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Impact of gene polymorphisms of gonadotropins and their receptors on human reproductive success.

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Short Title

Gonadotropin SNPs and human reproductive success.

Abstract

Gonadotropins and their receptors' genes carry several single-nucleotide polymorphisms resulting in endocrine genotypes modulating reproductive parameters, diseases and lifespan leading to important implications for reproductive success and potential relevance during human evolution. Here we illustrate common genotypes of the gonadotropins and gonadotropin receptors' genes and their clinical implications in phenotypes relevant for reproduction such as ovarian cycle length, age of menopause, testosterone levels, polycystic ovary syndrome and cancer. We then discuss their possible role in human reproduction and adaptation to the environment. Gonadotropins and their receptors' variants are differently distributed among human populations. Some hints suggest that they may be the result of natural selection occurred in ancient times, increasing the individual chance of successful mating, pregnancy, and effective post-natal parental cares. The gender-related differences in regulation of the reproductive endocrine systems imply that many of these genotypes may lead to sex-dependent effects, increasing the chance of mating and reproductive success in one sex at the expenses of the other sex. Also, we suggest that sexual conflicts within the follicle-stimulating and luteinizing hormone-choriogonadotropin receptor genes contributed to maintain genotypes linked to subfertility among humans. Since the distribution of polymorphic markers results in a defined geographical pattern due to human migrations rather than natural selection, these polymorphisms may have had only a weak impact on reproductive success. On the contrary, such genotypes could acquire relevant consequences in the modern, developed societies, in which parenthood attempts often occur at later age, during a short, suboptimal reproductive window, making clinical fertility treatments necessary.

Introduction.

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are glycoproteins produced by the pituitary regulating development and reproductive functions in both men and women. On the contrary, choriogonadotropin (hCG) is the human placental hormone managing pregnancy. Gonadotropins share a common α subunit together with the thyroid-stimulating hormone (TSH), while having a unique β subunit, specific for the receptor located in the gonads. The FSH receptor (FSHR) and the common LH/hCG receptor (LHCGR) belong to the superfamily of the G protein-coupled receptors (GPCRs). They are characterized by an extracellular domain, 7 trans-membrane domains joined by 3 intra- and extra-cellular loops, and an intracellular, C-terminal domain. Upon hormone binding with the extracellular portion, the intracellular domain triggers the activation of multiple signaling pathways by interacting with specific molecules, such as G proteins or β-arrestins (Simoni et al., 1997; Ascoli et al., 2002; Gloaguen et al., 2011).

Gonadotropins and their receptor genes carry several single-nucleotide polymorphisms (SNPs), resulting in several genotypes differently distributed among human populations and affecting sex-related reproductive features and diseases by modulating signal transduction (Casarini et al., 2011). These genotypes are evolutionarily old and have accompanied humans during their ancient migrations throughout the continents. However, the impact of these SNPs on human reproductive success and evolution is unclear and was recently debated (Grigorova et al., 2007; Simoni and Casarini, 2014).

Polymorphisms of the *FSHR* and *FSHB* genes.

The *FSHR* carries about two thousands SNPs but only a few of these are known as modulators of gonadal response. One of the most common *FSHR* polymorphisms is rs6166 (NCBI SNPs database ID; http://www.ncbi.nlm.nih.gov) consisting in the nucleotide change A to G at position 2039 from the gene transcription start codon (c.2039A>G), and resulting in the amino acid change N to S at position 680 of the protein chain (p.N680S). rs6166 is in strong *linkage disequilibrium* with the SNP rs6165 (c.919A>G, p.T307A), at least in Caucasians and Asians, resulting in two discrete FSHR isoforms. p.N680S is close to the C-terminal intracellular region of the receptor and modulates serum FSH levels and gonadal response in both women and men (Lledo, et al. 2013; Grigorova et al., 2014; Simoni and Casarini, 2014). Women carriers of the p.N680S S homozygous genotype have higher serum FSH levels during the follicular phase and lower progesterone levels in the luteal phase than the carriers of different genotypes, while p.N680S N homozygous males are characterized by higher testes volume than p.N680S S homozygous men. It was suggested that the FSHR p.N680S S variant is functionally ‘’resistant’’ to FSH stimulation; The p.N680S polymorphism modulates cell signaling resulting in differential gene expression and steroidogenesis in cultured human lutein granulosa cells as recently demonstrated *in vitro* (Casarini et al., 2014).

Interestingly, the cumulative effect of p.N680S together with other FSHR polymorphisms (e.g. rs1394205; -29G>A) was proposed, leading to genotypes linked with lower fertility (Casarini and Simoni, 2014; Grigorova et al., 2014). The -29G>A SNP falls within the 5’-untranslated region of the *FSHR* gene, 29 nucleotide upstream the ATG codon. The *in vitro* transcriptional activity of the -29G>A A variant is lower than that of the -29G>A G genotype in Chinese hamster ovary (CHO) cells transfected with the *FSHR* promoter and was found to be associated with hypertension (Nakayama et al., 2006), lower estradiol levels in women (Achrekar et al., 2009) and higher serum FSH levels (Achrekar et al., 2009; Grigorova et al., 2014).

The FSHβ subunit is encoded by the *FSHB* gene, which carries about twenty-four SNPs, but only the rs10835638 (-211G>T), located in the promoter region of the gene (-211G>T, rs10835638), was extensively studied in association with serum FSH levels and reproductive parameters in males (Grigorova et al. 2008). In particular, -211G>T T homozygous Baltic, Italian and German men have lower FSH levels and testis volume compared to carriers of other genotypes (Grigorova et al., 2008; Tüttelmann et al., 2012; Grigorova et al., 2014). The promoter region of the *FSHB* gene is a putative target of a transcription regulatory element and is highly conserved among placental mammals (Grigorova et al. 2008), suggesting that the T nucleotide at position -211 affects the *FSHB* gene transcription leading to low hormone levels. Interestingly, the studies performed in males and females are contradictory; -211G>T T homozygous women were shown to have elevated FSH, LH, and reduced progesterone levels compared with carriers of other genotypes, suggesting a gender-specific, compensatory regulation of the gonadotropin secretion (Schüring et al., 2013). Further elucidations may be provided by genotype-phenotype association studies focusing on the cumulative effect of *FSHB* together with *FSHR* gene SNPs, revealing how they affect the sex-related modulation of hormone levels and reproductive parameters. Taken together, the combination of SNPs within the *FSHB* and *FSHR* genes account for a substantial proportion of the total normal phenotypic variance in male and female reproductive parameters (Tüttelmann et al., 2012; La Marca et al., 2013; Grigorova et al., 2014; Simoni and Casarini, 2014).

Polymorphisms of the *LHCGR* gene and *LHB*/*CGB* gene cluster.

Several inactivating mutations of the *LHCGR* were associated with peculiar phenotypes such as 46,XY disorder of sex development (DSD), primary amenorrhea and anovulation in women (Powell et al. 2003), and undescended testes and androgen deficiency in men (Simoni et al. 2008), revealing the crucial role of this receptor in human sex development and reproduction. *LHCGR* harbors at least 300 known polymorphisms but only few of them lead to relevant effects (Casarini et al., 2011).

The LHCGR variant 18insLQ, consisting in the insertion of 6 nucleotides in frame in exon 1 and falling near the N-terminus of the mature receptor, was associated with early onset of breast cancer and short disease-free survival. This is consistent with increased LHCGR 18insLQ sensitivity and plasma membrane expression (1.9 fold lower hCG half-effective concentration and 1.4 fold higher expression levels than wild-type LHCGR, respectively) (Piersma et al., 2006). Interestingly, LHCGR 18insLQ has a high frequency among Northern-European Caucasians which are characterized by higher prevalence of breast cancer compared to other ethnic groups, leading to the speculation that the *LHCGR* genotype may be linked to disease risk (Casarini et al., 2011).

Only few other *LHCGR* SNPs provided significant clinical findings so far. The SNP rs2293275 (c.942G>A, p.S312N), which falls within exon 10 of the *LHCGR* gene, might affect the trafficking and stability of the receptor resulting in impaired spermatogenesis in men (Simoni et al., 2008) and increased risk of developing polycystic ovary syndrome (PCOS) in women (Thathapudi et al., 2015). Lastly, the polymorphic LHCGR variant rs4073366 (c.3442-20797C>G) occurr about 142 base pairs downstream of LHCGR18insLQ. The C allele was associated with an approximately 3-fold increased risk of developing ovarian hyperstimulation syndrome (OHSS) in adult women undergoing procedures for assisted reproduction (O'Brien et al., 2013).

Few *LHB* gene variants are known. The so-called "V-LH" variant was discovered in Finland and consists in the double amino acid exchange p.W8R and p.I15T of *LHB* (Pettersson et al., 1992). V-LH shows a lower circulatory half time and bioactivity *in vivo* than the "classical" LH, possibly compensated by increased transcriptional levels of the LH beta subunit due to SNPs within the promoter *LHB* region, which are in *linkage disequilibrium* with p.W8R and p.I15T (Jiang et al., 1999). Curiously, V-LH may be a protective agent from symptomatic PCOS in obese women, among which it is less frequent compared to healthy women and *non*-obese PCOS patients (Tapanainen et al., 1999).

While the genes encoding the FSHβ and LHβ are present in all vertebrates, the CGβ-coding genes exist only in primates and equids, likely as result of repeated duplications of an ancestral *LHB* gene (Henke and Gromoll, 2008). The human genome carries eight *CGB* genes contiguous with the LHB gene on chromosome 19; subsequently frame-shift mutations and nucleotide insertions resulted in 24 additional codons for *CGB*. The *LHB*/*CGB* gene cluster spans about 40 Kbase-pairs and carries several SNPs; especially, polymorphic variants of the *CGB5* were associated with recurrent spontaneous abortions in Chinese and Caucasian women (Rull et al., 2008; Sun and Ji, 2014).

Gonadotropin variants and implications in disease and menopause

Although further investigations are needed to elucidate the molecular mechanisms underlying the modulatory effects of SNPs within *FSHR* and *FSHB* genes on reproductive parameters and diseases, their pathophysiological relevance and clinical outcomes were widely described in the literature. On the contrary, the pathophysiological implications of SNPs belonging to the *LHCGR* gene and the *LHB*/*CGB* gene cluster are poorly understood.

Polycystic ovarian syndrome. PCOS is a common endocrine disorder affecting 4-10% of women in reproductive-age. A wide number of candidate genes were found to be potential markers of the disease (Chen et al., 2011; Shi et al., 2012). PCOS women are characterized by heterogenenous sub-fertile phenotypes and related clinical features. Hyperandrogenism, metabolic syndrome, insulin resistance and anovulation are some of the main clinical aspects of PCOS, which may be the result of endocrine adaptation to ancestral environmental conditions (Corbett and Morin-Papunen, 2013; Casarini and Brigante, 2014). Several studies searched evolutionary explanations for the origin of PCOS, suggesting that the energy saving resulting from less-ovulatory reproductive systems and insulin resistant phenotypes may be advantageous during seasons of food shortage or high energy demand, when indeed the anovulation risk increases (Vitzthum et al., 2004; Vitzthum, 2009; Corbett and Morin-Papunen, 2013). However, theories supporting natural selection of PCOS phenotypes were downsized in favor of genetic drift; this issue is still debated and need further investigation (Casarini and Brigante, 2014). Gonadotropins and their receptors are logical candidate genes involved in the pathogenesis of the disease due to their crucial role in folliculogenesis and hormone regulation. However, conflicting data exist in the literature, because of the polygenic nature of the disease and the ethnic differences in the prevalence of lifestyle-related symptoms.

Alzheimer's disease. The Alzheimer's disease is a progressive, neurodegenerative disorder characterized by neuronal and synaptic loss, neurofibrillary tangles located in neuronal cytoplasm and deposition of amyloid in neuritic plaques. Genoma wide association studies (GWAS) suggested that SNPs within the *FSHR* and *LHCGR* genes may contribute to the pathogenesis of the disease (Sun et al., 2014). Especially, the polymorphism rs4073366 (c.161+28G>C) located within the first intron of the *LHGCR* gene was associated with a protective effect from the disease risk in the male (Haasl et al., 2008).

Cancer. Gonadotropins activate multiple intra-cellular signaling pathways which may result in proliferative or anti-apoptotic events in primary cells and cell lines; also, gonadotropin receptors are expressed in several tumor cells (Mertens-Walker et al., 2012), thus, the possible link between hormone level and cancer risk was proposed.

FSHR p.N680S was indicated as possible modulator of ovarian cancer (Yang et al., 2006; Ludwig et al., 2009) as well as LHCGR polymorphism 18insLQ, which may be linked with breast cancer risk (Powell et al., 2003). Some studies suggested that *LHB* SNPs are risk factors for cryptorchidism (Kaleva et al., 2005) and testicular cancer (Elkins et al., 2003). Interestingly, SNPs within gonadotropin genes were linked to papillary thyroid cancer risk (Schonfeld, et al. 2012), revealing possible cross-activity among these molecules and their receptors.

Menopausal age. A link between menopausal age and SNPs in gonadotropins and their receptors' genes was suggested, providing a wide spectrum of candidate markers and conflicting, ethnicity-related results. Several loci associated with age at natural menopause were identified by meta-analyzing 22 GWAS in women of European ancestry (Stolk et al., 2012, Perry et al., 2014). This statistically powerful analysis identified top SNPs located within 3 out of 17 genomic regions in strong *linkage disequilibrium* with *FSHB*, *STARD1* and *BCAR4* genes in Caucasians, suggesting that they are involved in hormonal regulation of follicle recruitment and exhaustion, but further confirmation in other ethnic groups are required. Interestingly, women with PCOS have a later onset of menopause compared to normo-ovulatory women (Tehrani et al., 2010), likely resulting from the protective effect of high anti-Mullerian hormone levels for ovarian reserve, extending the reproductive lifespan in spite of less ovulatory cycles.

Taken together, SNPs in the gonadotropins and their receptors' genes modulate fertility of both sexes and may affect lifespan and reproductive health.

Limitations

Due to the polygenic regulation and the modulatory effects of lifestyle on reproductive traits (Sharma et al., 2013), genotype-phenotype associations need to be well-characterized in different, appropriately sized sample groups and independently confirmed to avoid methodological biases. However, the medical literature often provides conflicting results. Although the link between the FSHR SNP p.N680S and serum FSH levels or ovarian response was repeatedly observed (Simoni and Casarini, 2014), other studies failed to find the same associations (Binder et al., 2012; Mohiyiddeen et al., 2013; Trevisan et al., 2014), suggesting that the endocrine features are modulated by several factors such as age or ethnicity. However, studies using suboptimal sample groups characterized by subfertility or endocrine dysfunction (e.g. pre-menopausal women or poor responders to gonadotropin treatments) should be carefully evaluated. Proper sample sizes and combined genotype analysis are required to detect significant and clinically relevant associations. For example, to unmask the effects of the p.N680S polymorphism on serum FSH levels in men, a combined model taking into account the *FSHB* promoter SNP -211G>T may be necessary (Tüttelmann et al., 2012). Association studies of polygenic traits should be replicated in different sample groups rigorously established, and corroborated by *in vitro* evidences. Finally, mathematical corrections weighting the sample size from different investigations should provide the optimal verification, therefore, meta-analyzes may be a safe and reliable tool to further confirm *in vivo* association studies.

Population genetics of of gonadotropins and gonadotropin receptors' polymorphisms

Previous studies demonstrated that the African continent holds the highest human genetic variability worldwide (Cann et al., 2002; Ramachandran et al., 2005; Li et al., 2008). Consistently with the routes of ancient human migrations, genetic variability decreases together with the distance from Africa, and oppositely to the genetic diversity, determining the current distribution of several sex-related genetic markers (Casarini and Brigante, 2014). Since natural selection contributed poorly to the distribution of human genotypes worldwide (Li et al., 2008), it is reasonable that slightly different hormonal levels and menstrual cycle duration may have only a marginal impact on the selection of sex-related genotypes, compared to other, more determinant phenotypic features, such as skin pigmentation or sickle cell anemia (Liu et al., 2013).

On the other hand, a full explanation of human reproductive success may not merely rely on human migrations or genetic drift, and the evolutionary role of the SNPs in gonadotropin and their receptors' genes was debated (Grigorova et al., 2007; Simoni and Casarini, 2014). It was estimated that about 20% of Caucasians carry a "less favorable" *FSHB*/*FSHR* genotype, in terms of serum FSH levels and FSHR expression and activity, which are enriched in sub-fertile subjects previously studied (Simoni and Casarini, 2014). Especially, ovarian cycle length depends, at least in part, on the combination of *FSHB* and *FSHR* genotypes, which affect the sensibility threshold to FSH. This results in heterogeneity in menstrual cycle length and, consequently, a theoretical difference in the total number of cycles which can be calculated in about ±30-40 ovarian cycles during the reproductive lifespan depending on the *FSHR* genotype. FSHR p.N680S S homozygous women have longer ovarian cycle than p.N680S N homozygous women (Greb et al., 2005). In fact the FSHR variant carrying the amino acid serine at position 680 is more abundant in South-Central Asians and Oceanians (Simoni and Casarini, 2014) who are characterized by an overall longer cycle duration than women of East Asian, European or African ancestry (Vitzhum, 2009). This is consistent with the lower steroidogenic potential of the FSHR p.N680S homozygous S compared to the homozygous N genotype (Casarini et al., 2014). Most importantly, this suggests that some women have a lower number of ovulations for months of exposure, potentially resulting in slightly lower reproductive potential, but preserving the individual from unnecessary energy expenditure to maintain overall fitness (Simoni and Casarini, 2014). However, since women with low cycle variability have a higher conception rate than those with longer but irregular cycle duration, pregnancy success depends on cycle quality rather than length (Vitzthum, 2009).

Prenatal maternal investments give a key contribution in maintaining progeny (Vitzhum, 2009), suggesting that the genotype of *LHB*/*CGB* gene cluster is important to optimize the birth rate across human evolution.

Protective effect from recurrent miscarriage was associated with some SNPs located in both the *CGB5* e *CGB8* genes, which encode the major fraction of *CGB*-mRNA transcripts (Rull et al., 2008) reflecting their importance in physiological adaptation to pregnancy. The genomic region embedding the *CGB2*, *CGB5* and *CGB8* promoter genes is featured by high heterozygosity and increased frequencies of the derived alleles in *non*-African populations (figure 1). On the contrary, ancestral alleles of *CGB2*, *CGB5* and *CGB8* promoter genes achieve the highest frequencies among individuals of African ancestry (figure 1). Moreover, high heterozygosity in *non*-Africans suggests that balancing selection accompanied ancient human migrations (Rull et al., 2008). Taken together, this is consistent with the concept that genotypic (thus phenotypic) variability improves the persistence of a population in a given habitat (Forsman, 2014), providing more flexible reproductive features, such as endocrine adaptation to the new environmental conditions (Cornelius et al., 2013) reasonably encountered out from Africa. Interestingly, the analysis of the *LHB*/*CGB* cluster sequences from several human populations revealed selective pressures among Africans compared to humans in other continents (figure 2). Cross Population Extended Haplotype Homozygosity test (XP-EHH) (Sabeti et al., 2007), a measure of natural selection which takes into account the SNPs frequencies within a genomic region, is higher when calculated for the *LHB*/*CGB* gene cluster of individuals from Africa compared to other populations. Since African populations maintained high homozygosity for the *LHB* gene and *CGB2*, *CGB5* and *CGB8* promoter genes (figure 1), this was likely an advantageous condition in (but not out from) Africa. This conflicts with the concept that Africa, where human species arose, holds the highest heterozygosity and genetic variability (Cann et al., 2002; Ramachandran et al., 2005; Li et al., 2008). Also, since chorionic gonadotropin is massively produced exclusively in pregnant females, the *CGB* gene cluster is reasonably the result of selection acting only in women, providing an interesting model to study sex-related aspects of the human evolution. However, the contribution of males in the selection of *LHB*/*CGB* cluster genotypes should not to be excluded, at least in Africans; paternal transmission of methylated SNPs within *CGB5* promoter results in the loss of bi-allelic expression, leading to failure of pregnancy by impairment of placental-maternal interface (Uusküla et al., 2011). In addition, a role of certain CGβ transcripts in the male reproductive system was proposed (Parrott et al., 2011) suggesting that paternal inheritance of *LHB*/*CGB* cluster genotypes was important for pregnancy in daughters.

An evolutionary role of pregnancy may consists in protecting from disease risk due to long-term exposure to physiologic pituitary gonadotropins (Meier-Abt et al., 2015) and a link between fertility and lifespan was indeed observed (Kuningas et al., 2011); it is plausible, even if speculative, that a longer lifespan could provide a wider reproductive window. However the impact of life duration in human evolution remains unclear, since the mean life expectancy was overall less than 40 years worldwide until the beginning of the twentieth century, mainly due to causes unrelated to hormonal features (e.g. infectious diseases, famines, etc) (Christensen et al., 2009), thus suggesting that the reproductive lifespan had mild beneficial effects for human reproduction.

Post-natal parental care is important for progeny growth, improving reproductive success (Vitzhum, 2009). Since sexual behavior and fatherhood are linked to testosterone levels in men (Gettler et al. 2013), the functional significance of hormonal changes in mammalian males was debated (Saltzman and Ziegler, 2014). While high testosterone levels favors the male in acquiring sex partners, increased paternal care was associated with low testosterone levels in humans (Pollet et al., 2013; Perini et al., 2012). Therefore, genotypes linked to low fertility may have provided an evolutionary advantage, especially when the adaptation to new environmental factors favored the need of cooperative behaviors among kin (Apicella et al., 2012), which should be plausibly strengthened during ancient migration of relatively small human groups. This may explain why the relatively recent SNP variants associated with lower fertile phenotypes, such as rs1394205 (-29G>A, *FSHR*) and rs10835638 (-211G>T, *FSHB*) (Grigorova et al., 2008; Tüttelmann et al., 2012), have higher frequencies among Northern European and native American populations than in Africa where humans arose (Simoni and Casarini, 2014). However, the current distribution of genotypes evolutionarily disadvantageous among humans may be due, at least in part, to social issues, e.g. patrilineal populations, which affect the genetic diversity by sex-biased transmission of reproductive success (Heyer et al., 2015).

Reproductive conflicts.

Intralocus sexual conflict occurs when traits encoded by the same genetic locus result in opposite effects in males and females, in terms of reproductive success (Pennell and Morrow, 2013). This was experimentally demonstrated in animal models, revealing that high levels of the sex hormone testosterone result in different, sex-related reproductive success in the bank vole *Myodes glareolus* (Mills et al., 2012). In this model, high testosterone levels were oppositely associated with the reproductive success of sons and daughters; thus, genetic benefits of selecting reproductively successful males with high testosterone levels were lost with daughters. This may explain why genetic variants linked to sub-fertile phenotypes in females did not disappeared during evolution. Since risk alleles may have been maintained in a population due to their beneficial effect in one sex (Gilks et al., 2014), GWAS of sex-specific reproductive disorders could be improved by including both sexes, rather than separate-sex analysis. Unfortunately, sex-related genetic disorders (e.g. PCOS) are usually investigated by excluding male samples. Using human genotypic data from both males and females we recently observed that sexual conflict might explain the geographic distribution of PCOS risk alleles and the overall constant prevalence of the disease (Casarini and Brigante, 2014). In particular, we observed that genotypes linked to hyperandrogenic phenotypes could have been evolutionarily favorable for males in challenging for food resources, although disadvantageous for females in which they are involved in PCOS pathogenesis. PCOS markers are SNPs located within several genomic regions, including *FSHR* and *LHCGR* genes (Chen et al., 2011; Shi et al., 2012); since gonadotropin receptor genes are linked to testosterone levels and testes volume in men (Grigorova et al., 2014), they may be hot spots for intralocus sexual conflicts by oppositely modulating the reproductive parameters in a sex-dependent manner.

Even if speculative, the evolution of the *LHB*/*CGB* gene cluster may be a case of solved intralocus sexual conflict occurred *via* sexual dimorphism by gene duplication (Assis and Bachtrog, 2013), resulting in the independent evolution of novel functions of the derived genes. In this sense, gestation and embryo development in primates are controlled by several copies of the *CGB* gene derived from the original *LHB* gene (Henke and Gromoll, 2008; Nagirnaja et al., 2010), which, in turn, maintains the original physiologic functions exerted in development, folliculogenesis, ovulation and spermatogenesis in all animals but the primates. In primates, the number of *CGB* genes increase together with complexity of hemochorial placentation (Cole et al., 2009), revealing that they have different, widely unknown roles in pregnancy and that evolved separately. The *CGB1* and *CGB2* genes are highly conserved in humans and great apes, and a low number of SNPs maps in the proximity of these genes. Due to the low genetic variation of *CGB1* and *CGB2* genes, it is plausible that they are dedicated to the regulation of delicate stages such as embryo implantation and placental development (Hallast et al., 2007), which are crucial for pregnancy in all primates. Other *CGB* genes are abundantly transcribed in different gestational periods, suggesting that they may serve for further, species-specific adaptations to later stages of pregnancy.

Phylogenesis

Due to the polygenic modulation of the sexual features, it is overall difficult to quantify the real impact of each genotypic variant of the gonadotropins and their receptors' genes in human reproductive success (Casarini et al., 2011). The overall, worldwide distribution of genotypic markers results in a geographical pattern due to human migrations rather than selection (Ramachandran et al., 2005; Li et al., 2008). Human phylogenetic trees produced using SNP frequencies of the whole *FSHR* and *LHCGR* genes from the HapMap database (International HapMap Consortium, 2003) by the POPTREE2 software (Takezaki et al., 2010) (figure 3) revealed indeed that the genotypic variants of both the genes are embedded in continent-specific groups, depending on the genetic ancestry of the populations (Jia et al., 2014). This suggests that human populations may be represented by three main *FSHR* and *LHCGR* genotypes peculiar of Africa, Eurasian and East Asian-American continents, supporting that ancient human migrations gave the main contribution to the current genetic diversity. This analysis did not take into account that few SNPs may have contributed to the selection of peculiar phenotypes (e.g. FSHR p.N680S; rs6166) more than others (e.g. non-synonymous or intronic polymorphic variants). However, the *FSHR* and *LHCGR* genes are characterized by genomic regions in high *linkage disequilibrium* (Simoni and Casarini, 2014), except in Africans, suggesting that they were inherited together. Taken together, gonadotropin receptor gene variants seem to have accompanied humans during ancient migrations only weakly contributing to their reproductive success.

Socio-economic and cultural aspects of human reproduction.

It is unclear how the endocrine genotypes and phenotypes affect human reproductive success in the modern, developed societies, in which family structure, lifestyle and healthcare deeply changed during the last century and appear now profoundly different from those of ancient times. Currently, different world regions differ widely in fertility rate. The number of births *per* woman is inversely related with socio-economical indexes (*per* capita income, health expenditure and life expectancy) (figure 4), so that highest income countries have the lowest fertility rate and this is not depending on ethnicity (data available at the World Bank Group website; http://www.worldbank.org). In low income countries the mean fertility rate achieves 6-8 births *per* woman. This means that reproductive success in current, developed human societies is merely depending on social and cultural aspects reflected by richness, health, trust in the future, etc., while it is poorly affected by the endocrine phenotype of the individuals. Couples of developed countries currently begin to search fertility and parenthood at late reproductive age, e.g. 35-40 years, when the reproductive success and birth rate are naturally low, mainly due to decreased ovarian reserve and/or metabolic disturbances which amplify the effects of sub-fertile phenotypes. This explains why several developed countries are currently characterized by population aging and demographic decline as compared to high fertility rate observed in the poorest countries (Bongaarts, 2015). Therefore, the socio-economic *status* is currently linked to reproductive success. In addition, in ancient human societies sexual activity aiming at conception were concomitant with the beginning of the fertile age and persisted for longer times, plausibly increasing the chance for parenthood as it continues to occur in the poorest countries. Endocrine and metabolic disorders, such as hyperandrogenism or insulin resistance, which result in sub-fertile female phenotypes (Corbett and Morin-Papunen, 2013), might significantly affect fertility in the modern, developed societies where the conception attempts *per* individual are reasonably fewer compared to the ancient times. If so, then the genotypic features, irrelevant in the past, may be relevant to optimize fertility management in the modern societies, when an increasing number of "reproductively aged" couples, characterized by a reduced fertile window, undergo clinical treatments for assisted reproduction.

Conclusions.

An increasing number of studies progressively elucidate how polymorphic variants of gonadotropins and their receptors' genes modulate the human reproductive functions and diseases. Although traces of selective pressure on genes related to endocrine functions were found, the effects of gonadotropins and their receptors' SNPs should normally have relatively weak impact in human reproductive success. Peculiar endocrine genotypes may be linked to phenotypes leading to opposite, sex-related reproductive success, resulting in intralocus sexual conflicts and favoring the inheritance of alleles disadvantageous for one sex through the ancient human history. Thus, individuals from both sexes and proper sample-sizes should be required in GWAS and evolutionary studies in the field of reproduction. The endocrine phenotypes related to sub-fertility may strengthen the decline of fertility in modern societies, in which parenthood attempts are relegated in the last, short period of the fertile age.

Declaration of interest.

The authors have no conflict of interests.

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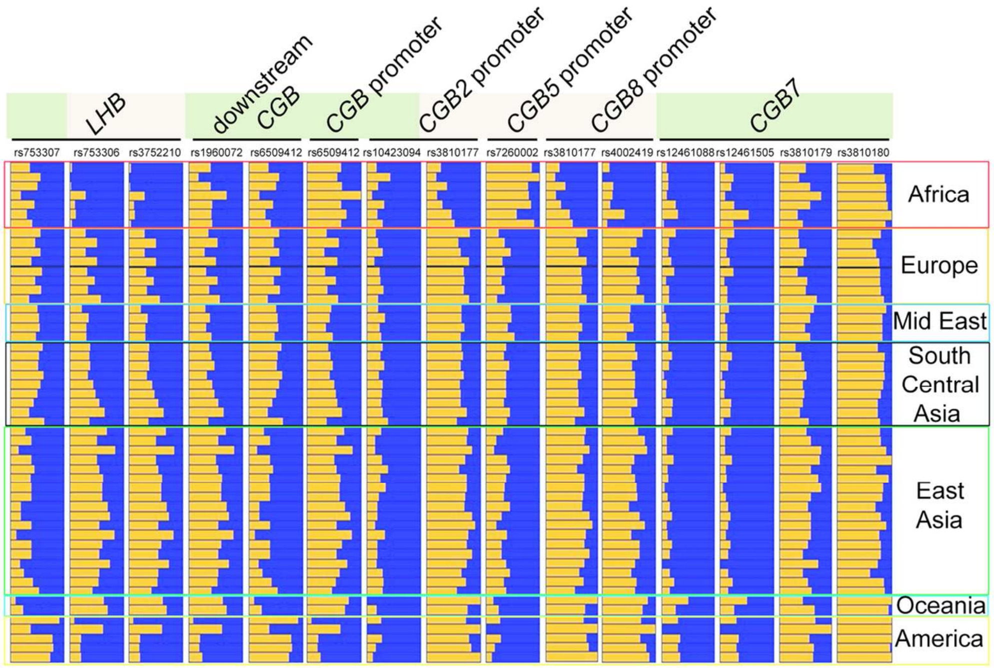

Allele frequencies of SNPs within LHB/CGB gene cluster in human populations. Orange/blue bars indicate the proportion in percentage of the two alleles in the different human groups, which are represented by the colored lines in each column (please refer to the web browser for the populations order and name). The populations belonging the same geographical area were grouped as indicated on the right side of the panel. SNPs ID are shown above each column and grouped by gene. Pink panels above the bars indicate when mean SNP frequencies of African are significantly different versus that of all other continents (Kruskal-Wallis and Dunn's post-test; $p<0.001$); non-significant differences are indicated by green panels (exceptions: Africa versus America for SNPs rs753306 and rs3752210, $p\geq0.001$). Data were obtained using the Human Genome Diversity Project (HGDP) selection browser (http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP).
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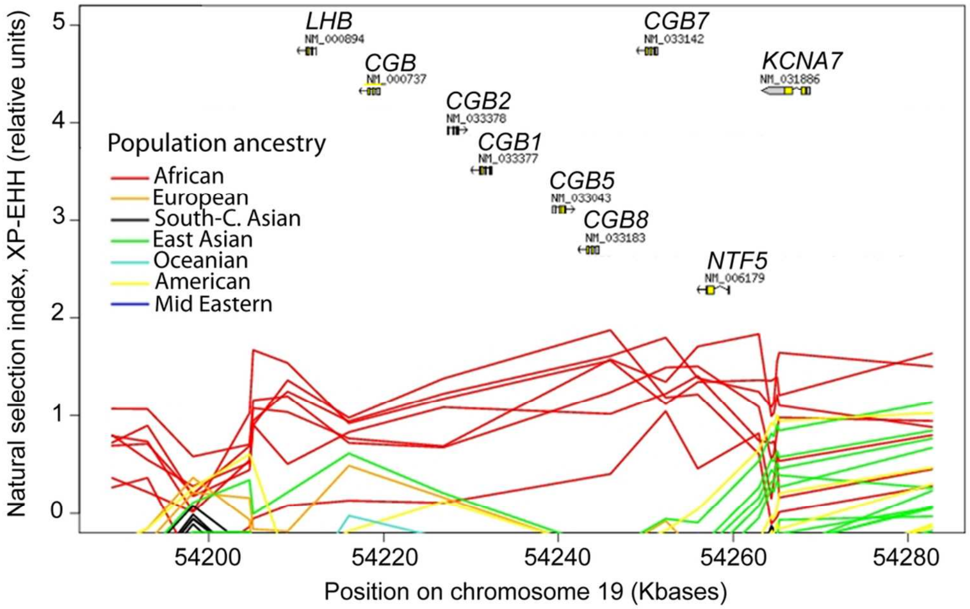

Analysis of the natural selection pressure sustained by the LHB/CGB gene cluster. The measure of natural selection was inferred from the gene cluster sequences of several human populations using the XP-EHH index (Sabeti et al., 2007) and represented on the Y-axis (relative units). The name, ID and exon sequences (boxes and arrows) of each genes are indicated on the panel, in proximity of their genomic position on chromosome 19 (X-axis). Red lines corresponding to measures of natural selection of the LHB/CGB cluster in African achieve higher levels than that of other populations, indicating that stronger natural selection occurs in African compared to other populations. The population belonging the same geographical area were grouped and colored as indicated in the legend (top-left side of the panel); please refer to the web browser for the population name list (http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP). The calculation of the XP-EHH index was performed by the proper online tool available at the HGDP selection browser website.
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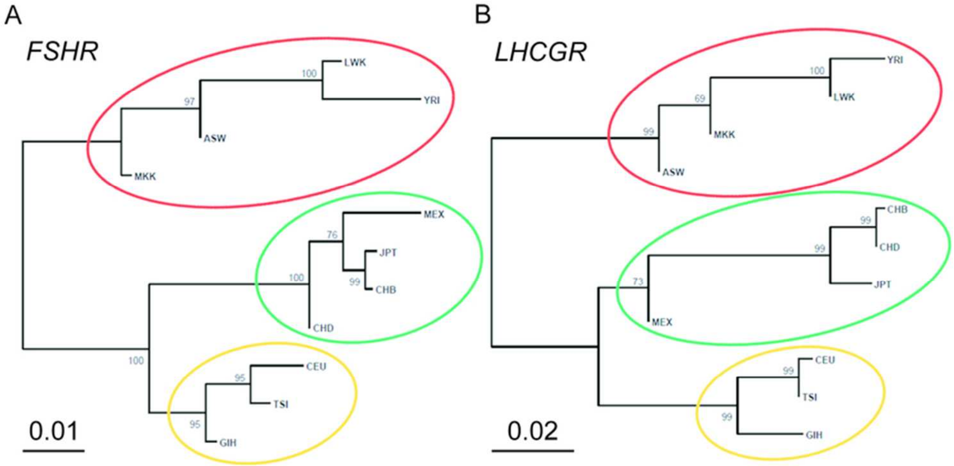

Phylogenic analysis of the FSHR (A) and LHCGR (B) genes. SNPs frequencies were extracted from HapMap populations (http://hapmap.ncbi.nlm.nih.gov) and analyzed by the POPTREE2 software (Takezaki et al., 2010). The population belonging the same geographical area were grouped by colored ovals (Red=populations of African ancestry; Green=East Asian/American; Yellow=European Caucasian/Central Asian), resulting in phylogenetic pattern of both the FSHR and LHCGR genotypes according to the continental distribution of the human groups. The populations were assigned to each continents depending on the major genetic component of their ancestry (Jia et al., 2014); ASW were assumed as African, CHD as East Asian, GIH as Central Asian, CEU as Caucasian from Europe despite they are from USA residents. The measure of genetic distance Fst is indicated by the bars below the trees (relative frequency; please refer to the author’s software and article for references about genetic distance); the numbers throughout the trees are percentage values representing an index of reliability of the analysis, which is assumed significantly reliable when ≥70-75 (relative units) (Takezaki et al., 2010). POPTREE2 software was used with these default settings: Fixation index (Fst) Uncorrected, NJ, Bootstrap 100000.

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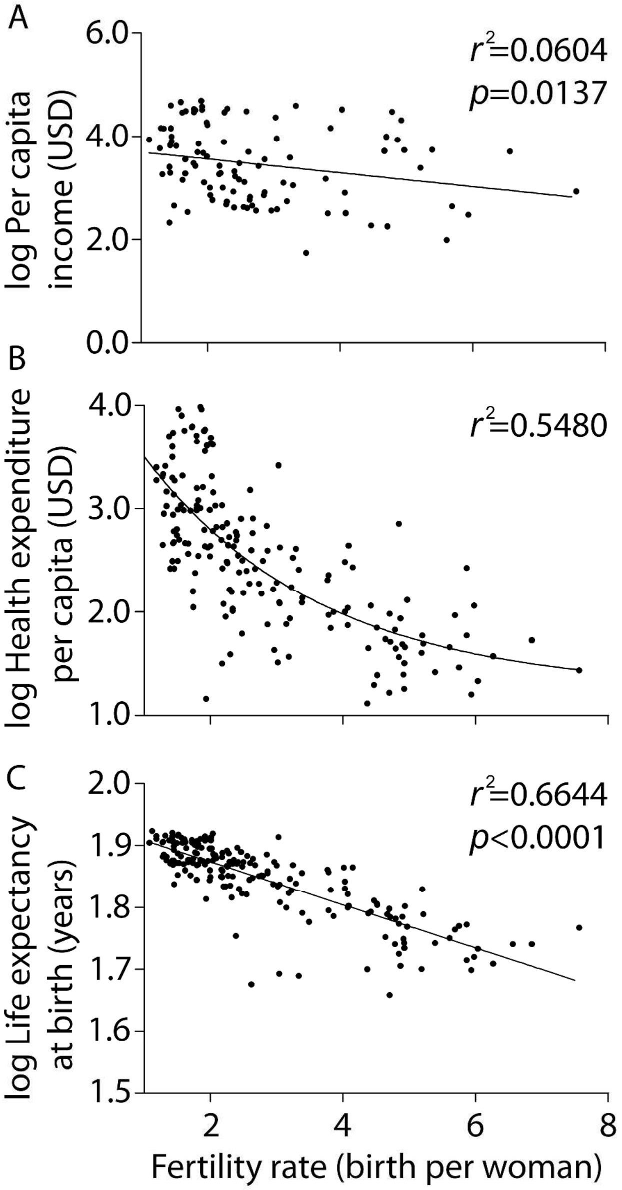

Relationship between fertility rate and socio-economical current indexes in World countries. Fertility rate is represented as "birth per woman" (X-axis) and plotted against measures of socio-economic status, i.e. per capita income (A), health expenditure per capita (B) and life expectancy at birth (C) (logarithmic Y-axis). Fertility rate is inversely related to all these indexes, demonstrating that the countries in which people has high standard of living are featured by low number of births, and vice versa (linear or non-linear regression were used where appropriate as best-fitting model; p<0.005; calculation by GraphPad Prism, GraphPad Software Inc., La Jolla, CA, USA). The graphs were obtained using data available at the World Bank Group website (http://www.worldbank.org), an observer at the United Nations Development Group.
143x273mm (600 x 600 DPI)

Figure Legends

Figure 1: Allele frequencies of SNPs within LHB/CGB gene cluster in human populations. Orange/blue bars indicate the proportion in percentage of the two alleles in the different human groups, which are represented by the colored lines in each column (please refer to the web browser for the populations order and name). The populations belonging the same geographical area were grouped as indicated on the right side of the panel. SNPs ID are shown above each column and grouped by gene. Pink panels above the bars indicate when mean SNP frequencies of African are significantly different versus that of all other continents (Kruskal-Wallis and Dunn's post-test; $p<0.001$); non-significant differences are indicated by green panels (exceptions: Africa versus America for SNPs rs753306 and rs3752210, $p\geq0.001$). Data were obtained using the Human Genome Diversity Project (HGDP) selection browser (http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP).

Figure 2: Analysis of the natural selection pressure sustained by the LHB/CGB gene cluster. The measure of natural selection was inferred from the gene cluster sequences of several human populations using the XP-EHH index (Sabeti et al., 2007) and represented on the Y-axis (relative units). The name, ID and exon sequences (boxes and arrows) of each genes are indicated on the panel, in proximity of their genomic position on chromosome 19 (X-axis). Red lines corresponding to measures of natural selection of the LHB/CGB cluster in African achieve higher levels than that of other populations, indicating that stronger natural selection occurs in African compared to other populations. The population belonging the same geographical area were grouped and colored as indicated in the legend (top-left side of the panel); please refer to the web browser for the population name list (http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP). The calculation of the XP-EHH index was performed by the proper online tool available at the HGDP selection browser website.

Figure 3: Phylogenic analysis of the FSHR (A) and LHCGR (B) genes. SNPs frequencies were extracted from HapMap populations (http://hapmap.ncbi.nlm.nih.gov) and analyzed by the POPTREE2 software (Takezaki et al., 2010). The population belonging the same geographical area were grouped by colored ovals (Red=populations of African ancestry; Green=East Asian/American; Yellow=European Caucasian/Central Asian), resulting in phylogenetic pattern of both the FSHR and LHCGR genotypes according to the continental distribution of the human groups. The populations were assigned to each continents depending on the major genetic component of their ancestry (Jia et al., 2014); ASW were assumed as African, CHD as East Asian, GIH as Central Asian, CEU as Caucasian from Europe despite they are from USA residents. The measure of genetic distance Fst is indicated by the bars below the trees (relative frequency; please refer to the author's software and article for references about genetic distance); the numbers throughout the trees are percentage values representing an index of reliability of the analysis, which is assumed significantly reliable when ≥70-75 (relative units) (Takezaki et al., 2010). POPTREE2 software was used with these default settings: Fixation index (Fst) Uncorrected, NJ, Bootstrap 100000.

Figure 4: Relationship between fertility rate and socio-economical current indexes in World countries. Fertility rate is represented as “birth per woman” (X-axis) and plotted against measures of socio-economic status, i.e. per capita income (A), health expenditure per capita (B) and life expectancy at birth (C) (logarithmic Y-axis). Fertility rate is inversely related to all these indexes, demonstrating that the countries in which people has high standard of living are featured by low number of births, and vice versa (linear or non-linear regression were used where appropriate as best-fitting model; $p<0.005$; calculation by GraphPad Prism, GraphPad Software Inc., La Jolla, CA, USA). The graphs were obtained using data available at the World Bank Group website (http://www.worldbank.org), an observer at the United Nations Development Group.