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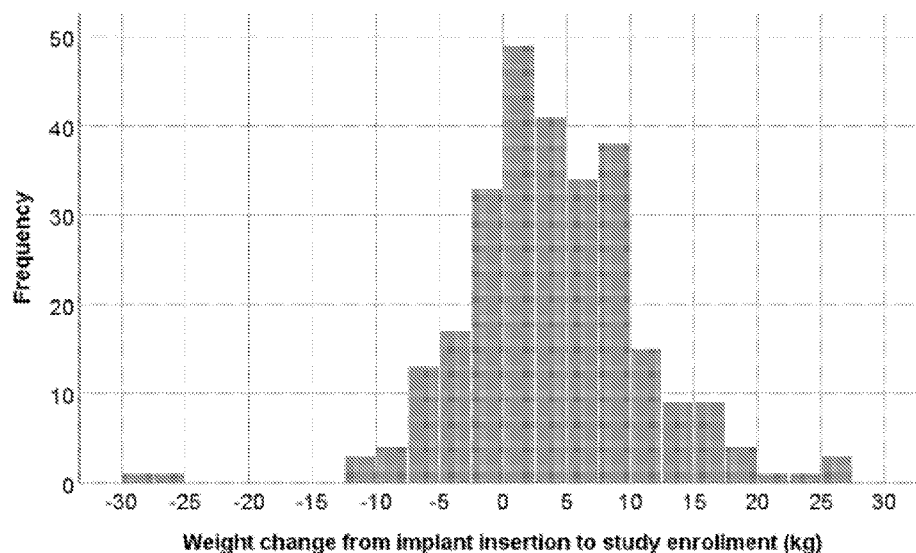


FIG. 1

(57) Abstract: The present inventive concept relates to genetic variants, for example, *ESR1* rs9340799, and the association of such genetic variants with increased weight gain among contraceptive users, such as contraceptive implant users. Embodiments of the inventive concept may include selecting, recommending, and/or prescribing contraception based on whether a subject/patient has/is homozygous for a particular genetic variant.

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GENETIC VARIANTS AND METHODS OF SELECTING CONTRACEPTION RELATED TO THE SAME

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 63/186,936 filed on May 11, 2021, the content of which is hereby incorporated herein in its entirety.

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BACKGROUND

[0003] Weight gain remains a rare primary reason for etonogestrel contraceptive implant (NEXPLANON®, Merck & Co., Whitehouse Station NJ) discontinuation [1,2]. However, etonogestrel implant users experience wide inter-individual variability in weight changes [3–7]. The largest study with a non-hormonal comparison group found etonogestrel implant users had a greater mean weight increase (3.0 kg versus 1.1 kg) after 36 months of use, but similarly wide variability in individual weight changes (24 kg to +25 kg versus 18 kg to +20 kg) [3]. Although the average weight gain associated with etonogestrel implant use may not be clinically significant, reproductive-aged women experiencing a 25 kg weight gain likely face additional health risks [8].

[0004] Means to determine the risk of weight gain are currently lacking for an individual considering an etonogestrel implant or other hormonal contraceptive methods, though individual patient characteristics like race can affect this risk [7]. Pharmacogenomics, the study of how genetic variants affect drug metabolism and side effects, may help elucidate if and how individual genetic differences contribute to variability in hormonal drug-related weight gain [9]. For example, studies using pharmacogenomic methods have identified genetic variants associated with antipsychotic drug-related weight gain [10].

[0005] Accordingly, there remains a need for improved methods of selecting, recommending, and/or prescribing contraception for subjects seeking and/or in need of the same.

SUMMARY

[0006] Aspects of the present inventive concept relate to methods of selecting, recommending, and/or prescribing contraceptives, methods of minimizing side effects associated with a contraceptive selected, recommended, and/or prescribed, methods of administering a contraceptive, and methods of minimizing side effects associated with administering a contraceptive in a subject based on analysis of genetic background/variation possessed by the subject.

[0007] According to an aspect of the inventive concept, provided is a method for selecting, recommending, and/or prescribing a contraceptive for a subject including: identifying any genetic variant or genetic variants of, for example, any one gene, or a set of genes including *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2*, *PXR*, *CYP3A4*, and/or *CYP2C9*, or a combination of more than one thereof the subject possesses; and selecting, recommending, and/or prescribing the contraceptive depending on the genetic variant or the genetic variants of *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2*, *PXR*, *CYP3A4*, and/or *CYP2C9*, or a combination of more than one thereof the subject possesses. In some aspects of the inventive concept, the gene is *ESR1*, and/or genetic variants thereof. In some aspects of the inventive concept, the gene is *CYP2C19*, and/or genetic variants thereof.

[0008] According to another aspect of the inventive concept, provided is a method of minimizing side effects of a contraceptive selected, recommended, and/or prescribed for a subject including: identifying any genetic variant or genetic variants of, for example, any one gene, or a set of genes including *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2*, *PXR*, *CYP3A4*, and/or *CYP2C9*, or a combination of more than one thereof the subject possesses; and selecting, recommending, and/or prescribing the contraceptive depending on the genetic variant or the genetic variants of *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2*, *PXR*, *CYP3A4*, and/or *CYP2C9*, or a combination of more than one thereof the subject possesses. In some aspects of the inventive concept, the gene is *ESR1*, and/or genetic variants thereof. In some aspects of the inventive concept, the gene is *CYP2C19*, and/or genetic variants thereof.

[0009] According to another aspect of the inventive concept, provided is a method of administering a contraceptive to a subject including: identifying any genetic variant or genetic variants of, for example, any one gene, or a set of genes including *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2*, *PXR*, *CYP3A4*, and/or *CYP2C9*, or a combination of more than one thereof the subject possesses; and selecting a contraceptive depending on the genetic variant or the genetic variants of *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2*, *PXR*, *CYP3A4*, and/or *CYP2C9*, or a combination of more than one thereof the subject possesses; and administering the contraceptive to the subject. In some aspects of the inventive concept, the gene is *ESR1*, and/or genetic variants thereof. In some aspects of the inventive concept, the gene is *CYP2C19*, and/or genetic variants thereof.

[0010] According to another aspect of the inventive concept, provided is a method of minimizing side effects associated with administering a contraceptive to a subject including: identifying any genetic variant or genetic variants of, for example, any one gene, or a set of genes including *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2*, *PXR*, *CYP3A4*, and/or *CYP2C9*, or a combination of more than one thereof the subject possesses; and selecting a contraceptive depending on the genetic variant or genetic variants of *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2*, *PXR*, *CYP3A4*, and/or *CYP2C9*, or a combination of more than one thereof the subject possesses; and administering the contraceptive to the subject. In some aspects of the inventive concept, the gene is *ESR1*, and/or genetic variants thereof. In some aspects of the inventive concept, the gene is *CYP2C19*, and/or genetic variants thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] **FIG. 1.** Histogram of weight change from implant insertion to study enrollment for all 276 participants with available medical records.

[0012] **FIG. 2.** Box plots of weight change from implant insertion to study enrollment among participants with the *CYP2C19* rs7088784 wild-type genotype (n = 248) compared with *CYP2C19* rs7088784 variant carriers (n = 48), excluding those with unknown genotype for this single nucleotide polymorphism (n = 54). The box represents the first and third quartiles with the band inside the box representing the median for each respective group. The whiskers represent the data within 1.5 interquartile range of the upper and lower quartile. Circles indicate outliers

with values between 1.5 times and 3 times the IQR and asterisks indicate outliers with values greater than 3 times the IQR.

[0013] FIG. 3. Box plots of weight change from implant insertion to study enrollment among participants homozygous for the *ESR1* rs9340799 variant allele (n = 7) versus carriers of at least one wild-type allele (n = 324), excluding those with unknown genotype for this single nucleotide polymorphism (n = 19). The box represents the first and third quartiles with the band inside the box representing the median for each respective group. The whiskers represent the data within 1.5 interquartile range of the upper and lower quartile. Circles indicate outliers with values between 1.5 times and 3 times the IQR and asterisks indicate outliers with values greater than 3 times the IQR.

DETAILED DESCRIPTION

[0014] The foregoing and other aspects of the present invention will now be described in more detail with respect to other embodiments described herein. It should be appreciated that the invention can be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

[0015] The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used in the description of the invention and the appended claims, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Additionally, as used herein, the term "and/or" includes any and all combinations of one or more of the associated listed items and may be abbreviated as "/".

[0016] The term "comprise," and its equivalents, as used herein, in addition to its regular meaning, may also include, and, in some embodiments, may specifically refer to the expressions "consist essentially of" and/or "consist of." Thus, the expression "comprise" can also refer to, in some embodiments, the specifically listed elements of that which is claimed and does not include further elements, as well as embodiments in which the specifically listed elements of that which is claimed may and/or does encompass further elements, or embodiments in which the specifically listed elements of that which is claimed may encompass further elements that do not

materially affect the basic and novel characteristic(s) of that which is claimed. For example, that which is claimed, such as a composition, formulation, method, system, etc. "comprising" listed elements also encompasses, for example, a composition, formulation, method, kit, etc.

"consisting of," i.e., wherein that which is claimed does not include further elements, and a composition, formulation, method, kit, etc. "consisting essentially of," i.e., wherein that which is claimed may include further elements that do not materially affect the basic and novel characteristic(s) of that which is claimed.

[0017] The term "about" generally refers to a range of numeric values that one of skill in the art would consider equivalent to the recited numeric value or having the same function or result. For example, "about" may refer to a range that is within $\pm 1\%$, $\pm 2\%$, $\pm 5\%$, $\pm 10\%$, $\pm 15\%$, or even $\pm 20\%$ of the indicated value, depending upon the numeric values that one of skill in the art would consider equivalent to the recited numeric value or having the same function or result. Furthermore, in some embodiments, a numeric value modified by the term "about" may also include a numeric value that is "exactly" the recited numeric value. In addition, any numeric value presented without modification will be appreciated to include numeric values "about" the recited numeric value, as well as include "exactly" the recited numeric value. Similarly, the term "substantially" means largely, but not wholly, the same form, manner or degree and the particular element will have a range of configurations as a person of ordinary skill in the art would consider as having the same function or result. When a particular element is expressed as an approximation by use of the term "substantially," it will be understood that the particular element forms another embodiment.

[0018] "Administering" and methods of administration of, for example, a contraceptive or contraceptives as described herein are not particularly limited, and any method that would be appreciated by one of skill in the art may be used for administering a contraceptive or contraceptives, such as a progestin-only contraceptive including, but not limited to desogestrel, etonogestrel, levonorgestrel, and/or norethindrone as described herein without departing from the spirit and scope of the inventive concept. In some embodiments, the contraceptive, for example, etonogestrel, may be administered by an implant, such as NEXPLANON®. In some embodiments, the contraceptive may be administered, for example, orally, parenterally, subcutaneously, topically/transdermally, and/or vaginally to the subject.

[0019] "Subject" as used herein may be a patient, such as a patient seeking and/or in need of contraception. In some embodiments, the subject is a human; however, a subject of this disclosure can include animal subjects, particularly mammalian subjects such as canines, felines, bovines, caprines, equines, ovines, porcines, rodents (*e.g.*, rats and mice), lagomorphs, primates (including non-human primates), etc., including domesticated animals, companion animals and wild animals for veterinary medicine, for treatment, or for research and development purposes.

[0020] The subjects relevant to this disclosure may be human, more particularly female, and may be of any race or ethnicity, including, but not limited to, Caucasian, African-American, Native American, African, Asian, Hispanic, Indian, etc., as well as of combined backgrounds. Although human subjects within the scope of the inventive concept may conceivably be of any age, *e.g.*, newborn, neonate, infant, child, adolescent, adult, and geriatric, embodiments of the inventive concept generally relate to subjects that are adolescent and/or adult female human subjects, *i.e.*, female human subjects of child-reproducing age, for example, female human subjects between about 15–49 years in age.

[0021] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0022] Embodiments of the present inventive concept provide methods of selecting, recommending, and/or prescribing contraceptives, and methods of administering contraceptives to a subject based on genetic variations that the subject may have. Embodiments of the inventive concept include, for example, selecting, recommending, and/or prescribing particular contraceptives dependent on may have, for example, two copies of a variant allele is homozygous in genotype for a genetic variant vs., for example, the wild-type genotype, and/or having one copy of a variant allele, *i.e.*, heterozygous in genotype for a particular gene or particular genes. A subject having a "variant" or "genetic variant" for a particular gene or particular genes, as referred to herein, in some embodiments, means that the subject has: two copies of/is homozygous in genotype for, a genetic variant of the particular gene, as opposed to having a wild-type genotype for the particular gene. Nevertheless, it will also be appreciated that, in some embodiments, a subject having a variant or genetic variant for a particular gene or particular genes may mean that the subject has: one copy of/is heterozygous in genotype for a genetic variant of the particular gene, as opposed to having a wild-type genotype for the

particular gene, and, in some embodiments, a subject having a variant or genetic variant for a particular gene or particular genes may mean that the subject has at least one copy of/is either heterozygous or homozygous in genotype for a genetic variant of the particular gene, as opposed to having a wild-type genotype for the particular gene. Genes that are encompassed by the inventive concept include, for example, any one gene, at least one gene, or a set of genes including, but not necessarily limited to: *ESR1*; *CYP2C19*; *CYP3A7*; *HSD3B1*; *LRP2*, *PXR*, *CYP3A4*, and/or *CYP2C9*, or any combination of more than one thereof, and in some embodiments, identifying any genetic variant or genetic variants thereof that a subject may have. In some embodiments, the gene is *ESR1*, and/or genetic variants thereof. In some embodiments, the gene is *CYP2C19*, and/or genetic variants thereof. In some embodiments, the gene is *CYP3A7*, and/or genetic variants thereof. In some embodiments, the gene is *HSD3B1*, and/or genetic variants thereof. In some embodiments, the gene is *LRP2*, and/or genetic variants thereof. In some embodiments, the gene is *PXR*, and/or genetic variants thereof. In some embodiments, the gene is *CYP3A4*, and/or genetic variants thereof. In some embodiments, the gene is *CYP2C9*, and/or genetic variants thereof. Genetic variants of these genes include, but are not necessarily limited to, for example: *ESR1* rs9340799; *CYP2C19* rs7088784; *LRP2* rs2075252; *HSD3B1* rs7553527; *CYP2C19*19*; *CYP3A7*1C*; and *PXR* rs2471817, *CYP3A4*1G* (rs2242480); *CYP2C9* rs9332238; or a combination of more than one thereof, such as, in some embodiments, *CYP2C19*19*, *CYP3A7*1C*, and *PXR* rs2471817.

[0023] Determining, methods of determination of, and methods of identifying, for example, the genotype of a subject and/or identifying any genetic variant or variants a subject may possess may be accomplished/performed by any method available and/or known to one of skill in the art without departing from the spirit and scope of the inventive concept.

[0024] In some embodiments, the contraceptive selected, recommended, and/or prescribed may be a hormonal contraceptive. Hormonal contraceptives include hormone active agents such as estrogens and/or progestins, and may be in the form of, for example, combined contraceptives, such as combined oral contraceptives containing both estrogens and progestins ("the pill"); and progestin-only contraceptives, such as pills containing only progestins (progestin-only pills), progestin-only implants, such as the etonogestrel contraceptive implant NEXPLANON®, and the vaginal ring (NUVARING®). In some embodiments, the contraceptive, such as a hormonal contraceptive, that is selected, recommended, and/or prescribed is a progestin-only contraceptive

that is and/or includes a progestin, such as, but not limited to, for example, desogestrel, etonogestrel, levonorgestrel, and/or norethindrone. It will be appreciated that selecting, recommending, and/or prescribing a contraceptive to a subject may refer to, in some embodiments, selecting, recommending, and/or prescribing an active agent, such as a progestin, for example, etonogestrel, or the like, as well as may refer to, in some embodiments, selecting, recommending, and/or prescribing compositions, formulations, and/or devices that may deliver/administer said active agent, such as a contraceptive implant, for example, the etonogestrel contraceptive implant NEXPLANON®, as would be understood in the context of the discussion and/or description.

[0025] In some embodiments, a contraceptive, such as a progestin, for example, but not limited to desogestrel, etonogestrel, levonorgestrel, and/or norethindrone is selected, recommended and/or prescribed for the subject, and in some embodiments administered to the subject if the subject has, for example, a wild-type genotype for a gene or genes from the set of genes as set forth herein. In some embodiments, the contraceptive selected, recommended, and/or prescribed is a progestin-only contraceptive, such as a progestin-only contraceptive that includes etonogestrel. In some embodiments, the contraceptive selected, recommended, and/or prescribed if the subject has, for example, a wild-type genotype for a gene or genes from the set of genes as set forth herein is etonogestrel. In some embodiments, an alternative contraceptive, such as an alternative contraceptive to, a progestin, for example, but not limited to desogestrel, etonogestrel, levonorgestrel, and/or northindrone is selected, recommended, and/or prescribed for the subject, and in some embodiments administered to the subject if the subject has, for example, a variant genotype for a gene or genes from the set of genes as described herein. The alternative contraceptive selected, recommended, and/or prescribed is not particularly limited. In some embodiments, the alternative contraceptive selected, recommended, and/or prescribed is a non-hormonal contraceptive, such as an IUD, cervical cap, diaphragm, and/or is phexxi. In some embodiments, the IUD selected, recommended and/or prescribed include copper-containing IUDs. However, the IUD selected, recommended, and/or prescribed as an alternative contraceptive to, for example, etonogestrel, may also include, in some embodiments, levonorgestrel-releasing IUDs, in that the much lower systemic hormone release associated with a levonorgestrel-releasing IUD may result in lower side effects than those associated with administration of levonorgestrel/progestins in pill, implant, and/or vaginal ring form. In some

embodiments, a contraceptive, such as a hormonal contraceptive, for example, a progestin, such as, but not limited to desogestrel, etonogestrel, levonorgestrel, and/or norethindrone is selected, recommended and/or prescribed for the subject, and in some embodiments administered to the subject if the subject has, for example, a variant genotype for a gene from the set of genes as described herein. In some embodiments, a contraceptive, such as a hormonal contraceptive, for example, a progestin, such as, but not limited to desogestrel, etonogestrel, levonorgestrel, and/or norethindrone is selected, recommended, and/or prescribed for the subject, and in some embodiments administered to the subject if the subject has, for example, a variant genotype for a gene from the set of genes as described herein, in combination a wild-type genotype of another gene or genes from the set of genes as described herein.

[0026] In some embodiments, a hormonal contraceptive, such as a hormonal contraceptive, for example, a progestin, such as desogestrel, etonogestrel, levonorgestrel, and/or northindrone is selected, recommended, and/or prescribed for the subject, and in some embodiments administered to the subject if the subject has, for example, a wild-type *ESR1* or *LRP2* genotype. In some embodiments, a contraceptive, such as a hormonal contraceptive, for example, a progestin, such as desogestrel, etonogestrel, levonorgestrel, and/or northindrone is selected, recommended, and/or prescribed for the subject if the subject has, for example, a variant *LRP2* rs2075252 genotype or a *CYP2C19* rs7088784 genotype. In some embodiments, a contraceptive, such as a hormonal contraceptive, for example, a progestin, such as desogestrel, etonogestrel, levonorgestrel, and/or northindrone is selected, recommended, and/or prescribed for the subject if the subject has, for example, a wild-type *CYP2C19*19*, and wild-type *PXR* rs2471817 genotypes. In some embodiments, a contraceptive, such as a hormonal contraceptive, for example, a progestin, such as desogestrel, etonogestrel, levonorgestrel, and/or northindrone is selected, recommended, and/or prescribed for a subject, and in some embodiments administered to the subject if the subject has a variant *CYP3A7*1C* genotype and a wild-type *CYP2C19*19* genotype. In some embodiments, a contraceptive, such as a hormonal contraceptive, for example, a progestin, such as desogestrel, etonogestrel, levonorgestrel, and/or northindrone is selected, recommended, and/or prescribed for a subject, and in some embodiments administered to the subject if the subject has a variant *CYP2C9* rs9332238 genotype.

[0027] In some embodiments, an alternative contraceptive, such as an alternative to etonogestrel, such as a non-hormonal contraceptive, is selected, recommended, and/or

prescribed, for example, if the subject has a variant *ESRI* rs9340799 genotype. In some embodiments, an alternative contraceptive to etonogestrel, such as a non-hormonal contraceptive, is selected, recommended, and/or prescribed for a subject, and in some embodiments administered to the subject, for example, if the subject has a variant *LRP2* rs2075252 genotype. In some embodiments, an alternative contraceptive to etonogestrel, such as a non-hormonal contraceptive, is selected, recommended, and/or prescribed, for example, if the subject has a variant *HSD3B1* rs7553527 genotype. In some embodiments, an alternative contraceptive to etonogestrel, such as a non-hormonal contraceptive, is selected, recommended, and/or prescribed for a subject, and in some embodiments administered to the subject, for example, if the subject has a variant *CYP2C19*19*, and variant *PXR* rs2471817 genotypes. In some embodiments, an alternative contraceptive to etonogestrel, such as a non-hormonal contraceptive, is selected, recommended, and/or prescribed for a subject, and in some embodiments administered to the subject, for example, if the subject has a variant *CYP3A4*1G* rs2242480 genotype.

[0028] Further embodiments of the inventive concept include methods of minimizing side effects, such as, for example, weight gain, mood changes, abnormal bleeding, and/or breast tenderness, of a contraceptive selected, recommended, and/or prescribed for a subject, methods of administering a contraceptive to a subject, and methods of minimizing side effects associated with administering a contraceptive to a subject including selecting, for example, etonogestrel or an alternative to etonogestrel as described herein.

[0029] The present invention is more particularly described in the following examples, which is intended to be illustrative only, since numerous modifications and variations therein will be apparent to those skilled in the art.

EXAMPLE 1 - ANALYSIS OF THE INFLUENCE OF GENETIC VARIANTS ON WEIGHT GAIN AMONG ETONOGESTREL CONTRACEPTIVE IMPLANT USERS

Materials and Methods

[0030] This was a retrospective analysis of a parent pharmacogenomic association study [11]. The candidate gene methodology for this study has been previously published [11]. Participants were reproductive aged women (18–45 years old) with an etonogestrel contraceptive implant in

place for at least 12 months and no more than 36 months at the time of enrollment (the steady-state period of the implant's pharmacokinetics) [12,13]. Of particular pertinence to this study, the parent study excluded women with a measured body-mass index (BMI) less than 18.5 kg/m² due to concerns for altered etonogestrel metabolism among underweight women; there was no upper BMI limit. The hypotheses tested in this analysis were pre-specified secondary outcomes of the parent study [11]. The protocol was approved by the Colorado Multiple Institutional Review Board and all participants gave written informed consent before study initiation. Participants were recruited through community advertising and contraceptive clinics at the University of Colorado Anschutz Medical Campus.

[0031] At the time of enrollment, participants' height and weight were measured and their BMI was calculated. For all participants, an electronic medical record review was performed to capture their weight at the time of implant insertion if available. The weight change was then calculated from time of implant insertion to study enrollment for participants with documented weight in the electronic medical record for the initial time point. Participants also completed a brief questionnaire to obtain self-reported demographics and side effect information, including if participants had experienced "weight gain" (yes/no) while using their contraceptive implant.

[0032] The full genotyping methodology for this study has been published [11]. See Table 5 for the full list of single nucleotide polymorphisms (SNPs) selected for this study and their reference (rs) ID numbers. Allele and genotype frequencies and Hardy-Weinberg equilibrium calculations were also included in this publication [11]. Ultimately, included 99 genetic variants were included for statistical analysis after excluding nineteen variants not present in this study population and two variants with significant deviation from Hardy-Weinberg equilibrium [11]. The methodology for serum etonogestrel concentration analysis is previously described [11,13].

[0033] IBM SPSS version 25 statistical software was used for these analyses. Descriptive frequencies were performed, and Cohen's kappa coefficient was utilized to determine agreement between objective and subjective weight gain. A simple linear modeling was then conducted to identify genetic variants associated with objective weight change. For multivariable analysis of objective weight change, a generalized linear model was used due to the non-normal distribution of this outcome [14]. In the generalized linear modeling, genetic variants significantly associated with weight change in simple linear modeling (p-value cut-off of 0.05 for inclusion) and the continuous variables of age, BMI, duration of implant use, and serum etonogestrel concentration,

and the categorical variables of self-reported race and ethnicity were included. A backward-stepwise approach was utilized to create the final linear model based on Akaike's Information Criterion [15]. Given the multiple hypothesis testing performed using simple logistic regression (99 independent analyses), a Bonferroni corrected p-value cut-off of 5.0×10^{-4} was determined. This corrected p-value was used to determine overall significance for the variables included in the final generalized linear model. The sample size ($N = 350$) was determined a priori based on the primary outcome of the parent pharmacogenomic study [11].

[0034] Clinical Trial Registration: Clinicaltrials.gov, NCT03092037.

Results

[0035] The available medical records were reviewed for all 350 participants from the parent study for inclusion in this analysis [11]. Weight at the time of implant insertion was available for 276 participants (78.9%). Table 1 shows pertinent patient characteristics and demographics for these 276 participants. Participants' median age was 22.3 years (range 18.0–39.1) and median duration of implant use was 27.0 months (range 12–36). Approximately 57% reported their ethnicity as Hispanic/Latina and 43% self-reported their race as White or Caucasian. The median BMI at enrollment was 25.8 kg/m^2 (range 18.5–48.1). Table 1 demonstrates the BMI categories of participants [8]. The median duration of implant use and median BMI at the time of enrollment were not statistically different between participants from the parent study with or without insertion weight data (Independent-samples median test, $p = 0.42$ and $p = 0.36$, respectively).

[0036] Simple linear regression was performed to test for genetic associations with objective weight change from contraceptive implant insertion to study enrollment. Seven genetic variants had significant associations ($p < 0.05$): *CYP2C19* rs11568732, *AKR1C3* rs12529, *ESR1* rs2077647, *ESR1* rs2228480, *CYP2C19* rs7088784, *ESR1* rs9322335, and *ESR1* rs9340799. Table 2 shows the unadjusted beta-coefficients for all patient characteristics and demographics and Table 3 contains the unadjusted beta-coefficients for these seven genetic variants. Using generalized linear modeling BMI at time of enrollment and two genetic variants (*CYP2C19* rs7088784 and *ESR1* 9340799) remained in the final model (Table 4). For every 1 kg/m^2 increase in enrollment BMI, the average weight gain from contraceptive implant insertion to study enrollment increased by 0.54 kg.

[0037] The median weight change from implant insertion to study enrollment was +3.2 kg (range 27.6 kg to +26.5 kg) with an interquartile range of 8.3. Fig. 1 shows the distribution of weight changes. Though only 43.1% (119/276) of participants with objective weight data reported subjective weight gain with the implant, the majority of participants had objective weight gain since implant insertion (73.9%, 204/276). A report of subjective weight gain had only minimal agreement with measured weight gain during implant use (Cohen's kappa = 0.21).

[0038] *CYP2C19* rs7088784 had an overall variant allele frequency of 8.3% among the participants successfully genotyped for this SNP (n = 296) with 15.9% (n = 47) having one variant allele (heterozygous) and 0.3% (n = 1) having two variant alleles. *ESR1* rs9340799 had an overall variant allele frequency of 22.5% among the participants successfully genotyped for this SNP (n = 331) with 40.8% (n = 135) having one variant allele (heterozygous) and 2.1% (n = 7) having two variant alleles (homozygous variant). Participants with at least one *CYP2C19* rs7088784 variant allele (carriers) had on average less weight gain compared to participants homozygous for the wild-type genotype (mean weight change +0.97 kg versus +4.28 kg, respectively, Fig. 2). Participants with two copies of the *ESR1* rs9340799 variant (homozygous variant) on average gained 14.1 kg more weight than participants with at least one wild-type allele (Fig. 3). Using a conservative Bonferroni corrected p-value of 5.0×10^{-4} , only the associations with BMI and *ESR1* rs9340799 remained statistically significant.

[0039] Multivariable logistic regression was also performed to test for genetic associations with subjective weight gain during contraceptive implant use. Given the differences between primary objective weight change outcome and subjective weight gain, these data are presented in EXAMPLE 2.

Discussion

[0040] This study was undertaken to assess a pharmacogenomic approach could help our understanding of contraceptive-associated weight changes. Among a large, diverse group of etonogestrel contraceptive implant users, unique genetic variants were identified with clinically significant associations for objective weight change. Objective weight changes were found among our participants that closely match the findings of prior studies [2,3]. As measured weight gain has only minimal agreement with reporting subjective weight gain, our study highlights that the perception of weight gain with the contraceptive implant may be inaccurate, which is

consistent with previously published data [16]. BMI at time of enrollment was the only non-genetic variable found to be significantly associated with objective weight change while controlling for all other patient characteristics and demographics.

[0041] One genetic variant associated with objective weight change in contraceptive implant users that met our conservative threshold for statistical significance was identified: *ESR1* rs9340799. Estrogen receptor 1 (*ESR1*) found on chromosome 6, encodes an estrogen receptor involved in cellular hormone binding and DNA transcription when activated [17]. Though *ESR1* rs9340799 is located in the intronic region of *ESR1*, prior studies have found pharmacogenomic associations between this variant and other medications. Postmenopausal women with the homozygous variant *ESR1* rs9340799 genotype (GG) treated with conjugated estrogens and medroxyprogesterone had greater increases in spine bone mineral density as compared to similar women with the homozygous wild-type genotype (AA) [18]. In this same study, women with the homozygous variant genotype (GG) also had smaller decreases in spine bone mineral density when left untreated compared to women with the homozygous wild-type genotype (AA) [18]. These findings demonstrate an increased response to both exogenous and endogenous estrogen among women with the *ESR1* rs9340799 variant genotype, supporting an association between this variant and either upregulation of *ESR1* transcription or a more responsive estrogen receptor 1 protein. Additional pharmacogenomic studies further support that the *ESR1* rs9340799 variant genotype confers an increased clinical response to estrogen agonists [19,20].

[0042] *ESR1* rs9340799 has no known associations with obesity or metabolic syndrome and large studies have failed to find a consistent association between any *ESR1* SNPs and obesity [21,22]. The negative findings of these studies with the general population lend support to our discovery of a progestin-dependent effect between variants in *ESR1* and weight gain. Though etonogestrel has no binding affinity to estrogen receptors [23], research on breast cancer has found that the activated progesterone receptor can directly modulate estrogen receptor 1 transcriptional activity [24]. Thus, these *ESR1* intronic variants or an exonic variant in linkage disequilibrium may lead to an estrogen receptor 1 protein with greater sensitivity to this progesterone receptor modulated relationship. As etonogestrel has a high binding affinity to the progesterone receptor (3 times higher than endogenous progesterone) [23], this ligand-mediated effect may be dependent on this increased binding affinity, thus potentially accounting for the lack of association between *ESR1* variants and obesity in the absence of exogenous progestins.

However, this research on the interplay between the progesterone and estrogen receptors primarily comes from tumor cell lines, which may not accurately reflect the signaling and expression patterns of normal cells [24]. More mechanistic research is needed to elucidate the functional impact of these genetic variants on the transcription and activity of the estrogen receptor 1 protein, particularly as related to interactions with the progesterone receptor.

[0043] The other genetic variant found that was associated with objective weight change (*CYP2C19* rs7088784). *CYP2C19* encodes a metabolic enzyme that may play a role in steroid hormone metabolism, however, this variant was not associated with serum etonogestrel concentrations among our study participants [11]. *CYP2C19* rs7088784 currently has no published pharmacogenomic associations.

[0044] The major strength of this study was in the selection of SNPs from genes encoding proteins with known involvement in steroid hormone pharmacokinetics and pharmacodynamics. Also, a diverse cohort of contraceptive implant users was able to be recruited, and sufficient power was available to detect significant associations between genetic variants and our outcomes of interest. The participants were also representative of the general population of etonogestrel implant users, being relatively young with a wide distribution in BMI [25].

[0045] The BMI cut-off for the parent study (18.5 kg/m^2) and exclusion of underweight implant users limits the generalizability of our findings to such women. Underweight women were excluded to avoid issues of altered metabolism for the parent study's primary pharmacokinetic outcome [11]. The lack of a control group also limits our ability to state that these genetic variants have an association with weight change dependent on etonogestrel exposure. As non-hormonal intrauterine device users also demonstrate wide variability in weight changes [3], more research on the genetic variants associated with weight change among both hormonal and non-hormonal contraceptive users is needed to substantiate a progestin-dependent effect. In addition, weight intention (i.e., gain, loss, maintain) was not collected from the participants, and potential confounding due to diet and exercise differences during implant use was not controlled. Only participants with at least 12 months of contraceptive implants use were enrolled, thus limiting data on early implant discontinuers, however ensuring at least 12 months of opportunity for weight changes. Finally, the candidate gene approach used in this study prevented the identifying novel genetic loci associated with progestin-mediated weight changes

and did not allow for controlling for population stratification, which is confounding by genetic ancestry.

[0046] In this analysis, variants in *ESR1* accounted for four of the seven SNPs associated with weight change, all associated with increased objective weight gain. The *ESR1* rs9340799 variant, in particular, was associated with both a statistically and clinically significant increase in average weight gain of 14 kg among contraceptive implant users with two copies of this variant allele. Exogenous steroid hormone medications are used for various indications throughout a woman's lifespan. It is imperative to understand how individual genetic variation may influence a woman's risk of adverse weight gain while using them. Our results support the pursuit of definitive mechanistic and clinical research to determine the actual influence of *ESR1* variants on progestin-associated weight gain. As our understanding of pharmacogenomics in women's health expands, individualized counseling can be developed that can reduce the incidence of hormone-related adverse effects, improve patient satisfaction, and help prevent future health risks associated with weight gain.

Table 1

Characteristics and demographics of etonogestrel implant users included in this exploratory analysis (N = 276).

	Median (Range)
Age (years)	22.3 (18.0–39.1)
Months of implant use	27.0 (12.0–36.0)
BMI (kg/m ²)	25.8 (18.5–52.0)
Serum etonogestrel concentration (pg/mL)	136.1 (61.0–434.1)
Weight change [*] (kg)	3.2 (-27.6, 26.5)
	n (%)
BMI category [†]	
Normal (18.5 to <25)	116 (42.0)
Overweight (25.0 to <30)	85 (30.8)
Obese (30.0 or higher)	75 (27.2)
Class 1 Obesity (30 to <35)	47 (17.0)
Class 2 Obesity (35 to <40)	20 (7.2)
Class 3 Obesity (40 or higher)	8 (2.9)
Race	
White or Caucasian	119 (43.1)
Black or African American	33 (12.0)
Asian or Pacific Islander	15 (5.4)
Native American or Alaskan	6 (2.2)
More than one	34 (12.3)
No response or Unknown	69 (25.0)
Ethnicity	
Hispanic or Latina	158 (57.2)
Non-Hispanic	118 (42.8)

^{*} Calculated by: (measured weight at time of study enrollment) minus (measured weight at time of implant insertion).

[†] Based on the Centers for Disease Control and Prevention definitions [8].

Table 2

Associations between patient characteristics and demographics and objective weight change in a study of etonogestrel implant users.

	Objective weight change	
	β	95% CI
Age (per year)	-0.16	-0.44, 0.12
Duration of implant use (per month)	0.04	-0.06, 0.13
BMI (at enrollment, per 1 kg/m ²)	0.54 [†]	0.40, 0.67
Serum etonogestrel concentration (per 1 pg/mL)	-0.01	-0.02, 0.01
Race*		
White or Caucasian	-0.43	-2.15, 1.28
Black or African American	-2.5	-5.09, 0.11
Asian or Pacific Islander	-0.75	-4.50, 2.99
Native American or Alaskan	3.51	-2.29, 9.32
More than one	-0.78	-3.36, 1.80
Hispanic or Latina	2.37 [‡]	0.68, 4.07

Performed using simple linear regression. Weight change defined as measured weight at time of study enrollment minus measured weight at time of implant insertion.

* Each race category was dummy coded for comparison to all other participants.

[†] Statistically significant using *p*-value cut-off <0.05.

Table 3

Associations between genetic variants and objective weight change in a study of etonogestrel implant users.

	Objective weight change	
	β	95% CI
Genetic Variants		
CYP3A5 rs15524*	0.75	−0.71, 2.21
CYP3A4*1G [§]	0.38	−1.39, 2.16
CYP3A4 rs2246709 [§]	−0.93	−2.09, 0.24
CYP3A4 rs4646440 [§]	1.53	−0.05, 3.09
ESR1 rs9322335*	1.64 [†]	0.27, 3.01
CYP2C19 rs11568732 [§]	−2.82 [†]	−5.62, −0.02
AKR1C3 rs12529*	1.17 [†]	0.03, 2.31
ESR1 rs2077647*	1.29 [†]	0.05, 2.53
ESR1 rs2228480*	2.68 [†]	1.13, 4.24
CYP2C19 rs7088784 [§]	−2.99 [†]	−5.46, −0.52
ESR1 rs9340799*	1.95 [†]	0.34, 3.55

Performed using simple linear regression.

[†] Statistically significant using p -value cut-off <0.05.

* Genetic variant grouped as participants with two copies of variant allele (homozygous variant) versus all others.

[§] Genetic variant grouped as participants with at least one copy of the variant allele (carriers) versus all others.

Table 4

Final associations between patient characteristics and genetic variants and objective weight change in a study of etonogestrel implant users.

Variable	β	95% CI	p -value
BMI (at enrollment) [†]	0.54	0.39, 0.70	9.39×10^{-12}
CYP2C19 rs7088784 carrier	−3.74	−6.12, −1.35	0.002
ESR1 rs9340799 homozygous variant [†]	14.10	6.86, 21.35	1.36×10^{-4}

Performed using generalized linear modeling with a backward-stepwise approach.

[†] Statistically significant using *Bonferroni* corrected p -value cut-off < 5.0×10^{-4} .

Table 5

List of single nucleotide polymorphisms (SNPs) included in this study

Gene	Variant (rs #)	Designation (if available)	Functional Consequence per dbSNP*
<i>CYP3A4</i>	rs2740574	CYP3A4*1B	Upstream transcript
	rs2242480	CYP3A4*1G	Intron
	rs55785340	CYP3A4*2	Missense
	rs55951658	CYP3A4*4	Missense
	rs56324128	CYP3A4*7	Missense
	rs72552799	CYP3A4*8	Missense
	rs72552798	CYP3A4*9	Missense
	rs67784355	CYP3A4*11	Missense
	rs12721629	CYP3A4*12	Missense
	rs4987161	CYP3A4*17	Missense
	rs67666821	CYP3A4*20	Frameshift
	rs35599367	CYP3A4*22	Intron
	rs2246709		Intron
	rs28371759		Missense
	rs3735451		Intron
	rs4646437		Intron
	rs4646438		Frameshift
	rs4646440		Intron
	rs4986909		Missense
	rs4986910		Missense
<i>CYP3A5</i>	rs28365083	CYP3A5*2	Missense
	rs776746	CYP3A5*3A/*10/*11	Downstream transcript
	rs15524	CYP3A5*3F/*1D/*10	Non coding transcript
	rs10264272	CYP3A5*6	Synonymous
	rs41303343	CYP3A5*7	Non coding transcript
	rs17161788		Non coding transcript
	rs41279854		Missense
	rs55965422		Splice donor
<i>CYP3A7</i>	rs11568824	CYP3A7*1C	Upstream transcript
	rs45446698	CYP3A7*1C	Upstream transcript
	rs11568826	CYP3A7*1C	Upstream transcript
	rs28451617	CYP3A7*1E	5 prime UTR
	rs2257401	CYP3A7*2	Missense
	rs10211		Intron
	rs45465393		Upstream transcript
<i>CYP2C9</i>	rs1799853	CYP2C9*2	Missense
	rs1057910	CYP2C9*3	Missense
	rs56165452	CYP2C9*4	Missense
	rs28371686	CYP2C9*5	Missense
	rs7900194	CYP2C9*8	Missense
	rs28371685	CYP2C9*11	Missense

	rs10509680		Intron
	rs12782374		None
	rs1934969		Intron
	rs4086116		Intron
	rs4918758		Upstream transcript
	rs7089580		Intron
	rs9332096		Upstream transcript
	rs9332098		Upstream transcript
	rs9332238		Intron
<i>CYP2C19</i>	rs17885098	CYP2C19*1B/2C/5B	Synonymous
	rs4244285	CYP2C19*2	Synonymous
	rs3758580	CYP2C19*2B/2C	Synonymous
	rs17878459	CYP2C19*2B/2C	Missense
	rs12571421	CYP2C19*2C	Intron
	rs28399513	CYP2C19*2C	Intron
	rs7916649	CYP2C19*2C/3B	Intron
	rs12769205	CYP2C19*2C/2D	Intron
	rs4417205	CYP2C19*2C/2D	Intron
	rs4986894	CYP2C19*2C/2D	Upstream transcript
	rs4986893	CYP2C19*3	Stop gained
	rs11568732	CYP2C19*3B	Upstream transcript
	rs17884832	CYP2C19*3B	Intron
	rs7088784	CYP2C19*3B	Intron
	rs28399504	CYP2C19*4	Initiator codon
	rs56337013	CYP2C19*5	Missense
	rs72552267	CYP2C19*6	Missense
	rs11188072	CYP2C19*17	None
	rs12248560	CYP2C19*17	Upstream transcript
	rs4917623	CYP2C19*19	Intron
	rs7902257	CYP2C19*27	Upstream transcript
<i>POR</i>	rs1057868	POR*28	Missense
	rs2868177		Intron
<i>NR1I2 (PXR)</i>	rs10934498		Intron
	rs1464602		Intron
	rs1464603		Intron
	rs1523127		5 Prime UTR
	rs1523130		5 Prime UTR
	rs2276706		Intron
	rs2276707		Intron
	rs2461817		Intron
	rs2472677		Intron
	rs3732359		3 Prime UTR
	rs3732360		3 Prime UTR
	rs3814055		5 Prime UTR
	rs3814058		3 Prime UTR

	rs4688040		Intron
	rs6785049		Intron
	rs72551372		Missense
	rs72551374		Missense
	rs7643645		Intron
<i>AKRIC3</i>	rs12529		Missense
	rs1937840		Intron
	rs34186955		Missense
	rs35575889		Missense
	rs4987102		Missense
<i>AKRIC4</i>	rs11253043		Missense
	rs17134592		Missense
<i>HSD3B1</i>	rs7553527		None
<i>LRP2</i>	rs2075252		Stop gained
	rs2228171		Missense
<i>UGT1A1</i>	rs887829	UGT1A1*80	Intron
<i>ESR1</i>	rs2077647		Synonymous
	rs2207396		Intron
	rs2228480		Synonymous
	rs2234693		Intron
	rs2813543		Intron
	rs3020314		Intron
	rs3798577		Intron
	rs4870061		Intron
	rs9322335		Intron
	rs9322336		Intron
	rs9340799		Intron
<i>PGR</i>	rs11224556		None
	rs3740751		Non coding transcript
	rs471767		Non coding transcript
	rs537681		Intron
	rs555653		Intron
	rs561610		Non coding transcript
	rs572943		Intron

* Information obtained from <https://www.ncbi.nlm.nih.gov/snp/>

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EXAMPLE 2 - THE INFLUENCE OF GENETIC VARIANTS ON WEIGHT GAIN AMONG ETONOGESTREL CONTRACEPTIVE IMPLANT USERS - OBJECTIVE VS. SUBJECTIVE WEIGHT GAIN

Methods

[0047] For these additional analyses, subjective weight gain responses (yes/no) were utilized from all 350 participants in the parent study [11]. Participants were given a questionnaire at time of enrollment that included a specific question asking if they had experienced "weight gain" while using their contraceptive implant. IBM SPSS® version 25 statistical software was used for these supplemental analyses. Descriptive frequencies were performed and conducted simple logistic regression was conducted to identify genetic variants associated with patient-reported subjective weight gain (yes/no). Multivariable logistic regression analysis was then performed including genetic variants significantly associated with subjective weight gain (p-value cut-off of 0.05 for inclusion) and the continuous variables of age, BMI, duration of implant use, and serum etonogestrel concentration, and the categorical variables of self-reported race and ethnicity. A backward-stepwise conditional approach was then utilized to create our final logistic regression model where all variables were entered into the model initially. variables without significant

associations ($p < 0.05$) until a model with maximum log-likelihood value was obtained. Given the multiple hypothesis testing performed using simple logistic regression (99 independent analyses), a Bonferroni corrected p-value cut-off of 5.0×10^{-4} was determined. This corrected p-value was used to determine overall significance for the variables included in our final logistic regression model.

Results

[0048] Overall, 41.4% of participants (141/350) reported having experienced "weight gain" during contraceptive implant use. The median BMI at enrollment for all 350 participants was 25.7 kg/m^2 (range 18.5-52.0). Simple logistic regression was performed with the 99 genetic variants included in this analysis to test for associations with patient-reported weight gain. Five genetic variants had significant associations ($p < 0.05$): *CYP3A5* rs15524, *CYP3A4* rs2242480 (designated *CYP3A4*1G*), *CYP3A4* rs2246709, *CYP3A4* rs4646440, and *ESR1* rs9322335. Table 6 shows the crude odds ratios for all patient characteristics and demographics and Table 7 contains the crude odds ratios for these five genetic variants. Using multivariable logistic regression modeling as outlined in the methods, BMI at time of enrollment, Hispanic/Latina ethnicity, and two genetic variants (*CYP3A4*1G* and *ESR1* rs9322335) remained in the final model (Table 8). For every 1 kg/m^2 increase in enrollment BMI, the odds of reporting weight gain increased by 1.1 times, and participants who self-reported as Hispanic/Latina had 2.7 times the odds of reporting weight gain as compared to all other individuals.

[0049] *CYP3A4*1G* had an overall variant allele frequency of 33.3% among our participants successfully genotyped for this SNP ($n=329$) with 41% ($n=135$) having one variant allele (heterozygous) and 12.8% ($n=42$) having two variant alleles. *ESR1* rs9322335 had an overall variant allele frequency of 68.1% among our participants successfully genotyped for this SNP ($n=332$) with 47.6% ($n=158$) having one variant allele (heterozygous) and 44.3% ($n=147$) having two variant alleles (homozygous variant). Participants that had at least one *CYP3A4*1G* allele (carriers) had 2.6 times the odds of reporting weight gain compared to participants homozygous for the wild-type gene, while participants with two copies of the *ESR1* rs9322335 variant (homozygous variant) had 2.7 times the odds of subjective weight gain compared to all other Participants were then sequentially removed. All of these associations remained significant using a Bonferroni corrected p-value cut-off of 5.0×10^{-4} .

[0050] Linkage disequilibrium was evaluated for between *ESRI* rs9340799 (SNP associated with objective weight gain) and *ESRI* rs9322335 (SNP associated with subjective weight gain) using Ensembl 94 (https://www.ensembl.org/Homo_sapiens/Tools/LD). A D' (coefficient of linkage disequilibrium) of 0.59 and r^2 of 0.05 was found between these two SNPs. This D' value indicates at least moderate linkage disequilibrium between these variants, but with a relatively low correlation (r^2) between the two due to the lower variant allele frequency of *ESRI* rs9340799. Thus, these variants are often inherited together, but do not provide identical information.

Table 7

Unadjusted associations between all patient characteristics and demographics and subjective weight gain in a study of etonogestrel implant users

	Subjective weight gain (yes/no)	
	OR	95% CI
Age	0.93 [†]	0.86, 0.99
Duration of implant use	1.01	0.99, 1.04
BMI (at enrollment)	1.12 [†]	1.08, 1.17
Serum etonogestrel concentration	1.00	1.00, 1.00
Race*		
White or Caucasian	0.52 [†]	0.34, 0.81
Black or African American	0.74	0.37, 1.46
Asian or Pacific Islander	1.03	0.40, 2.63
Native American or Alaskan	1.91	0.42, 8.67
More than one	1.21	0.64, 2.28
Hispanic or Latina	2.97 [†]	1.91, 4.63

Performed by simple logistic regression

* Each race category was dummy coded for comparison to all other participants

† Statistically significant using p-value cut-off <0.05

Table 8

Unadjusted associations between genetic variants and subjective weight gain in a study of etonogestrel implant users

Genetic Variants	Subjective weight gain	
	OR	95% CI
<i>CYP3A5</i> rs15524*	0.62 [†]	0.43, 0.90
<i>CYP3A4</i> *1G [§]	2.60 [†]	1.64, 4.11
<i>CYP3A4</i> rs2246709 [§]	0.62 [†]	0.46, 0.86
<i>CYP3A4</i> rs4646440 [§]	2.29 [†]	1.48, 3.55
<i>ESR1</i> rs9322335*	1.54 [†]	1.07, 2.20
<i>CYP2C19</i> rs11568732 [§]	0.60	0.30, 1.20
<i>AKR1C3</i> rs12529*	1.30	0.98, 1.74
<i>ESR1</i> rs2077647*	1.36	0.99, 1.87
<i>ESR1</i> rs2228480*	1.26	0.85, 1.87
<i>CYP2C19</i> rs7088784 [§]	0.61	0.32, 1.16
<i>ESR1</i> rs9340799*	1.03	0.69, 1.55

Performed by simple logistic regression

[†] Statistically significant using p-value cut-off <0.05

* Genetic variant grouped as participants with two copies of variant allele (homozygous variant) versus all others

§ Genetic variant grouped as participants with at least one copy of the variant allele (carriers) versus all others

Table 9

Adjusted associations between patient characteristics and genetic variants and subjective weight gain in a study of etonogestrel implant users

Variable	aOR	95% CI	p-value
BMI (at enrollment) [†]	1.10	1.05, 1.15	2.9×10^{-5}
Hispanic or Latina ethnicity [†]	2.65	1.56, 4.52	3.28×10^{-4}
<i>CYP3A4*1G</i> carrier [†]	2.59	1.52, 4.40	4.53×10^{-4}
<i>ESRI</i> rs9322335 homozygous variant [†]	2.69	1.59, 4.57	2.38×10^{-4}

Performed by multivariable logistic regression using a backward-stepwise approach

[†] Statistically significant using Bonferroni corrected p-value cut-off of $< 5.0 \times 10^{-4}$

[0051] The foregoing is illustrative of the present inventive concept and is not to be construed as limiting thereof. Further embodiments of the present inventive concept are exemplified in the following claims.

THAT WHICH IS CLAIMED IS:

1. A method for selecting, recommending, and/or prescribing a contraceptive for a subject comprising:

identifying any genetic variant or genetic variants of any one gene, or a set of genes comprising *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2*, *PXR*, *CYP3A4*, and/or *CYP2C9*, or a combination of more than one thereof the subject possesses; and

selecting, recommending, and/or prescribing the contraceptive depending on the genetic variant or the genetic variants of *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2*, *PXR*, *CYP3A4*, and/or *CYP2C9*, or a combination of more than one thereof the subject possesses.

2. The method of claim 1, wherein the contraceptive is a progestin.

3. The method of claim 2, wherein the progestin is etonogestrel.

4. The method of claim 3, wherein the etonogestrel is provided by a contraceptive implant.

5. The method of claim 1, wherein the contraceptive is an alternative to etonogestrel.

6. The method of claim 5, wherein the alternative to etonogestrel is a non-hormonal contraceptive.

7. The method of any one of claims 1–6, wherein the genetic variant is *ESR1* rs9340799, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has a wild-type *ESR1* rs9340799 genotype, and an alternative to etonogestrel is selected, recommended, and/or prescribed if the subject has two copies of a variant allele for *ESR1* rs9340799.

8. The method of any one of claims 1–6, wherein the genetic variant is *CYP2C19* rs7088784, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has at least one variant allele for *CYP2C19* rs7088784.

9. The method of any one of claims 1–6, wherein the genetic variant is *LRP2* rs2075252, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has a wild-type *LRP2* rs2075252 genotype, and an alternative to etonogestrel is selected, recommended, and/or prescribed if the subject has a variant *LRP2* rs2075252 genotype.

10. The method of any one of claims 1–6, wherein the genetic variant is *HSD3B1* rs7553527, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has a variant *HSD3B1* rs7553527 genotype.

11. The method of any one of claims 1–6, wherein the genetic variant is *CYP2C19*19*, *CYP3A7*1C* and *PXR* rs2471817, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has wild-type *CYP2C19*19*, and wild-type *PXR* rs2471817 genotypes, and an alternative to etonogestrel is selected, recommended, and/or prescribed if the subject has a variant *CYP2C19*19*, and variant *PXR* rs2471817 genotypes.

12. The method of any one of claims 1–6, wherein the genetic variant is *CYP2C19*19*, *CYP3A7*1C* and *PXR* rs2471817, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has a variant *CYP3A7*1C* genotype and a wild-type *CYP2C19*19* genotype.

13. A method of minimizing side effects of a contraceptive selected, recommended, and/or prescribed for a subject comprising:

identifying any genetic variant or genetic variants of any one gene, or a set of genes comprising *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2*, *PXR*, *CYP3A4*, and/or *CYP2C9*, or a combination of more than one thereof the subject possesses; and

selecting, recommending, and/or prescribing the contraceptive depending on the genetic variant or the genetic variants of *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2*, *PXR*, *CYP3A4*, and/or *CYP2C9* or a combination thereof the subject possesses.

14. The method of claim 13, wherein the contraceptive is a progestin.

15. The method of claim 14, wherein the contraceptive is etonogestrel.
16. The method of claim 14, wherein the etonogestrel is provided by a contraceptive implant.
17. The method of claim 13, wherein the contraceptive is an alternative to etonogestrel.
18. The method of claim 17, wherein the alternative to etonogestrel is a non-hormonal contraceptive.
19. The method of any one of claims 13–18, wherein the genetic variant is *ESR1* rs9340799, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has a wild-type *ESR1* rs9340799 genotype, and an alternative to etonogestrel is selected, recommended, and/or prescribed if the subject has two copies of a variant allele for *ESR1* rs9340799.
20. The method of any one of claims 13–18, wherein the genetic variant is *CYP2C19* rs7088784, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has at least one variant allele for *CYP2C19* rs7088784.
21. The method of any one of claims 13–18, wherein the genetic variant is *LRP2* rs2075252, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has a wild-type *LRP2* rs2075252 genotype, and an alternative to etonogestrel is selected, recommended, and/or prescribed if the subject has a variant *LRP2* rs2075252 genotype.
22. The method of any one of claim 13–18, wherein the genetic variant is *HSD3B1* rs7553527, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has a variant *HSD3B1* rs7553527 genotype.

23. The method of any one of claims 13–18, wherein the side effects are any one side effect selected from the group consisting of weight gain, mood changes, abnormal bleeding, and breast tenderness.

24. The method of claim 23, wherein the side effect is weight gain, and the genetic variant is *ESR1* rs9340799, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has a wild-type *ESR1* rs9340799 genotype, and an alternative to etonogestrel is selected, recommended, and/or prescribed if the subject has a variant *ESR1* rs9340799 genotype.

25. The method of claim 23, wherein the side effect is mood changes, and the genetic variant is *LRP2* rs2075252, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has a wild-type *LRP2* rs2075252 genotype, and an alternative to etonogestrel is selected, recommended, and/or prescribed if the subject has a variant *LRP2* rs2075252 genotype.

26. The method of claim 23, wherein the side effect is mood changes, and the genetic variant is *HSD3B1* rs7553527, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has a variant *HSD3B1* rs7553527 genotype.

27. The method of claim 23, wherein the side effect is abnormal bleeding, and the genetic variants are *CYP2C19**19, *CYP3A7**1C and *PXR* rs2471817, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has wild-type *CYP2C19**19, and *PXR* rs2471817 genotypes, and an alternative to etonogestrel is selected, recommended, and/or prescribed if the subject has a variant *CYP2C19**19, and variant *PXR* rs2471817 genotypes.

28. The method of claim 23, wherein the side effect is abnormal bleeding, and the genetic variants are *CYP2C19**19, *CYP3A7**1C and *PXR* rs2471817, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has a variant *CYP3A7**1C genotype and a wild-type *CYP2C19**19 genotype.

29. The method of claim 23, wherein the side effect is breast tenderness, and the genetic variant is *CYP3A4*IG* (rs2242480), and wherein an alternative to etonogestrel is selected if the subject has a variant *CYP3A4*IG* (rs2242480) genotype.

30. The method of claim 23, wherein the side effect is breast tenderness, and the genetic variant is *CYP2C9* rs9332238, wherein the side effect is breast tenderness, and wherein etonogestrel is selected if the subject has a variant *CYP2C9* rs9332238 genotype.

31. A method of administering a contraceptive to a subject comprising:
identifying any genetic variant or genetic variants of any one gene, or a set of genes comprising *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2* and/or *PXR*, or a combination of more than one thereof the subject possesses; and
selecting a contraceptive depending on the genetic variant or the genetic variants of *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2* and/or *PXR*, or a combination of more than one thereof the subject possesses; and
administering the contraceptive to the subject.

32. The method of claim 31, wherein the contraceptive is a progestin.

33. The method of claim 32, wherein the progestin is etonogestrel.

34. The method of claim 33, wherein the etonogestrel is provided by a contraceptive implant.

35. The method of claim 31, wherein the contraceptive is an alternative to etonogestrel.

36. The method of claim 35, wherein the alternative to etonogestrel is a non-hormonal contraceptive.

37. The method of any one of claims 31–36, wherein the genetic variant is *ESR1* rs9340799, and wherein etonogestrel is selected if the subject has a wild-type *ESR1* rs9340799 genotype, and an alternative to etonogestrel is selected if the subject has two copies of a variant allele for *ESR1* rs9340799.

38. The method of any one of claims 31–36, wherein the genetic variant is *CYP2C19* rs7088784, and wherein etonogestrel is selected, recommended, and/or prescribed if the subject has at least one variant allele for *CYP2C19* rs7088784.

39. The method of any one of claims 31–36, wherein the genetic variant is *LRP2* rs2075252, and wherein etonogestrel is selected if the subject has a wild-type *LRP2* rs2075252 genotype, and an alternative to etonogestrel is selected if the subject has a variant *LRP2* rs2075252 genotype.

40. The method of any one of claims 31–36, wherein the genetic variant is *HSD3B1* rs7553527, and wherein etonogestrel is selected if the subject has a variant *HSD3B1* rs7553527 genotype.

41. The method of any one of claims 31–36, wherein the genetic variants are *CYP2C19*19*, *CYP3A7*1C* and *PXR* rs2471817, and wherein etonogestrel is selected if the subject has wild-type *CYP2C19*19*, and *PXR* rs2471817 genotypes, and an alternative to etonogestrel is selected if the subject has a variant *CYP2C19*19*, and variant *PXR* rs2471817 genotypes.

42. The method of any one of claims 31–36, wherein the genetic variants are *CYP2C19*19*, *CYP3A7*1C* and *PXR* rs2471817, and wherein etonogestrel is selected if the subject has a variant *CYP3A7*1C* genotype and a wild-type *CYP2C19*19* genotype.

43. A method of minimizing side effects associated with administering a contraceptive to a subject comprising:

identifying any genetic variant or genetic variants of any one gene, or a set of genes comprising *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2*, *PXR*, *CYP3A4*, and/or *CYP2C9* the subject possesses; and

selecting a contraceptive depending on the genetic variant or genetic variants of *ESR1*, *CYP2C19*, *CYP3A7*, *HSD3B1*, *LRP2*, *PXR*, *CYP3A4*, and/or *CYP2C9* the subject possesses; and administering the contraceptive to the subject.

44. The method of claim 43, wherein the side effects are any one side effect selected from the group consisting of weight gain, mood changes, abnormal bleeding, and breast tenderness.

45. The method of claim 44, wherein the side effect is weight gain, and the genetic variant is *ESR1* rs9340799, and wherein etonogestrel is selected if the subject has a wild-type *ESR1* rs9340799 genotype, and an alternative to etonogestrel is selected if the subject has a variant *ESR1* rs9340799 genotype.

46. The method of claim 44, wherein the side effect is mood changes, and the genetic variant is *LRP2* rs2075252, and wherein etonogestrel is selected if the subject has a wild-type *LRP2* rs2075252 genotype, and an alternative to etonogestrel is selected if the subject has a variant *LRP2* rs2075252 genotype.

47. The method of claim 44, wherein the side effect is mood changes, and the genetic variant is *HSD3B1* rs7553527, and wherein etonogestrel is selected if the subject has a variant *HSD3B1* rs7553527 genotype.

48. The method of claim 44, wherein the side effect is abnormal bleeding, and the genetic variants are *CYP2C19**19, *CYP3A7**1C and *PXR* rs2471817, and wherein etonogestrel is selected if the subject has wild-type *CYP2C19**19, and *PXR* rs2471817 genotypes, and an alternative to etonogestrel is selected if the subject has a variant *CYP2C19**19, and variant *PXR* rs2471817 genotypes.

49. The method of claim 44, wherein the side effect is abnormal bleeding, and the genetic variants are *CYP2C19**19, *CYP3A7**1C and *PXR* rs2471817, and wherein etonogestrel is selected if the subject has a variant *CYP3A7**1C genotype and a wild-type *CYP2C19**19 genotype.

50. The method of claim 44, wherein the side effect is breast tenderness, and the genetic variant is *CYP3A4**1G (rs2242480), and wherein an alternative to etonogestrel is selected if the subject has a variant *CYP3A4**1G (rs2242480) genotype.

51. The method of claim 44, wherein the side effect is breast tenderness, and the genetic variant is *CYP2C9* rs9332238, wherein the side effect is breast tenderness, and wherein etonogestrel is selected if the subject has a variant *CYP2C9* rs9332238 genotype.

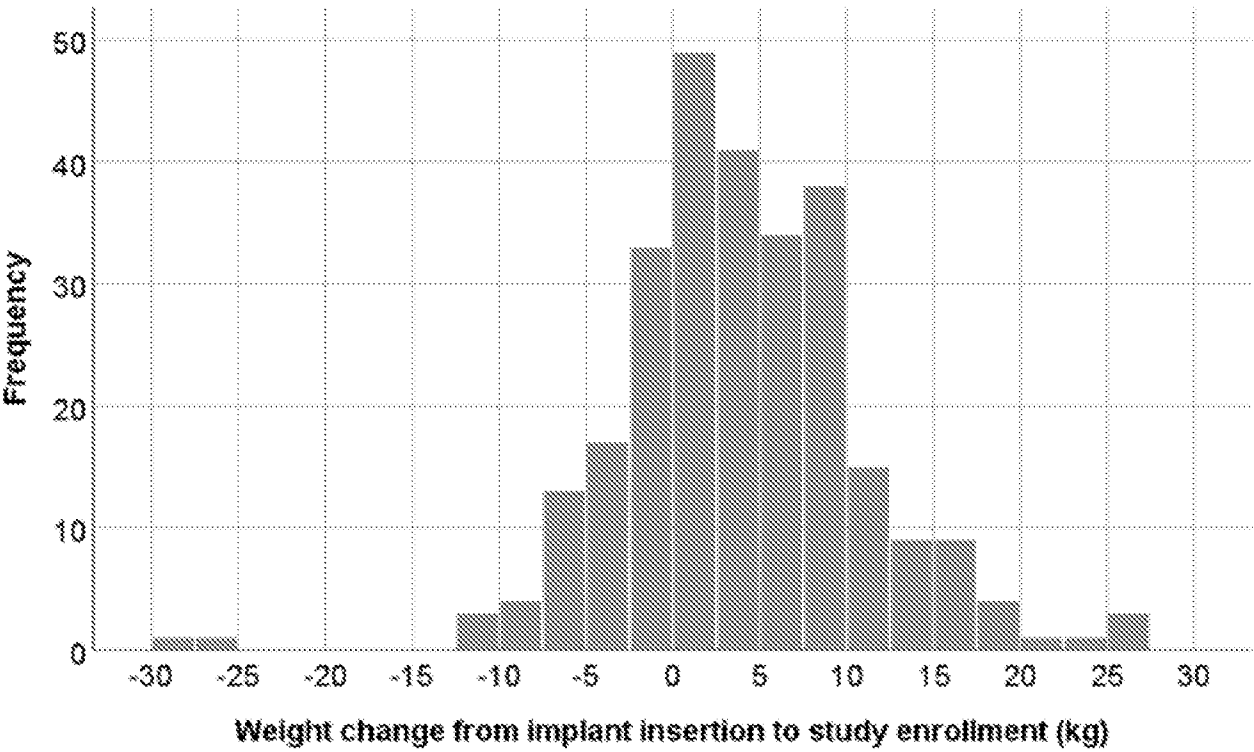


FIG. 1

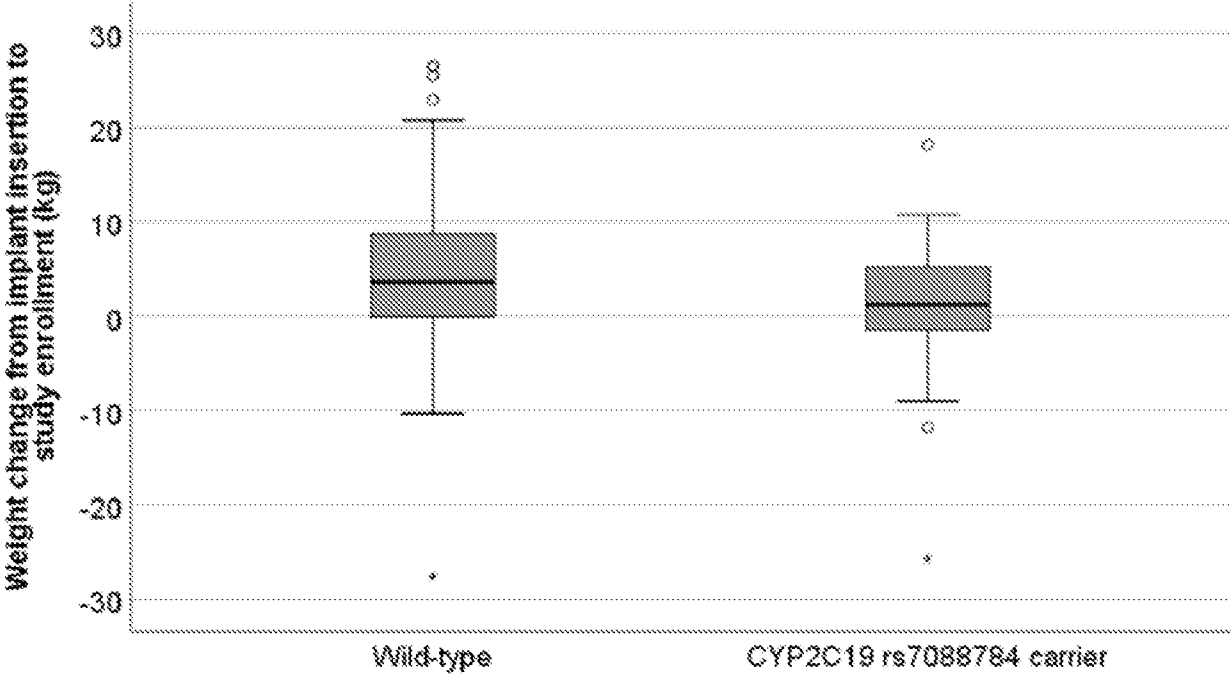
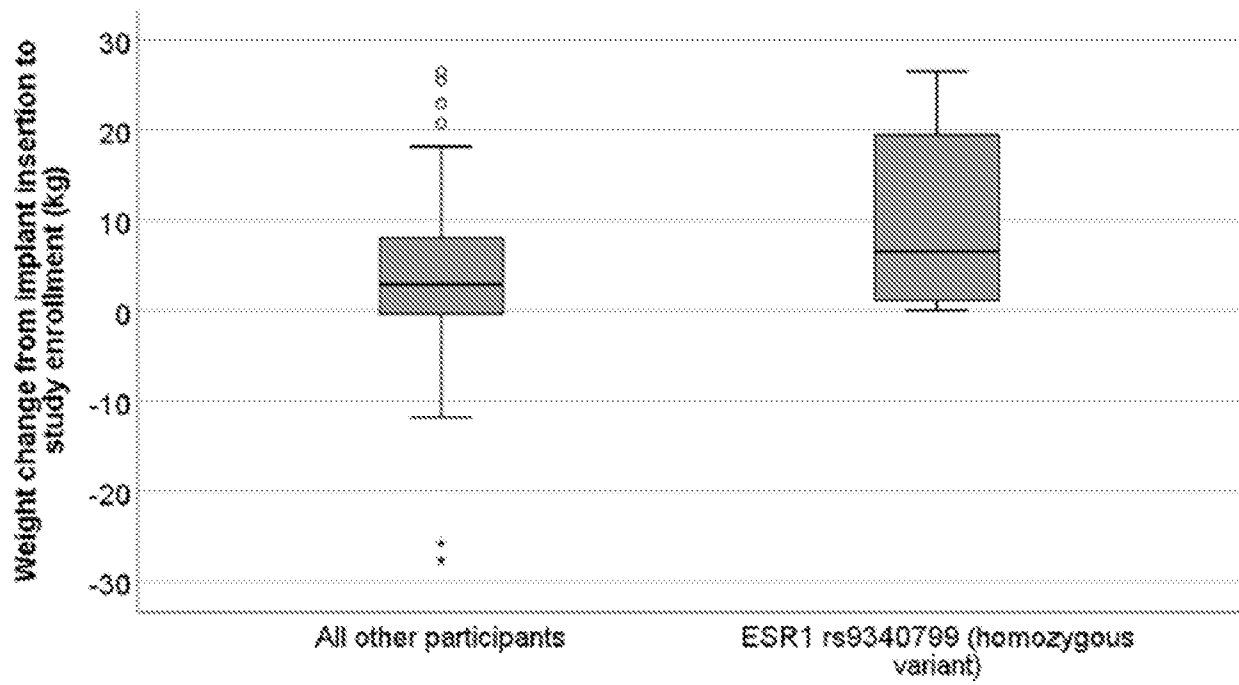


FIG. 2

***FIG. 3***

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 22/28760

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C12Q 1/68, C12Q 1/6827, A61P 15/18; ADD. C12Q 1/6841 (2022.01)

CPC - INV. C12Q 1/6883, C12Q 1/6876, C12Q 1/6827, A61P 15/18; ADD. C12Q 2600/156

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LAZORWITZ et al., An exploratory analysis on the influence of genetic variants on weight gain among etonogestrel contraceptive implant users. Contraception, September 2020, Vol. 102, No. 3, pg. 180-185. full text, especially pg 181, col 1, para 3; pg 181, col 1, para 4; pg 182, col 1, para 3; pg 182, col 1, para 4; pg 184, col 2, para 1	1-7, 13-19, 23-24, 31-37, 43-45
A	US 2002/0123095 A1 (KALUSH et al.) 05 September 2002 (05.09.2002) whole doc.	1

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

22 July 2022

Date of mailing of the international search report

SEP 28 2022

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 22/28760

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

----- See extra sheet -----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-7, 13-19, 23-24, 31-37, 43-45 limited to ESR1 genetic variant

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 22/28760

--continued from: Box No. III Observations where unity of invention is lacking--

Groups I+: Claims 1-50, drawn to a method for selecting a contraceptive for a subject comprising: identifying any genetic variant or genetic variants. The method will be searched to the extent that the genetic variant encompasses ESR1 (see claim 1). This first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. It is believed that claims 1-7, 13-19, 23-24, 31-37, 43-45 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass ESR1 genetic variant. Additional genetic variant(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected genetic variant(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a genetic variant encompassing CYP2C19 (Claims 1-6, 8, 13-18, 20, 23, 31-36, 38 43-44).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

The inventions of Groups I+ each include the special technical feature of a specific genetic variant, not required by any of the other inventions of Groups I+.

Common Technical Features

The inventions of Groups I+ share the technical feature of a method for selecting, recommending, and/or prescribing a contraceptive for a subject comprising: identifying any genetic variant or genetic variants.

However, these shared technical features do not represent a contribution over prior art in view of the article entitled "An exploratory analysis on the influence of genetic variants on weight gain among etonogestrel contraceptive implant users" by Lazorwitz et al. (hereinafter 'Lazorwitz') (Contraception, 2020, Vol. 102, pp. 180-185).

Lazorwitz teaches (instant claim 1) a method for selecting, recommending, and/or prescribing a contraceptive for a subject (pg 184, col 2, para 1 - "As our understanding of pharmacogenomics in women's health expands, we can develop individualized counseling that may reduce the incidence of hormone-related adverse effects, improve patient satisfaction, and help prevent future health risks associated with weight gain") comprising:

identifying any genetic variant or genetic variants of any one gene, or a set of genes comprising ESR1, CYP2C19, CYP3A7, HSD3B1, LRP2, PXR, CYP3A4, and/or CYP2C9, or a combination of more than one thereof the subject possesses; and selecting, recommending, and/or prescribing the contraceptive depending on the genetic variant or the genetic variants of ESR1, CYP2C19, CYP3A7, HSD3B1, LRP2, PXR, CYP3A4, and/or CYP2C9, or a combination of more than one thereof the subject possesses (pg 181, col 1, para 3 - "To explore potential pharmacogenomic associations between hormonal contraceptive use and weight gain, we used a candidate gene approach to identify genetic variants associated with measured objective weight change among etonogestrel contraceptive implant users"; pg 182, col 1, para 3 - "We performed simple linear regression to test for genetic associations with objective weight change from contraceptive implant insertion to study enrollment. Seven genetic variants had significant associations ($p < 0.05$): CYP2C19 rs11568732, AKR1C3 rs12529, ESR1 rs2077647, ESR1 rs2228480, CYP2C19 rs7088784, ESR1 rs9322335, and ESR1 rs9340799").

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.