for GPR54 is encoded by the gene *KISS1* [5-7]. Its full-length gene product, kisspeptin, is a 154-amino-acid protein that is proteolytically processed to a 54-amino-acid peptide, kisspeptin 68-121, initially named metastin based on its ability to inhibit tumor metastasis. Smaller C-terminal fragments of 14, 13, and 10 amino acids can also activate GPR54. The 10-amino-acid (decapeptide) isoform of kisspeptin (kisspeptin 112-121) is the isoform used in this protocol. Although the terms “kisspeptin” and “metastin” can both be found throughout the literature, there have been recent proposals to standardize the nomenclature to “kisspeptin.” The term “kisspeptin” will be used throughout this protocol.

Kisspeptin is a Powerful Stimulator of GnRH-Induced Gonadotropin Release *in vivo*

Kisspeptin peptides are the most powerful stimulators of gonadotropin secretion on a molar basis that have ever been studied in mammalian species including rodents, [8-14] sheep, [14] and monkeys.[15]

- The stimulatory effect of kisspeptin is extraordinarily potent, as intracerebral doses as low as 1 fmol evoke significant LH responses. [8]
- The effects of kisspeptin on LH secretion can be completely abrogated by a GnRH antagonist. [8,15] Thus, kisspeptin acts to stimulate GnRH release, which in turn stimulates LH secretion.
- Kisspeptin is unable to stimulate LH release when given to *Gpr54* knockout mice, suggesting that the stimulatory effects of this peptide are mediated only through its cognate receptor. [16]

- However, kisspeptin is able to stimulate LH release when given to *Kiss1* knockout mice, demonstrating its powerful stimulating effects even in the face of abiding hypogonadism.[16]
- Kisspeptin may have a subtle effect on gonadotropin secretion directly at the pituitary. This is a controversial point in the literature and the effect is modest at best, predominantly limited to synergism with GnRH. [17]
- In hyperprolactinemic mice, which exhibit a form of acquired hypogonadotropism, kisspeptin is able to restore GnRH and LH release. [18]

What does the Discovery of Kisspeptin Mean from a Physiologic Perspective?
Probe of the GnRH Neuron

Because kisspeptin is a powerful stimulus for GnRH secretion, administration of kisspeptin offers a method to directly interrogate the function of GnRH neurons in humans, just as GnRH administration has served as a probe of pituitary gonadotrope function in humans for decades. We hypothesize that, in order to respond to kisspeptin with an increase in serum LH, humans must have a certain "threshold" number of GnRH neurons positioned properly within the hypothalamus, adequate GnRH synthetic capacity, and normal secretory mechanisms of GnRH and LH. Thus, in pathophysiologic states that compromise the integrity of the GnRH neurons, the response to kisspeptin would be reduced or attenuated. Therefore, our goal is to use kisspeptin administration as the first direct probe of GnRH neuronal integrity in human reproductive biology to gain new insights into GnRH neuronal function (or dysfunction) in the context of gene variants that impact central regulation of the reproductive axis.

Kisspeptin Administration to Humans:

The following section 1) lists the various isoforms, doses, and modes of the worldwide experience with administration of kisspeptin in human studies to date, and 2) summarizes key findings from these studies.

Kisspeptin 112-121 (the 10-amino-acid isoform used in this protocol) has been given by our group and others in the following scenarios:

A) Single Boluses (doses: 0.01 – 32 nmol/kg; routes: intravenous (IV), subcutaneous (SC))
- Healthy men[19-22]
- Healthy women (including those taking oral contraceptives and with progestin implants)[19,23,24]
- Healthy postmenopausal women[24]
- Patients with low testosterone and type 2 diabetes mellitus[22]
- Patients with IHH[25,26,27,28]

B) Multiple Boluses (doses: 0.24 – 2.4 nmol/kg IV q1-2 hours for up to 5 boluses)
- Patients with IHH[25,28,29]

C) Brief Infusions (doses: 0.02-0.72 nmol/kg/hr IV for 30 min followed by 0.01-0.36 nmol/kg/hr IV for 60 min)
- Healthy cycling women[19]

C) Long Infusions (doses: 1.1 – 9.5 nmol/kg/hr IV for 7 – 24 hours)
- Healthy cycling women [30]
- Healthy men[20,31]
- Healthy postmenopausal women[31]

- Patients with low testosterone and type 2 diabetes mellitus[22]
- Patients with IHH[26,29,32]
- Women with hyperprolactinemia[33]

In addition, the 54-amino-acid isoform of kisspeptin (kisspeptin 68-121) has also been given in the following scenarios:

A) Single Boluses (doses: 0.15-12.8 nmol/kg SC)
- Healthy women (in all phases of the menstrual cycle)[34,35]
- Women with hypothalamic amenorrhea[36,37]
- Women undergoing ovulation induction[38]
- Women with or at risk of ovarian hyperstimulation syndrome[39,40]

B) Multiple boluses (dose: 6.4 nmol/kg SC for up to 8 weeks)
- Healthy women[41,42]
- Women with hypothalamic amenorrhea[36,37]

C) Brief Infusions (doses: 0.0075-2.4 nmol/kg/hr IV for 1.25-3.75 hours)
- Healthy men[43,44,45,46]

D) Long Infusions (doses: 0.01 – 1 nmol/kg IV and SC for up to 10 hours)
- Healthy cycling women[47]
- Women with hypothalamic amenorrhea[48]

Kisspeptin Safety Data in Humans:

Kisspeptin has a track record of safety in humans. It has been given to over 150 subjects, both healthy volunteers and patients with reproductive conditions, in studies at 3 centers using 2 different isoforms at a wide range of doses.[19-21,34,36,37,43]

Importantly, no serious adverse events related to kisspeptin have occurred in our studies or, to our knowledge, in studies conducted by other investigators. Kisspeptin has been given to women undergoing ovulation induction for *in vitro* fertilization which has resulted in 12 successful pregnancies with no adverse effects[38,49] Kisspeptin has also been given with no adverse effects to subjects with medical conditions such as type 2 diabetes, hypothyroidism, hypertension, hyperlipidemia, and anxiety/depression, and who were taking prescription medications for these conditions.[50] (SB Seminara, unpublished results, J. George, personal communication, March 24, 2013, and C. Jayasena, personal communication, June 15, 2013). Full details are available in the Kisspeptin Investigator Brochure.

Design of the Study

To assess the physiologic response to the IV administration of the C-terminal decapeptide of human kisspeptin (kisspeptin 112-121), frequent blood sampling (12 h) will be performed to provide detailed neuroendocrine characterization of endogenous LH secretion prior to kisspeptin administration and to evaluate the response after kisspeptin administration.

II. SPECIFIC AIM

To employ exogenous administration of kisspeptin to directly interrogate human GnRH neurons *in vivo* using GnRH-induced LH secretion as a surrogate marker of adequate GnRH neuronal fate specification, migration, hormone synthesis, secretion, and action.

Hypotheses:

Patients with a positive but comparatively attenuated response to kisspeptin will have defects in GnRH neuronal fate specification or migration that reduce either the total complement of GnRH neurons or the coordinated activity of the GnRH neuronal population (e.g. mutations in the genes encoding fibroblast factor receptor 1 [*FGFR1*]).

Individuals ascertained from the MGB Biobank with rare variants in genes that have been linked to reproductive axis function will have attenuated endogenous LH pulse amplitude or pulse frequency.

III. SUBJECT SELECTION

The goal of this protocol is to administer kisspeptin to subjects carrying genetic variants of interest and relevant control subjects.

Enrollment Targets

Variant-carrying subjects:

- Variant-carrying men and women (n=120: 60 males, 60 females to have 50 males and 50 females complete the study)

Inclusion Criteria

All subjects will meet the following criteria

History:

- genetic variant(s) predicted to be linked to reproductive axis (dys)function
- all medical conditions stable and well controlled
- for men
    - age 18-60 years
- for women
    - age 18-40 years
- no condition (medical, mental, or behavioral) that, in the opinion of a study investigator, would likely interfere with participation in/completion of the protocol
- no current or recent use of a medication (including hormonal replacement) that, in the opinion of a study investigator, can modulate the reproductive axis or willing to complete an appropriate washout for that particular medication and its method of administration no active illicit drug use
    - any active use of marijuana will be evaluated by a study medical professional to determine if it may impact study participation; individuals who regularly use marijuana may be asked to washout as referenced in the medication washout section below
- no excessive alcohol consumption (< 10 drinks/week)

- for women
    - not breastfeeding
    - not pregnant
- if applicable, willing to use birth control methods (as approved by a study medical professional) during the entire study

Physical examination:

- normal blood pressure, (systolic BP < 140 mm Hg, diastolic < 90 mm Hg)

Laboratory studies:

- for women
    - negative serum hCG pregnancy test at the time of screening (additional urine pregnancy test will be conducted prior to drug administration)
- hemoglobin within sex-specific reference range

IV. SUBJECT ENROLLMENT

Recruitment Methods

Participants will be recruited from multiple sources:

MGB Biobank (IRB Protocol 2009P002312)

The MGBB maintains plasma, serum, and DNA samples from tens of thousands of patients who have given their consent for broad-based research. Subjects consent to allow storage of up to fifty milliliters of blood, linkage of the sample with their MGB electronic medical record, and storage of a health information questionnaire that collects additional information about health behaviors, lifestyle, and family history. Subjects also provide consent to be re-contacted by the MGBB in the future for additional information/samples, and to be asked if they would be interested in joining other research studies. All samples and linked information stored in the Mass General Brigham Biobank are accessible for broad research use by Mass General Brigham investigators.

In order to identify eligible candidates for this study, we may conduct fully identified chart reviews, RPDR queries, and queries in the Biobank Portal. The MGB Biobank will send an invitation letter, co-signed by the Biobank PI and our PI, and opt-in or opt-out letter to candidates. The Biobank will also provide us with an identified data set that includes patient MRNs, names, and contact information for all re-contacted patients. At least ten business days after the Biobank sends the recontact letters to patients, we will call each candidate on the list (who did not choose to opt-out) to invite them to participate in our study. Upon request, we may provide the Biobank with a list of the response status for each patient so that the Biobank may measure the success rate for the re-contact process.

Individuals Seeking Care in the Reproductive Endocrine Associates (REA) Clinic

We will review identifiable medical records to determine if patients are candidates for study participation. Candidates may be introduced to the study by their REA physician (via phone, email, letter, virtual visit or in-person) and will be recruited to the study by other study team members.

Referrals from Non-REA Clinical Providers

Clinical providers both inside and outside of MGB may introduce subjects to this study. With permission from the referred individual, the provider can share the candidate's contact

information with the study team to proceed with recruitment. We will review medical records to determine if these patients are potential candidates for study participation.

Other Reproductive Endocrine Unit (REU) Studies

We will review identifiable records of individuals who previously participated or expressed interest in other research studies in the REU. The study team may contact candidates (via phone, email, letter, or in-person) who have agreed to be contacted about future studies.

A. If these individuals have an established clinical relationship with any physician investigator on this study, that physician may introduce the study, and recruitment of that candidate will be led by other study team members.
B. If these potential subjects do not have a clinical relationship with one of the physician investigators, they will first be contacted via letter. Individuals who express interest in the study following the recruitment letter will be contacted by the study staff and recruited under guidance of MGB policies. At least ten business days after the letter is sent, we may call each individual (who did not choose to opt-out) to ask whether they would like to participate in our study.

Pre-Screening

Individuals recruited from MGB sources may have medical records reviewed for eligibility. In order to assess eligibility of all individuals, we may ask pre-screening questions via telephone or REDCap, and may request clinical notes and/or endocrine labs from their endocrinologist, PCP, or other health care provider. If outside records are requested, candidates will be asked to complete medical record release forms. Identifiable information on individuals who do not enroll may be retained but will not be linked to any collected health information without permission of the individual.

Consent

Consent may be obtained either in person (at Massachusetts General Hospital) or remotely. The consent form is provided for the subject to review prior to the consent process and study participation. Informed consent will be obtained by a licensed physician investigator or by non-physician investigators who are nurse practitioners or physician assistants with backup by a licensed physician.

The consent process will take place prior to the commencement of study procedures and will in all instances include a complete explanation of the study by one of the study medical professionals (physicians, physician assistants, or nurse practitioners). Study staff will go over the purpose of the study, study procedures, potential risks of participation, and alternatives to participation. Before signing an MGB HRC-approved consent form, potential participants will be given the opportunity to ask the study investigators any questions that they may have concerning the study. The voluntary nature of participation in these studies will be emphasized. It will be made clear that a decision not to participate will in no way affect the relationship of patient to physician, investigator, or institution regarding the ongoing provision of care. Finally, a copy of the signed consent form, containing all the information outlined above, as well as contact information for the principal investigator will be provided to the study participant in person, via mail, or electronically.

When consent is obtained in person, after all parts of the consent discussion are completed, the subject will sign the consent form in the presence of the study investigator. The study investigator will also sign the consent form at this time.

When consent is obtained remotely, the study investigator will complete the consent process, as described above, over the phone. The study investigator will confirm the subject's identity before signature is obtained. The subject and study investigator will sign the consent form at this time.

REDCap eConsent will be used for all signatures. All electronic logging information and an electronic copy of the consent will be automatically saved by REDCap. Subjects may complete electronic signatures using either their own device or a research-owned iPad.

If the individual's preferred language is not English, it is at investigator discretion to determine if participation is appropriate and raises no safety concerns due to potential communication difficulty. If the individual does participate, in accordance with MGB policy, we will work with a remote or in-person interpreter to ensure the consent form can be presented to the subject in their preferred language. The subject will sign a full consent form translated into that language.

V. STUDY PROCEDURES

General Outline of Protocol

The goal of these studies is to assess the GnRH response to kisspeptin individuals carrying genetic variants of interest. GnRH cannot be measured in the peripheral circulation, and so LH will be measured as a surrogate marker of GnRH secretion. LH is secreted by the pituitary in direct response to GnRH, and there is a well-established one-to-one concordance between GnRH and LH pulses. [51,52]

The protocol will consist of up to 2 visits:

- A screening visit
- A visit at the MGH Translational and Clinical Research Centers (TCRC) or REU to assess the subject's LH pulses and response to kisspeptin

The total amount of blood drawn across all parts of the study will not exceed MGB IRB guidelines.

Protocol Details

1. Screening:

Screening procedures will include a complete medical history, physical examination (including a breast exam for female subjects), and laboratory blood tests to ensure subjects meet the inclusion criteria. The total volume of blood drawn during screening is approximately 20 mL.

Screening laboratory tests

- Reproductive hormones including, but not limited to: luteinizing hormone, follicle stimulating hormone, progesterone, estradiol, testosterone
- Hemoglobin (with study medical professional approval, can be based on lab result from medical record, within past 6 months)
- hCG serum pregnancy test (only women who can get pregnant)

All components of screening may be performed at the MGH REU or remotely. Therefore, some participants might not need an in-person screening visit at MGH.

- The screening medical history may be obtained by phone.

- Documentation of a physical examination performed by a non-study physician within the previous 12 months may be used in lieu of an in-person physical examination to determine eligibility.
    - All participants will undergo a physical examination by a study investigator prior to frequent blood sampling.
- If non-MGB medical records will be obtained for documentation of eligibility, subjects will complete an MGH medical record release form.
- If applicable, remote screening laboratory tests will be performed at a local laboratory site (e.g., Quest Diagnostics, etc.); study funds will pay for all phlebotomy and shipping costs.

If all eligibility criteria are met, a member of the study team will contact the subject to schedule the study visits.

2. Pre-Study Visit Interventions
Subjects may undergo these interventions prior to either or both visits to the TCRC or REU.

Sex Steroid and Medication Discontinuation ("Washout")
Subjects on hormonal medication (including oral contraceptives or hormone replacement) or non-hormonal medication that may modulate the reproductive axis may be asked to undergo a washout period appropriate to that particular medication and its method of administration as follows:

- Pulsatile GnRH therapy > 2 weeks
- Gonadotropin injections > 4 weeks
- Transdermal testosterone > 2 weeks
- Testosterone injections > 6 weeks
- Oral contraceptive pills > 8 weeks
- Oral, vaginal, or transdermal estrogen (physiologic dosing) > 2 weeks
- Oral or vaginal progestin (physiologic dosing) > 2 weeks
- If not specified above, a length of time > 5 half-lives of the drug

Subjects will be advised to discuss the washout with their prescribing physician prior to beginning washout. Any subject who experiences intolerable side effects as a result of medication washout will end participation in the study and be instructed to follow recommendations by their prescribing physician regarding resuming any medications.

3. TCRC Visit:

The general structure of these visits is:

- a period of q10 minute blood sampling without intervention to assess endogenous hormone secretion,
- administration of kisspeptin followed by q10 minute blood sampling to assess the response to kisspeptin, and
- administration of GnRH followed by q10 minute blood sampling to assess the response to GnRH.

These may include the following procedures:

- Female subjects will undergo a urine pregnancy test. The results of the pregnancy test will return prior to any study drug administration and a positive pregnancy test will result in immediate termination of the study.

    - Female subjects with any well-documented method of surgical sterilization will not undergo a pregnancy test
- Frequent blood draws (3-5 mL) every 10 minutes for 12 hours; such that the total amount of blood drawn during the visits does not exceed 550 mL
- Kisspeptin administration of one IV bolus at hour 8
    - The dose of the kisspeptin bolus will not exceed 13.19 mcg/kg (10 nmol/kg)
- GnRH administration of one IV bolus at hour 11
    - The dose of the single GnRH bolus will be in the range of 25-75 ng/kg
- EMLA cream may be used to ease discomfort from needle insertion.

Subjects will receive isovolumetric replacement with saline during blood sampling. Kisspeptin will be administered by a TCRC RN or study medical professional. Vital signs (HR, BP, RR, and T) will be measured prior to beginning frequent sampling and at least once every 4 hours during sampling.

Study procedures will be halted if any of the following criteria are met at any point in the study.

- Blood pressure > 180 mmHg systolic or >105 mmHg diastolic at any point during the protocol
- Development or suspicion of an allergic reaction or serious adverse reaction
- Positive pregnancy test
- Temperature ≥101.5°F

Any subject who develops high blood pressure or an allergic reaction will receive medical care until the issue is resolved, with subsequent follow-up as needed.

Additional Study Procedure Information

Source, Preparation, and Storage of Kisspeptin

GMP-grade kisspeptin 112-121 powder and GMP-grade GnRH powder are synthesized by Polypeptide Laboratories (San Diego, CA). MGH Clinical Trials Pharmacy (CTP) receives and maintains the powder, reconstitutes powder into solution for use, and stores the solution. Each new batch of solution is sent for sterility (bacteria/fungi) testing at ARL Bio Pharma (Oklahoma City, OK), endotoxin testing at Dynalabs, LCC (Saint Louis, MO), and concentration testing at AAA Service Laboratory (Damascus, OR). We have previously found that the batches of kisspeptin and GnRH maintained sterility and stable concentration for at least 12 months. Each batch of kisspeptin or GnRH solution is given an expiration based on MGH CTP policy.

Prior to kisspeptin administration, a member of the study team will calculate the amount of kisspeptin that the participant will receive. The orders are sent to MGH CTP who double check the weight-based dose. MGH CTP prepares each participant's dose in accordance with MGH CTP guidelines. Study staff pick up the drug from pharmacy and bring it to the REU or the TCRC. Once there, the drug is logged in and dose, name, and date of birth are checked by study staff and, if applicable, TCRC staff. The drug is stored in a locked location until use.

Justification of Doses

- The bolus dose of kisspeptin to be given will not exceed 13.1 mcg/kg = 10 nmol/kg, which is equal to the largest IV dose of kisspeptin 112-121 used in human studies in the literature to date.[19]

- GnRH doses of 25-75 ng/kg give LH responses in the physiological range in most subjects. Under protocols in the Reproductive Endocrine Unit, subjects have been given GnRH at doses up to 800 ng/kg every 2 hours for up to 12 months with no significant adverse effects.[53,54]

Use of Samples and Data:

Any excess sera collected under this protocol may be coded and stored in the REU lab. Each sample will be given a unique identifying code and may be used for future research. Use of these specimens will be targeted at furthering our understanding of reproductive disorders and improving the management of subjects with these disorders and may include improving existing assays as well as developing new assays. IRB approval will be obtained if samples are used in the future for purposes not described in the protocol.

Samples may be shared with third parties, scientific collaborators, and laboratories. Study data may be shared with scientific collaborators at academic centers. Each collaboration will be driven by clear reproductive research questions and reviewed to ensure the proposed use is consistent with our consent form. When samples or data leave MGB, identifying information will be removed and samples/data will be coded using a unique identifying number. Identifiable data of subjects may be shared with other MGB investigators. Data will be sent to collaborators via secure methods such as secure email or secure file transfer.

Study data may be collected electronically (including via direct data entry into REDCap) or on paper. Electronic information will be stored on secure MGH provided storage systems. Passwords are required to access the information. Paper records are stored in the secure REU, which has restricted access, in double-locked file cabinets/rooms or stored at an offsite storage facility; paper records will also be scanned and stored electronically. After paper records are converted to electronic records the paper copy may be destroyed in compliance with MGB guidance on Electronic Storage of Research Documents. Ongoing access to data is restricted to members of the research team and access to identifiable information is restricted based on staff responsibilities.

Analysis performed under this study may be informed by data collected under other REU studies. All data from this study may be used for ongoing and future research and to better our understanding of reproductive disorders.

VI. BIOSTATISTICAL ANALYSIS

Assays

Given the long-term ongoing nature of this study, the assay methodologies employed to characterize the biochemical profile (both reproductive and metabolic) will be based on standard accepted methods as established by the MGH Pathology department and the Reproductive Endocrine Reference Laboratory. As such these methods may include immunoassays, measurement using standard laboratory platforms, radioimmunoassays, and mass spectroscopy. We anticipate that these methods will continue to evolve as the diagnostics field changes.

Data Analysis

The hypothalamic-pituitary axis will be analyzed using the following parameters:

a) Arithmetic mean levels for LH and FAS based on q10 min blood sampling

Analysis of pulsatile hormone secretion of LH and FAS using a modification of the Santen and Bardin Method [55] augmented by deconvolution analysis [21]. If evidence of endogenous GnRH secretion is present, GnRH pulse frequency will be determined by calculating the number of LH and FAS pulses during the sampling interval. LH pulse amplitude will be calculated from the difference between the nadir and peak hormone levels.

b) FSH and sex steroid levels from sample pools will be compared using analysis of variance with repeated measures to assess their responsiveness to kisspeptin.

STATISTICAL DESIGN AND POWER

Individuals recruited from the Biobank will undergo baseline neuroendocrine LH pulse pattern evaluation, as well as Kisspeptin and GnRH stimulation testing. Boys and girls with delayed puberty have kisspeptin-stimulated GnRH induced LH pulse amplitude 70% lower than healthy men and luteal phase women (144-145, 147). Therefore, we conservatively estimated a 50% reduction in kisspeptin-stimulated GnRH induced LH pulse amplitude in individuals at highest genetic risk compared to those at lowest risk. To detect a 50% decrease in the LH amplitude, 25 participants in each group would be required to provide a statistically meaningful result (80% power, one-sample exact binomial test). Therefore, the study would enroll 25 individuals (males and females combined) in the high risk group and 25 individuals (males and females combined) in the low risk group for a total of 50. To allow for a 15% estimated drop-out rate, 60 participants (males and females combined) will be enrolled.

The hypotheses will be accepted if subjects at high genetic risk demonstrate reduced endogenous and exogenous kisspeptin-induced LH pulse amplitude compared to individuals at low genetic risk.

Since healthy men and women in the luteal phase have similar responses to exogenous kisspeptin (144, 145), an interim analysis is planned to ensure that this remains true in men and luteal phase women in this study. If it is not the case, then the study would enroll 50 individuals (males and females combined) in the high risk group and 50 individuals (males and females combined) in the low risk group for a total of 100. This possibility is accounted for in study recruitment and analysis.

VII. RISKS AND DISCOMFORTS

Blood Draws and IV Lines:
There is a slight discomfort associated with the placement of IV lines and a small risk of infection at blood draw sites. There is a slight chance that an IV line placed will fail. In the event this occurs, a new IV line will be placed. Subjects may faint from blood drawing or develop bruise or irritation at the site of the blood draw. In addition, it is possible that subjects could develop anemia from blood-drawing. Subjects will be instructed to avoid donating blood or having large blood draws for 8 weeks before and after the study.

Subcutaneous Administration:
There is a possibility of a reaction at the site of SC administration including: pain, tenderness, warmth, itching, swelling, irritation, redness, or bruising.

EMLA Cream:
Repeated doses of EMLA cream may increase blood levels of lidocaine and prilocaine, although systemic adverse reactions following appropriate use of EMLA cream are unlikely due to the small dose absorbed. There have been reports of slight reddening, swelling, discoloration, rashes, and

hyperpigmentation at the application site, but these cases were generally mild and transient. No serious reactions have been reported.

Investigational New Drug (Kisspeptin IND, also known as Metastin 45-54, IND # 74, 977 and 74,978):

The following findings point to the safety of kisspeptin:

1) Kisspeptin is a peptide that is produced endogenously.
2) Blood levels of kisspeptin rise 7,000- to 10,000-fold across human gestation.[56] This clearly does not have long-term effects on women or their later reproductive capacity.
3) We have performed two years of pre-clinical toxicology testing giving kisspeptin to rats and dogs, and have not observed untoward effects.
4) Our group has acquired substantial experience with human administration of kisspeptin. To date, we have administered kisspeptin 112-121 to almost 200 subjects, both healthy volunteers and individuals with reproductive conditions. Subjects were monitored closely during the start of kisspeptin administration (for ambulatory studies) or the entire course of kisspeptin administration (for outpatient and inpatient studies).
    a. In about half of subjects receiving kisspeptin subcutaneously, we observed transient stinging or irritation at the site of subcutaneous delivery. When subcutaneous kisspeptin is delivered repeatedly (e.g., when using a pump device), stinging is not experienced during every bolus. Stinging is a known risk of subcutaneous delivery of fluid. Therefore, the relatedness to kisspeptin is unclear.
    b. We have observed no other IV kisspeptin-attributed adverse events.
5) In Europe, kisspeptin-54 has been used to trigger egg maturation in over 100 women undergoing IVF treatment. Over 30 healthy babies were delivered as a result. Kisspeptin was well tolerated in all participants.[39,57]

These points in combination with the experience of other groups with human kisspeptin administration demonstrate the safety and tolerability of kisspeptin. Additionally, participants have access to the study team at all times for questions or concerns.

Investigational New Drug (GnRH IND #93,353)

Administration of GnRH has been a cornerstone of physiologic investigation in the Reproductive Endocrine Unit here at MGH for the past 30 years. We have accumulated a large clinical experience and have treated many GnRH deficient men without any significant complications. In this protocol, the administration of Kisspeptin and GnRH will be separated by at least 1 hour; they would not be given simultaneously. In pre-clinical studies examining the stability of kisspeptin in human plasma, the half-life of kisspeptin is 55 seconds, with >99% of kisspeptin 112-121 degraded within 30 minutes [21]. Similarly, the *in vivo* half-life of kisspeptin in humans is 4 minutes. [58]

Medication Washout

Subjects who discontinue the use of their current medications may experience the return of symptoms that are controlled or mitigated by these medications. However, we do not expect our participants to experience a prolonged recurrence of symptoms, and we expect any short-term symptoms to be rapidly reversible. Subjects who experience intolerable side effects as a result of medication washout will end participation in the study. The likelihood of becoming pregnant increases for subjects who stop taking oral contraceptives or other forms of birth control. All subjects are advised to use multiple forms of birth control while on the study.

We will ask subjects with hyperprolactinemia being treated with bromocriptine to complete a washout period prior to the TCRC or REU visit(s). The washout period will last at least 2 weeks. A brief interruption in dopamine agonist therapy is standard of care for patients with hyperprolactinemia to determine if ongoing medication is needed.[59] We expect that some participants may experience a recurrence of their hyperprolactinemia symptoms.[59] We expect most of the recurrence cases to be mild; however, in instances where the symptoms are intolerable for the participant, they will be terminated from the study and asked to resume treatment with bromocriptine. We will ask participants to contact the study team should they experience a recurrence of their symptoms.

VIII. POTENTIAL BENEFITS

There are no direct benefits to patients that are anticipated from participating in this study. The only benefit from participating in this study comes from the altruistic contribution to furthering our understanding of the biology of human reproduction.

IX. MONITORING AND QUALITY ASSURANCE

Prior to initiation of the protocol, the Massachusetts General Brigham Human Research Committee (MGB HRC) will have reviewed and approved the protocol and consent form for this study. Following initial approval of the protocol, the MGB IRB regularly reviews the progress of the trial, and may require the investigator to provide information following reports of adverse events and protocol amendments.

The principal investigator and all key personnel involved in this study have completed human subjects research training, either the Collaborative IRB Training Initiative (CITI) or MGB Human Research Affairs Compliance and Education courses. These tutorials cover MGH policy and federal regulations governing human subject research commonly known as Good Clinical Practice. The tutorial and institutional policies and procedures governing the conduct of human subjects research are available on the MGB IRB website and may be accessed by the investigator and key study personnel as needed.

Adverse Event Reporting:

Adverse Event (AE) Reports:

The principal investigator will report adverse events experienced by study subjects to the FDA and MGB IRB in accordance with MGB IRB guidelines.

Unanticipated Problems and Serious Adverse Event (SAE) Reports:

As per regulations CFR 21 Part 312 and MGB IRB guidelines, the principal investigator will report unanticipated problems and SAEs to the MGB IRB and FDA by telephone, fax, or email within 5 working days/7 calendar days of becoming aware of the problem. In addition, SAEs that occur within 30 days after a subject's active participation in the study and that are categorized as definitely, probably, or possibly related to the study will be reported to the MGB IRB.

Investigational New Drug (IND): Kisspeptin IND #74,977 and #74,978, GnRH IND #93,353

The principal investigator, who is also the kisspeptin IND holder, will notify the FDA and all participating investigators in a written IND safety report of any adverse experience associated with the use of the drug that is both serious and unexpected or any finding from tests in laboratory animals that suggest a significant risk for human subjects, including reports of mutagenicity, teratogenicity, or carcinogenicity. Each notification will be made as soon as possible and no greater than 15 calendar days after the initial receipt of the information. Each written notification will be submitted on an FDA Form 3500A. The principal investigator will also notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-

threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days after the initial receipt of the information.

On an annual basis as part of the update to the study IND, the principal investigator will submit to the FDA:

- A summary of all IND safety reports submitted during the past year
- A list of all subjects who died during the participation in the investigation, listing cause of death for each
- A list of subjects who dropped out during the course of the investigation in association with any adverse experience, whether or not thought to be drug related

Monitoring:

The Principal Investigator is responsible for oversight of the trial. The study will also be monitored by an independent data safety monitoring board (DSMB) that will meet once a year.

The DSMB responsibilities will include:

- Review of the research protocol, informed consent documents and plans for safety and data monitoring of the study. This is to determine the risks and benefits to research subjects, protection and safety of the subjects and to offer suggestions for improving the study design.
- Review of all adverse events (expected, unexpected and serious), enrollment, dropout rates and protocol deviations conducted to ensure subject safety. The DSMB will be provided with reports of serious adverse events as they occur and may request additional information from the investigator in the course of its review.
- Evaluate the progress of the trial, including periodic assessments of data quality/completeness, recruitment goals, protocol adherence, accrual and retention of participants and other factors that may affect the study outcome. Protect confidentiality of the study participants, trial data, and results of the monitoring.
- Weigh efficacy and adverse events to determine if the trial should continue as originally designed, should be changed or should be stopped based on the data.

A copy of the minutes will be distributed to the DSMB members, the investigator, and the MGB IRB. The minutes will reflect which of the following three determinations the DSMB may make as a result of its review: 1) the study may continue without modification/amendment; 2) the study should be amended; 3) the study should terminate.

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