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

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12 **Short Title**

13 Gonadotropin SNPs and human reproductive success.

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16

17 **Abstract**

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

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43 Introduction.

44 Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are glycoproteins produced by the
45 pituitary regulating development and reproductive functions in both men and women. On the contrary,
46 choriogonadotropin (hCG) is the human placental hormone managing pregnancy. Gonadotropins share a
47 common α subunit together with the thyroid-stimulating hormone (TSH), while having a unique β subunit,
48 specific for the receptor located in the gonads. The FSH receptor (FSHR) and the common LH/hCG receptor
49 (LHCGR) belong to the superfamily of the G protein-coupled receptors (GPCRs). They are characterized by
50 an extracellular domain, 7 trans-membrane domains joined by 3 intra- and extra-cellular loops, and an
51 intracellular, C-terminal domain. Upon hormone binding with the extracellular portion, the intracellular
52 domain triggers the activation of multiple signaling pathways by interacting with specific molecules, such as
53 G proteins or β -arrestins (Simoni et al., 1997; Ascoli et al., 2002; Gloaguen et al., 2011).
54 Gonadotropins and their receptor genes carry several single-nucleotide polymorphisms (SNPs), resulting in
55 several genotypes differently distributed among human populations and affecting sex-related reproductive
56 features and diseases by modulating signal transduction (Casarini et al., 2011). These genotypes are
57 evolutionarily old and have accompanied humans during their ancient migrations throughout the
58 continents. However, the impact of these SNPs on human reproductive success and evolution is unclear
59 and was recently debated (Grigorova et al., 2007; Simoni and Casarini, 2014).

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

61 The *FSHR* carries about two thousands SNPs but only a few of these are known as modulators of gonadal
62 response. One of the most common *FSHR* polymorphisms is rs6166 (NCBI SNPs database ID;
63 <http://www.ncbi.nlm.nih.gov>) consisting in the nucleotide change A to G at position 2039 from the gene
64 transcription start codon (c.2039A>G), and resulting in the amino acid change N to S at position 680 of the
65 protein chain (p.N680S). rs6166 is in strong *linkage disequilibrium* with the SNP rs6165 (c.919A>G,
66 p.T307A), at least in Caucasians and Asians, resulting in two discrete FSHR isoforms. p.N680S is close to the
67 C-terminal intracellular region of the receptor and modulates serum FSH levels and gonadal response in
68 both women and men (Lledo, et al. 2013; Grigorova et al., 2014; Simoni and Casarini, 2014). Women

69 carriers of the p.N680S S homozygous genotype have higher serum FSH levels during the follicular phase
70 and lower progesterone levels in the luteal phase than the carriers of different genotypes, while p.N680S N
71 homozygous males are characterized by higher testes volume than p.N680S S homozygous men. It was
72 suggested that the FSHR p.N680S S variant is functionally “resistant” to FSH stimulation; The p.N680S
73 polymorphism modulates cell signaling resulting in differential gene expression and steroidogenesis in
74 cultured human lutein granulosa cells as recently demonstrated *in vitro* (Casarini et al., 2014).

75

76 Interestingly, the cumulative effect of p.N680S together with other FSHR polymorphisms (e.g. rs1394205;
77 -29G>A) was proposed, leading to genotypes linked with lower fertility (Casarini and Simoni, 2014;
78 Grigorova et al., 2014). The -29G>A SNP falls within the 5'-untranslated region of the *FSHR* gene, 29
79 nucleotide upstream the ATG codon. The *in vitro* transcriptional activity of the -29G>A A variant is lower
80 than that of the -29G>A G genotype in Chinese hamster ovary (CHO) cells transfected with the *FSHR*
81 promoter and was found to be associated with hypertension (Nakayama et al., 2006), lower estradiol levels
82 in women (Achrekar et al., 2009) and higher serum FSH levels (Achrekar et al., 2009; Grigorova et al., 2014).

83

84 The FSH β subunit is encoded by the *FSHB* gene, which carries about twenty-four SNPs, but only the
85 rs10835638 (-211G>T), located in the promoter region of the gene (-211G>T, rs10835638), was extensively
86 studied in association with serum FSH levels and reproductive parameters in males (Grigorova et al. 2008).
87 In particular, -211G>T T homozygous Baltic, Italian and German men have lower FSH levels and testis
88 volume compared to carriers of other genotypes (Grigorova et al., 2008; Tüttelmann et al., 2012; Grigorova
89 et al., 2014). The promoter region of the *FSHB* gene is a putative target of a transcription regulatory
90 element and is highly conserved among placental mammals (Grigorova et al. 2008), suggesting that the T
91 nucleotide at position -211 affects the *FSHB* gene transcription leading to low hormone levels. Interestingly,
92 the studies performed in males and females are contradictory; -211G>T T homozygous women were shown
93 to have elevated FSH, LH, and reduced progesterone levels compared with carriers of other genotypes,
94 suggesting a gender-specific, compensatory regulation of the gonadotropin secretion (Schüring et al.,

95 2013). Further elucidations may be provided by genotype-phenotype association studies focusing on the
96 cumulative effect of *FSHB* together with *FSHR* gene SNPs, revealing how they affect the sex-related
97 modulation of hormone levels and reproductive parameters. Taken together, the combination of SNPs
98 within the *FSHB* and *FSHR* genes account for a substantial proportion of the total normal phenotypic
99 variance in male and female reproductive parameters (Tüttelmann et al., 2012; La Marca et al., 2013;
100 Grigorova et al., 2014; Simoni and Casarini, 2014).

101

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

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

108

109 The *LHCGR* variant 18insLQ, consisting in the insertion of 6 nucleotides in frame in exon 1 and falling near
110 the N-terminus of the mature receptor, was associated with early onset of breast cancer and short disease-
111 free survival. This is consistent with increased *LHCGR* 18insLQ sensitivity and plasma membrane expression
112 (1.9 fold lower hCG half-effective concentration and 1.4 fold higher expression levels than wild-type *LHCGR*,
113 respectively) (Piersma et al., 2006). Interestingly, *LHCGR* 18insLQ has a high frequency among Northern-
114 European Caucasians which are characterized by higher prevalence of breast cancer compared to other
115 ethnic groups, leading to the speculation that the *LHCGR* genotype may be linked to disease risk (Casarini et
116 al., 2011).

117

118 Only few other *LHCGR* SNPs provided significant clinical findings so far. The SNP rs2293275 (c.942G>A,
119 p.S312N), which falls within exon 10 of the *LHCGR* gene, might affect the trafficking and stability of the
120 receptor resulting in impaired spermatogenesis in men (Simoni et al., 2008) and increased risk of

121 developing polycystic ovary syndrome (PCOS) in women (Thathapudi et al., 2015). Lastly, the polymorphic
122 LHCGR variant rs4073366 (c.3442-20797C>G) occurs about 142 base pairs downstream of LHCGR18insLQ.
123 The C allele was associated with an approximately 3-fold increased risk of developing ovarian
124 hyperstimulation syndrome (OHSS) in adult women undergoing procedures for assisted reproduction
125 (O'Brien et al., 2013).

126

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

134

135 While the genes encoding the FSH β and LH β are present in all vertebrates, the CGB β -coding genes exist only
136 in primates and equids, likely as result of repeated duplications of an ancestral *LHB* gene (Henke and
137 Gromoll, 2008). The human genome carries eight *CGB* genes contiguous with the *LHB* gene on chromosome
138 19; subsequently frame-shift mutations and nucleotide insertions resulted in 24 additional codons for *CGB*.
139 The *LHB/CGB* gene cluster spans about 40 Kbase-pairs and carries several SNPs; especially, polymorphic
140 variants of the *CGB5* were associated with recurrent spontaneous abortions in Chinese and Caucasian
141 women (Rull et al., 2008; Sun and Ji, 2014).

142

143 **Gonadotropin variants and implications in disease and menopause**

144 Although further investigations are needed to elucidate the molecular mechanisms underlying the
145 modulatory effects of SNPs within *FSHR* and *FSHB* genes on reproductive parameters and diseases, their
146 pathophysiological relevance and clinical outcomes were widely described in the literature. On the

147 contrary, the pathophysiological implications of SNPs belonging to the *LHCGR* gene and the *LHB/CGB* gene
148 cluster are poorly understood.

149

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

165

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

172

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

177

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

184

185 *Menopausal age*. A link between menopausal age and SNPs in gonadotropins and their receptors' genes
186 was suggested, providing a wide spectrum of candidate markers and conflicting, ethnicity-related results.
187 Several loci associated with age at natural menopause were identified by meta-analyzing 22 GWAS in
188 women of European ancestry (Stolk et al., 2012, Perry et al., 2014). This statistically powerful analysis
189 identified top SNPs located within 3 out of 17 genomic regions in strong *linkage disequilibrium* with *FSHB*,
190 *STARD1* and *BCAR4* genes in Caucasians, suggesting that they are involved in hormonal regulation of follicle
191 recruitment and exhaustion, but further confirmation in other ethnic groups are required. Interestingly,
192 women with PCOS have a later onset of menopause compared to normo-ovulatory women (Tehrani et al.,
193 2010), likely resulting from the protective effect of high anti-Mullerian hormone levels for ovarian reserve,
194 extending the reproductive lifespan in spite of less ovulatory cycles.

195

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

198

199 Limitations

200 Due to the polygenic regulation and the modulatory effects of lifestyle on reproductive traits (Sharma et al.,
201 2013), genotype-phenotype associations need to be well-characterized in different, appropriately sized
202 sample groups and independently confirmed to avoid methodological biases. However, the medical
203 literature often provides conflicting results. Although the link between the FSHR SNP p.N680S and serum
204 FSH levels or ovarian response was repeatedly observed (Simoni and Casarini, 2014), other studies failed to
205 find the same associations (Binder et al., 2012; Mohiyiddeen et al., 2013; Trevisan et al., 2014), suggesting
206 that the endocrine features are modulated by several factors such as age or ethnicity. However, studies
207 using suboptimal sample groups characterized by subfertility or endocrine dysfunction (e.g. pre-
208 menopausal women or poor responders to gonadotropin treatments) should be carefully evaluated. Proper
209 sample sizes and combined genotype analysis are required to detect significant and clinically relevant
210 associations. For example, to unmask the effects of the p.N680S polymorphism on serum FSH levels in men,
211 a combined model taking into account the *FSHB* promoter SNP -211G>T may be necessary (Tüttelmann et
212 al., 2012). Association studies of polygenic traits should be replicated in different sample groups rigorously
213 established, and corroborated by *in vitro* evidences. Finally, mathematical corrections weighting the sample
214 size from different investigations should provide the optimal verification, therefore, meta-analyzes may be
215 a safe and reliable tool to further confirm *in vivo* association studies.

216

217 Population genetics of of gonadotropins and gonadotropin receptors' polymorphisms

218 Previous studies demonstrated that the African continent holds the highest human genetic variability
219 worldwide (Cann et al., 2002; Ramachandran et al., 2005; Li et al., 2008). Consistently with the routes of
220 ancient human migrations, genetic variability decreases together with the distance from Africa, and
221 oppositely to the genetic diversity, determining the current distribution of several sex-related genetic
222 markers (Casarini and Brigante, 2014). Since natural selection contributed poorly to the distribution of
223 human genotypes worldwide (Li et al., 2008), it is reasonable that slightly different hormonal levels and
224 menstrual cycle duration may have only a marginal impact on the selection of sex-related genotypes,

225 compared to other, more determinant phenotypic features, such as skin pigmentation or sickle cell anemia
226 (Liu et al., 2013).

227

228 On the other hand, a full explanation of human reproductive success may not merely rely on human
229 migrations or genetic drift, and the evolutionary role of the SNPs in gonadotropin and their receptors'
230 genes was debated (Grigorova et al., 2007; Simoni and Casarini, 2014). It was estimated that about 20% of
231 Caucasians carry a "less favorable" *FSHB/FSHR* genotype, in terms of serum FSH levels and FSHR expression
232 and activity, which are enriched in sub-fertile subjects previously studied (Simoni and Casarini, 2014).
233 Especially, ovarian cycle length depends, at least in part, on the combination of *FSHB* and *FSHR* genotypes,
234 which affect the sensibility threshold to FSH. This results in heterogeneity in menstrual cycle length and,
235 consequently, a theoretical difference in the total number of cycles which can be calculated in about ± 30 -
236 40 ovarian cycles during the reproductive lifespan depending on the *FSHR* genotype. *FSHR* p.N680S S
237 homozygous women have longer ovarian cycle than p.N680S N homozygous women (Greb et al., 2005). In
238 fact the *FSHR* variant carrying the amino acid serine at position 680 is more abundant in South-Central
239 Asians and Oceanians (Simoni and Casarini, 2014) who are characterized by an overall longer cycle duration
240 than women of East Asian, European or African ancestry (Vitzthum, 2009). This is consistent with the lower
241 steroidogenic potential of the *FSHR* p.N680S homozygous S compared to the homozygous N genotype
242 (Casarini et al., 2014). Most importantly, this suggests that some women have a lower number of
243 ovulations for months of exposure, potentially resulting in slightly lower reproductive potential, but
244 preserving the individual from unnecessary energy expenditure to maintain overall fitness (Simoni and
245 Casarini, 2014). However, since women with low cycle variability have a higher conception rate than those
246 with longer but irregular cycle duration, pregnancy success depends on cycle quality rather than length
247 (Vitzthum, 2009).

248

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

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

276 reproductive system was proposed (Parrott et al., 2011) suggesting that paternal inheritance of *LHB/CGB*
277 cluster genotypes was important for pregnancy in daughters.

278

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

287

288 Post-natal parental care is important for progeny growth, improving reproductive success (Vitzhum, 2009).
289 Since sexual behavior and fatherhood are linked to testosterone levels in men (Gettler et al. 2013), the
290 functional significance of hormonal changes in mammalian males was debated (Saltzman and Ziegler,
291 2014). While high testosterone levels favors the male in acquiring sex partners, increased paternal care was
292 associated with low testosterone levels in humans (Pollet et al., 2013; Perini et al., 2012). Therefore,
293 genotypes linked to low fertility may have provided an evolutionary advantage, especially when the
294 adaptation to new environmental factors favored the need of cooperative behaviors among kin (Apicella et
295 al., 2012), which should be plausibly strengthened during ancient migration of relatively small human
296 groups. This may explain why the relatively recent SNP variants associated with lower fertile phenotypes,
297 such as rs1394205 (-29G>A, *F5HR*) and rs10835638 (-211G>T, *F5HB*) (Grigorova et al., 2008; Tüttelmann et
298 al., 2012), have higher frequencies among Northern European and native American populations than in
299 Africa where humans arose (Simoni and Casarini, 2014). However, the current distribution of genotypes
300 evolutionarily disadvantageous among humans may be due, at least in part, to social issues, e.g. patrilineal

301 populations, which affect the genetic diversity by sex-biased transmission of reproductive success (Heyer et
302 al., 2015).

303

304 **Reproductive conflicts.**

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

325

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

340

341 **Phylogenesis**

342 Due to the polygenic modulation of the sexual features, it is overall difficult to quantify the real impact of
343 each genotypic variant of the gonadotropins and their receptors' genes in human reproductive success
344 (Casarini et al., 2011). The overall, worldwide distribution of genotypic markers results in a geographical
345 pattern due to human migrations rather than selection (Ramachandran et al., 2005; Li et al., 2008). Human
346 phylogenetic trees produced using SNP frequencies of the whole *FSHR* and *LHCGR* genes from the HapMap
347 database (International HapMap Consortium, 2003) by the POPTREE2 software (Takezaki et al., 2010)
348 (figure 3) revealed indeed that the genotypic variants of both the genes are embedded in continent-specific
349 groups, depending on the genetic ancestry of the populations (Jia et al., 2014). This suggests that human
350 populations may be represented by three main *FSHR* and *LHCGR* genotypes peculiar of Africa, Eurasian and
351 East Asian-American continents, supporting that ancient human migrations gave the main contribution to

352 the current genetic diversity. This analysis did not take into account that few SNPs may have contributed to
353 the selection of peculiar phenotypes (e.g. *FSHR* p.N680S; rs6166) more than others (e.g. non-synonymous
354 or intronic polymorphic variants). However, the *FSHR* and *LHCGR* genes are characterized by genomic
355 regions in high *linkage disequilibrium* (Simoni and Casarini, 2014), except in Africans, suggesting that they
356 were inherited together. Taken together, gonadotropin receptor gene variants seem to have accompanied
357 humans during ancient migrations only weakly contributing to their reproductive success.

358

359 **Socio-economic and cultural aspects of human reproduction.**

360 It is unclear how the endocrine genotypes and phenotypes affect human reproductive success in the
361 modern, developed societies, in which family structure, lifestyle and healthcare deeply changed during the
362 last century and appear now profoundly different from those of ancient times. Currently, different world
363 regions differ widely in fertility rate. The number of births *per* woman is inversely related with socio-
364 economical indexes (*per* capita income, health expenditure and life expectancy) (figure 4), so that highest
365 income countries have the lowest fertility rate and this is not depending on ethnicity (data available at the
366 World Bank Group website; <http://www.worldbank.org>). In low income countries the mean fertility rate
367 achieves 6-8 births *per* woman. This means that reproductive success in current, developed human
368 societies is merely depending on social and cultural aspects reflected by richness, health, trust in the future,
369 etc., while it is poorly affected by the endocrine phenotype of the individuals. Couples of developed
370 countries currently begin to search fertility and parenthood at late reproductive age, e.g. 35-40 years, when
371 the reproductive success and birth rate are naturally low, mainly due to decreased ovarian reserve and/or
372 metabolic disturbances which amplify the effects of sub-fertile phenotypes. This explains why several
373 developed countries are currently characterized by population aging and demographic decline as compared
374 to high fertility rate observed in the poorest countries (Bongaarts, 2015). Therefore, the socio-economic
375 *status* is currently linked to reproductive success. In addition, in ancient human societies sexual activity
376 aiming at conception were concomitant with the beginning of the fertile age and persisted for longer times,
377 plausibly increasing the chance for parenthood as it continues to occur in the poorest countries. Endocrine

378 and metabolic disorders, such as hyperandrogenism or insulin resistance, which result in sub-fertile female
379 phenotypes (Corbett and Morin-Papunen, 2013), might significantly affect fertility in the modern,
380 developed societies where the conception attempts *per* individual are reasonably fewer compared to the
381 ancient times. If so, then the genotypic features, irrelevant in the past, may be relevant to optimize fertility
382 management in the modern societies, when an increasing number of “reproductively aged” couples,
383 characterized by a reduced fertile window, undergo clinical treatments for assisted reproduction.

384

385 **Conclusions.**

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

396

397 **Declaration of interest.**

398 The authors have no conflict of interests.

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406

407 **References.**

408 **Achrekar SK, Modi DN, Desai SK, Mangoli VS, Mangoli RV & Mahale SD** (2009) Poor ovarian response to
409 gonadotrophin stimulation is associated with FSH receptor polymorphism. *Reprod Biomed Online*. **18** 509-
410 515. (doi: [http://dx.doi.org/10.1016/S1472-6483\(10\)60127-7](http://dx.doi.org/10.1016/S1472-6483(10)60127-7))

411 **Apicella CL, Marlowe FW, Fowler JH & Christakis NA** (2012) Social networks and cooperation in hunter-
412 gatherers. *Nature*. **481** 497-501. (doi: [10.1038/nature10736](https://doi.org/10.1038/nature10736))

413 **Ascoli M, Fanelli F, Segaloff DL** (2002) The lutropin/choriogonadotropin receptor, a 2002 perspective.
414 *Endocr Rev*. **23** 141-74. (doi: [10.1210/er.23.2.141](https://doi.org/10.1210/er.23.2.141))

415 **Assis R & Bachtrog D** (2013) Neofunctionalization of young duplicate genes in *Drosophila*. *Proc Natl Acad*
416 *Sci U S A*. **110** 17409-14. (doi: [10.1073/pnas.1313759110](https://doi.org/10.1073/pnas.1313759110))

417 **Binder H, Strick R, Zaherdoust O, Dittrich R, Hamori M, Beckmann MW & Oppelt PG** (2012) Assessment of
418 FSHR variants and antimüllerian hormone in infertility patients with a reduced ovarian response to
419 gonadotropin stimulation. *Fertil Steril*. **97** 1169-75.e1. (doi: [10.1016/j.fertnstert.2012.02.012](https://doi.org/10.1016/j.fertnstert.2012.02.012))

420 **Bongaarts J** (2015) Global fertility and population trends. *Semin Reprod Med*. **33** 5-10. (doi: [10.1055/s-](https://doi.org/10.1055/s-0034-1395272)
421 [0034-1395272](https://doi.org/10.1055/s-0034-1395272))

422 **Cann HM, de Toma C, Cazes L, Legrand MF, Morel V, Piouffre L, Bodmer J, Bodmer WF, Bonne-Tamir B,**
423 **Cambon-Thomsen A, et al.** (2002) A human genome diversity cell line panel. *Science*. **296** 261-2. (doi:
424 [10.1126/science.296.5566.261b](https://doi.org/10.1126/science.296.5566.261b))

425 **Casarini L, Pignatti E, Simoni M.** (2011) Effects of polymorphisms in gonadotropin and gonadotropin
426 receptor genes on reproductive function. *Rev Endocr Metab Disord*. **12** 303-21. (doi: [10.1007/s11154-011-](https://doi.org/10.1007/s11154-011-9192-2)
427 [9192-2](https://doi.org/10.1007/s11154-011-9192-2))

- 428 **Casarini L & Brigante G** (2014) The polycystic ovary syndrome evolutionary paradox: a genome-wide
429 association studies-based, in silico, evolutionary explanation. *J Clin Endocrinol Metab.* **99** E2412-20. (doi:
430 10.1210/jc.2014-2703)
- 431 **Casarini L, Moriondo V, Marino M, Adversi F, Capodanno F, Grisolia C, La Marca A, La Sala GB & Simoni M**
432 (2014) FSHR polymorphism p.N680S mediates different responses to FSH in vitro. *Mol Cell Endocrinol.* **393**
433 83-91. (doi: 10.1016/j.mce.2014.06.013)
- 434 **Chen ZJ, Zhao H, He L, Shi Y, Qin Y, Shi Y, Li Z, You L, Zhao J, Liu J, et al.** (2011) Genome-wide association
435 study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3.
436 *Nat Genet.* 2011 **43** 55-9. (doi: 10.1038/ng.732)
- 437 **Christensen K, Doblhammer G, Rau R & Vaupel JW** (2009) Ageing populations: the challenges ahead.
438 *Lancet.* **374** 1196-208. (doi: 10.1016/S0140-6736(09)61460-4)
- 439 **Cole LA** (2009) hCG and hyperglycosylated hCG in the establishment and evolution of hemochorial
440 placentation. *J Reprod Immunol.* **82** 112-8. (doi: 10.1016/j.jri.2009.04.007)
- 441 **Corbett S & Morin-Papunen L** (2013) The Polycystic Ovary Syndrome and recent human evolution. *Mol Cell*
442 *Endocrinol.* **373** 39-50. (doi: 10.1016/j.mce.2013.01.001)
- 443 **Elkins DA, Yokomizo A, Thibodeau SN, J Schaid D, Cunningham JM, Marks A, Christensen E, McDonnell SK,**
444 **Slager S, J Peterson B, et al.** (2003) Luteinizing hormone beta polymorphism and risk of familial and
445 sporadic prostate cancer. *Prostate.* **56** 30-6. (doi: 10.1002/pros.10220)
- 446 **Gilks WP, Abbott JK & Morrow EH** (2014) Sex differences in disease genetics: evidence, evolution, and
447 detection. *Trends Genet.* **30** 453-63. (doi: 10.1016/j.tig.2014.08.006)
- 448 **Gloaguen P, Crépieux P, Heitzler D, Poupon A, Reiter E** (2011) Mapping the follicle-stimulating hormone-
449 induced signaling networks. *Front Endocrinol (Lausanne).* **2** 45. (doi: 10.3389/fendo.2011.00045)
- 450 **Grigorova M, Punab M, Ausmees K & Laan M** (2008) FSHB promoter polymorphism within evolutionary
451 conserved element is associated with serum FSH level in men. *Hum Reprod.* **23** 2160-2166. (doi:
452 10.1093/humrep/den216)

- 453 **Grigorova M, Punab M, Punab AM, Poolamets O, Vihljajev V, Zilaitienė B, Erenpreiss J, Matulevičius V &**
454 **Laan M** (2014) Reproductive physiology in young men is cumulatively affected by FSH-action modulating
455 genetic variants: FSHR -29G/A and c.2039 A/G, FSHB -211G/T. *PLoS One*. **9** e94244. (doi:
456 10.1371/journal.pone.0094244)
- 457 **Grigorova M, Rull K & Laan M** (2007) Haplotype structure of FSHB, the beta-subunit gene for fertility-
458 associated follicle-stimulating hormone: possible influence of balancing selection. *Ann Hum Genet*. **71** 18-
459 28. (doi: 10.1111/j.1469-1809.2006.00299.x)
- 460 **Forsman A** (2014) Effects of genotypic and phenotypic variation on establishment are important for
461 conservation, invasion, and infection biology. *Proc Natl Acad Sci U S A*. **111** 302-7. (doi:
462 10.1073/pnas.1317745111)
- 463 **Gettler LT, McDade TW, Agustin SS, Feranil AB & Kuzawa CW** (2013) Do testosterone declines during the
464 transition to marriage and fatherhood relate to men's sexual behavior? Evidence from the Philippines.
465 *Horm Behav*. 2013 **64** 755-63. (doi: 10.1016/j.yhbeh.2013.08.019)
- 466 **Greb RR, Grieshaber K, Gromoll J, Sonntag B, Nieschlag E, Kiesel L & Simoni M** (2005) A common single
467 nucleotide polymorphism in exon 10 of the human follicle stimulating hormone receptor is a major
468 determinant of length and hormonal dynamics of the menstrual cycle. *J Clin Endocrinol Metab*. **90** 4866-72.
469 (doi:10.1210/jc.2004-2268)
- 470 **Haasl RJ, Ahmadi MR, Meethal SV, Gleason CE, Johnson SC, Asthana S, Bowen RL & Atwood CS** (2008) A
471 luteinizing hormone receptor intronic variant is significantly associated with decreased risk of Alzheimer's
472 disease in males carrying an apolipoprotein E epsilon4 allele. *BMC Med Genet*. **9** 37. (doi: 10.1186/1471-
473 2350-9-37)
- 474 **Henke A & Gromoll J** (2008) New insights into the evolution of chorionic gonadotrophin. *Mol Cell*
475 *Endocrinol*. **291** 11-9. (doi: 10.1016/j.mce.2008.05.009)
- 476 **Heyer E, Brandenburg JT, Leonardi M, Toupance B, Balaesque P, Hegay T, Aldashev A & Austerlitz F**
477 (2015) Patrilineal populations show more male transmission of reproductive success than cognatic

478 populations in Central Asia, which reduces their genetic diversity. *Am J Phys Anthropol.* **157** 537-43. (doi:
479 10.1002/ajpa.22739)

480 **Jia J, Wei YL, Qin CJ, Hu L, Wan LH & Li CX** (2014) Developing a novel panel of genome-wide ancestry
481 informative markers for bio-geographical ancestry estimates. *Forensic Sci Int Genet.* **8** 187-94. (doi:
482 10.1016/j.fsigen.2013.09.004)

483 **Jiang M, Pakarinen P, Zhang FP, El-Hefnawy T, Koskimies P, Pettersson K & Huhtaniemi I** (1999) A
484 common polymorphic allele of the human luteinizing hormone beta-subunit gene: additional mutations and
485 differential function of the promoter sequence. *Hum Mol Genet.* **8** 2037-46. (doi: 10.1093/hmg/8.11.2037)

486 **Kaleva M, Virtanen H, Haavisto AM, Main K, Skakkebaek NE, Huhtaniemi I, Irjala K & Toppari J** (2005)
487 Does variant luteinizing hormone (V-LH) predispose to improper testicular position in late pregnancy?
488 *Pediatr Res.* **58** 447-50. (doi:10.1203/01.pdr.0000176918.68539.b4)

489 **Kuningas M, Altmäe S, Uitterlinden AG, Hofman A, van Duijn CM & Tiemeier H** (2011) The relationship
490 between fertility and lifespan in humans. *Age (Dordr)* **33** 615–622. (doi: 10.1007/s11357-010-9202-4)

491 **La Marca A, Papaleo E, Alviggi C, Ruvolo G, De Placido G, Candiani M, Cittadini E, De Michele F, Moriondo**
492 **V, Catellani V, et al.** (2013) The combination of genetic variants of the FSHB and FSHR genes affects serum
493 FSH in women of reproductive age. *Hum Reprod.* **28** 1369-74. (doi: 10.1093/humrep/det061)

494 **Li JZ, Absher DM, Tang H, Southwick AM, Casto AM, Ramachandran S, Cann HM, Barsh GS, Feldman M,**
495 **Cavalli-Sforza LL, et al.** (2008) Worldwide human relationships inferred from genome-wide patterns of
496 variation. *Science.* **319** 1100-4. (doi: 10.1126/science.1153717)

497 **Liu X, Ong RT, Pillai EN, Elzein AM, Small KS, Clark TG, Kwiatkowski DP & Teo YY** (2013) Detecting and
498 characterizing genomic signatures of positive selection in global populations. *Am J Hum Genet.* **92** 866-81.
499 (doi: 10.1016/j.ajhg.2013.04.021)

500 **Lledo B, Guerrero J, Turienzo A, Ortiz JA, Morales R, Ten J, Llacer J & Bernabeu R** (2013) Effect of follicle-
501 stimulating hormone receptor N680S polymorphism on the efficacy of follicle-stimulating hormone
502 stimulation on donor ovarian response. *Pharmacogenet Genomics* **23** 262-268. (doi:
503 10.1097/FPC.0b013e32835fe813.)

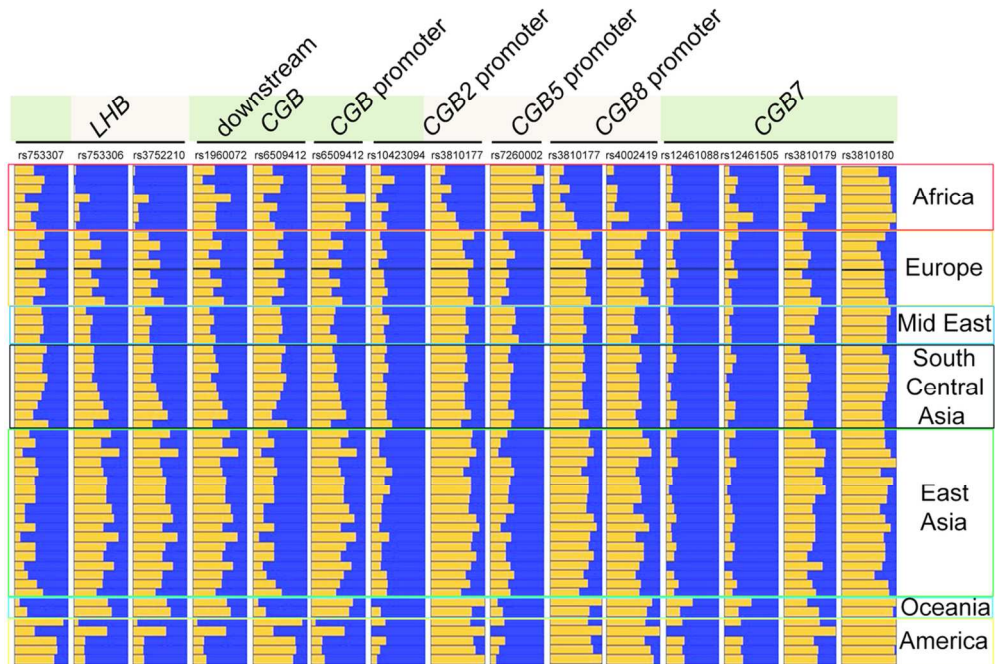
- 504 **Ludwig AH, Murawska M, Panek G, Timorek A & Kupryjanczyk J** (2009) Androgen, progesterone, and FSH
505 receptor polymorphisms in ovarian cancer risk and outcome. *Endocr Relat Cancer*. **16** 1005-16. (doi:
506 10.1677/ERC-08-0135)
- 507 **Meier-Abt F, Bentires-Alj M & Rochlitz C** (2015) Breast cancer prevention: lessons to be learned from
508 mechanisms of early pregnancy-mediated breast cancer protection. *Cancer Res*. **75** 803-7. (doi:
509 10.1158/0008-5472.CAN-14-2717)
- 510 **Mertens-Walker I, Baxter RC & Marsh DJ**. (2012) Gonadotropin signalling in epithelial ovarian cancer.
511 *Cancer Lett*. **324** 152-9. (doi: 10.1016/j.canlet.2012.05.017)
- 512 **Mills SC, Koskela E & Mappes T** (2012) Intralocus sexual conflict for fitness: sexually antagonistic alleles for
513 testosterone. *Proc Biol Sci*. **279** 1889-95. (doi: 10.1098/rspb.2011.2340)
- 514
- 515 **Mohiyiddeen L, Newman WG, Cerra C, McBurney H, Mulugeta B, Roberts SA & Nardo LG** (2013) A
516 common Asn680Ser polymorphism in the follicle-stimulating hormone receptor gene is not associated with
517 ovarian response to gonadotropin stimulation in patients undergoing in vitro fertilization. *Fertil Steril*. **99**
518 149-55. (doi: 10.1016/j.fertnstert.2012.08.037)
- 519 **Nagirnaja L, Rull K, Uusküla L, Hallast P, Grigorova M & Laan M** (2010) Genomics and genetics of
520 gonadotropin beta-subunit genes: Unique *FSHB* and duplicated *LHB/CGB* loci. *Mol Cell Endocrinol*. **329** 4-
521 16. (doi: 10.1016/j.mce.2010.04.024)
- 522 **Nakayama T, Kuroi N, Sano M, Tabara Y, Katsuya T, Ogihara T, Makita Y, Hata A, Yamada M, Takahashi N,**
523 **et al.** (2006) Mutation of the follicle-stimulating hormone receptor gene 5'-untranslated region associated
524 with female hypertension. *Hypertension*. **48** 512-8. (doi: 10.1161/01.HYP.0000233877.84343.d7)
- 525 **O'Brien TJ, Kalmin MM, Harralson AF, Clark AM, Gindoff I, Simmens SJ, Frankfurter D & Gindoff P** (2013)
526 Association between the luteinizing hormone/chorionic gonadotropin receptor (LHCGR) rs4073366
527 polymorphism and ovarian hyperstimulation syndrome during controlled ovarian hyperstimulation. *Reprod*
528 *Biol Endocrinol*. **11** 71. (doi: 10.1186/1477-7827-11-71)

- 529 **Parrott AM, Sriram G, Liu Y & Mathews MB** (2011) Expression of type II chorionic gonadotropin genes
530 supports a role in the male reproductive system. *Mol Cell Biol.* **31** 287-99. (doi: 10.1128/MCB.00603-10)
- 531 **Pennell TM & Morrow EH** (2013) Two sexes, one genome: the evolutionary dynamics of intralocus sexual
532 conflict. *Ecol Evol.* **3** 1819-34. (doi: 10.1002/ece3.540)
- 533 **Perini T, Ditzen B, Hengartner M & Ehlert U** (2012) Sensation seeking in fathers: the impact on
534 testosterone and paternal investment. *Horm Behav.* **61** 191-5. (doi: 10.1016/j.yhbeh.2011.12.004)
- 535 **Perry JR, Day F, Elks CE, Sulem P, Thompson DJ, Ferreira T, He C, Chasman DI, Esko T, Thorleifsson G, et al.**
536 (2014) Parent-of-origin-specific allelic associations among 106 genomic loci for age at menarche. *Nature.*
537 **514** 92-7. (doi: 10.1038/nature13545)
- 538 **Pettersson K, Ding YQ & Huhtaniemi I** (1992) An immunologically anomalous luteinizing hormone variant
539 in a healthy woman. *J Clin Endocrinol Metab.* **74** 164-71. (doi:
540 <http://dx.doi.org/10.1210/jcem.74.1.1727817>)
- 541 **Piersma D, Berns EM, Verhoef-Post M, Uitterlinden AG, Braakman I, Pols HA & Themmen AP** (2006) A
542 common polymorphism renders the luteinizing hormone receptor protein more active by improving signal
543 peptide function and predicts adverse outcome in breast cancer patients. *J Clin Endocrinol Metab.* **91** 1470-
544 6. (doi: <http://dx.doi.org/10.1210/jc.2005-2156>)
- 545 **Pollet TV, Cobey KD & van der Meij L** (2013) Testosterone levels are negatively associated with fatherhood
546 (corrected) in males, but positively related to offspring count in fathers. *PLoS One.* **8** e60018. (doi:
547 [10.1371/journal.pone.0060018](http://dx.doi.org/10.1371/journal.pone.0060018))
- 548 **Powell BL, Piersma D, Kevenaar ME, van Staveren IL, Themmen AP, Iacopetta BJ & Berns EM** (2003)
549 Luteinizing hormone signaling and breast cancer: polymorphisms and age of onset. *J Clin Endocrinol Metab.*
550 **88** 1653-1657. (doi: 10.1210/jc.2002-021585)
- 551 **Ramachandran S, Deshpande O, Roseman CC, Rosenberg NA, Feldman MW & Cavalli-Sforza LL** (2005)
552 Support from the relationship of genetic and geographic distance in human populations for a serial founder
553 effect originating in Africa. *Proc Natl Acad Sci U S A.* **102** 15942-7. (doi: 10.1073/pnas.0507611102)

- 554 **Rull K, Nagirnaja L, Ulander VM, Kelgo P, Margus T, Kaare M, Aittomäki K & Laan M** (2008) Chorionic
555 gonadotropin beta-gene variants are associated with recurrent miscarriage in two European populations. *J*
556 *Clin Endocrinol Metab.* 2008 **93** 4697-706. (doi: 10.1210/jc.2008-1101)
- 557 **Sabeti PC, Varilly P, Fry B, Lohmueller J, Hostetter E, Cotsapas C, Xie X, Byrne EH, McCarroll SA, Gaudet R,**
558 **et al.** (2007) Genome-wide detection and characterization of positive selection in human populations.
559 *Nature.* **449** 913-8. (doi: 10.1038/nature06250)
- 560 **Saltzman W & Ziegler TE** (2014) Functional significance of hormonal changes in mammalian fathers. *J*
561 *Neuroendocrinol.* **26** 685-96. (doi: 10.1111/jne.12176)
- 562 **Schonfeld SJ, Neta G, Sturgis EM, Pfeiffer RM, Hutchinson AA, Xu L, Wheeler W, Guénel P, Rajaraman P,**
563 **de Vathaire F, et al.** (2012) Common genetic variants in sex hormone pathway genes and papillary thyroid
564 cancer risk. *Thyroid.* **22** 151-6. (doi: 10.1089/thy.2011.0309)
- 565 **Schüring AN, Busch AS, Bogdanova N, Gromoll J & Tüttelmann F** (2013) Effects of the FSH- β -subunit
566 promoter polymorphism -211G->T on the hypothalamic-pituitary-ovarian axis in normally cycling women
567 indicate a gender-specific regulation of gonadotropin secretion. *J Clin Endocrinol Metab.* **98** E82-86. (doi:
568 10.1210/jc.2012-2780)
- 569 **Sharma R, Biedenharn KR, Fedor JM & Agarwal A** (2013) Lifestyle factors and reproductive health: taking
570 control of your fertility. *Reprod Biol Endocrinol.* **11** 66. (doi: 10.1186/1477-7827-11-66)
- 571 **Shi Y, Zhao H, Shi Y, Cao Y, Yang D, Li Z, Zhang B, Liang X, Li T, Chen J, et al.** (2012) Genome-wide
572 association study identifies eight new risk loci for polycystic ovary syndrome. *Nat Genet.* **44** 1020-5. (doi:
573 10.1038/ng.2384)
- 574 **Simoni M, Gromoll J, Nieschlag E** (1997) The follicle-stimulating hormone receptor: biochemistry,
575 molecular biology, physiology, and pathophysiology. *Endocr Rev.* **18** 739-73. (doi:
576 <http://dx.doi.org/10.1210/edrv.18.6.0320>)
- 577 **Simoni M, Tüttelmann F, Michel C, Böckenfeld Y, Nieschlag E & Gromoll J.** (2008) Polymorphisms of the
578 luteinizing hormone/chorionic gonadotropin receptor gene: association with maldescended testes and
579 male infertility. *Pharmacogenet Genomics.* **18** 193-200. (doi: 10.1097/FPC.0b013e3282f4e98c)

- 580 **Simoni M & Casarini L** (2014) Mechanisms in endocrinology: Genetics of FSH action: a 2014-and-beyond
581 view. *Eur J Endocrinol.* **170** R91-107. (doi: 10.1530/EJE-13-0624)
- 582 **Stolk L, Perry JR, Chasman DI, He C, Mangino M, Sulem P, Barbalic M, Broer L, Byrne EM, Ernst F, et al.**
583 (2012) Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and
584 immune pathways. *Nat Genet.* **44** 260-8. (doi: 10.1038/ng.1051)
- 585 **Sun Y & Ji X** (2014) Association of rs7260002 of chorionic gonadotrophin β 5 with idiopathic recurrent
586 spontaneous abortion in Chinese population. *J Assist Reprod Genet.* **31** 1497-1500. (doi: 10.1007/s10815-
587 014-0321-1)
- 588 **Sun J, Song F, Wang J, Han G, Bai Z, Xie B, Feng X, Jia J, Duan Y & Lei H** (2014) Hidden risk genes with high-
589 order intragenic epistasis in Alzheimer's disease. *J Alzheimers Dis.* **41** 1039-56. (doi: 10.3233/JAD-140054)
- 590 **Takezaki N, Nei M & Tamura K.** (2010) POPTREE2: Software for constructing population trees from allele
591 frequency data and computing other population statistics with Windows interface. *Mol Biol Evol.* **27** 747-
592 52. (doi: 10.1093/molbev/msp312)
- 593 **Tapanainen JS, Koivunen R, Fauser BC, Taylor AE, Clayton RN, Rajkova M, White D, Franks S, Anttila L,**
594 **Pettersson KS, et al.** (1999) A new contributing factor to polycystic ovary syndrome: the genetic variant of
595 luteinizing hormone. *J Clin Endocrinol Metab.* **84** 1711-5. (doi: <http://dx.doi.org/10.1210/jcem.84.5.5702>)
- 596 **Thathapudi S, Kodati V, Erukkambattu J, Addepally U & Qurratulain H** (2015) Association of luteinizing
597 hormone chorionic gonadotropin receptor gene polymorphism (rs2293275) with polycystic ovarian
598 syndrome. *Genet Test Mol Biomarkers.* **19** 128-132. (doi: 10.1089/gtmb.2014.0249)
- 599 **Tehrani FR, Solaymani-Dodaran M, Hedayati M & Azizi F** (2010) Is polycystic ovary syndrome an exception
600 for reproductive aging? *Hum Reprod.* **25** 1775-81. (doi: 10.1093/humrep/deq088)
- 601 **International HapMap Consortium** (2003) The International HapMap Project. *Nature.* 426 789-96. (doi not
602 available)
- 603 **Trevisan CM, Peluso C, Cordts EB, de Oliveira R, Christofolini DM, Barbosa CP & Bianco B** (2014) Ala307Thr
604 and Asn680Ser polymorphisms of FSHR gene in human reproduction outcomes. *Cell Physiol Biochem.* **34**
605 1527-35. (doi: 10.1159/000366356)

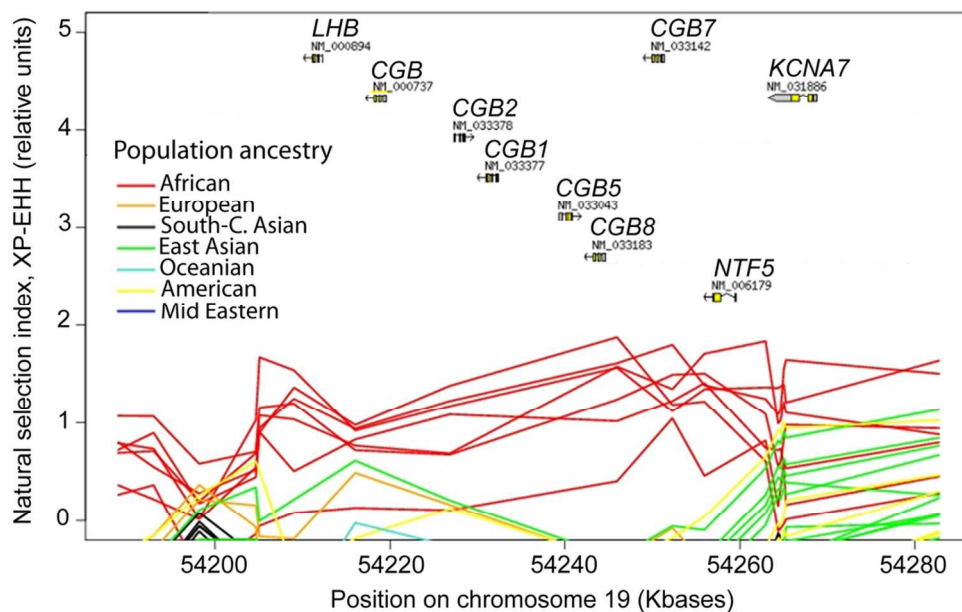
- 606 **Tüttelmann F, Laan M, Grigorova M, Punab M, Söber S & Gromoll J** (2012) Combined effects of the
607 variants FSHB -211G>T and FSHR 2039A>G on male reproductive parameters. *J Clin Endocrinol Metab.* **97**
608 3639-47. (doi: 10.1210/jc.2012-1761)
- 609 **Uusküla L, Rull K, Nagiraja L & Laan M** (2011) Methylation allelic polymorphism (MAP) in chorionic
610 gonadotropin beta5 (*CGB5*) and its association with pregnancy success. *J Clin Endocrinol Metab.* **96** E199-
611 207. (doi: 10.1210/jc.2010-1647)
- 612 **Vitzthum VJ, Spielvogel H & Thornburg J** (2004) Interpopulational differences in progesterone levels during
613 conception and implantation in humans. *Proc Natl Acad Sci U S A.* **101** 1443-8. (doi:
614 10.1073/pnas.0302640101)
- 615 **Vitzthum VJ** (2009) The ecology and evolutionary endocrinology of reproduction in the human female.
616 *Am J Phys Anthropol.* **140** 95-136. (doi: 10.1002/ajpa.21195)
- 617 **Yang CQ, Chan KY, Ngan HY, Khoo US, Chiu PM, Chan QK, Xue WC & Cheung AN** (2006) Single nucleotide
618 polymorphisms of follicle-stimulating hormone receptor are associated with ovarian cancer susceptibility.
619 *Carcinogenesis.* **27** 1502-6. (doi: 10.1093/carcin/bgl014)



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.

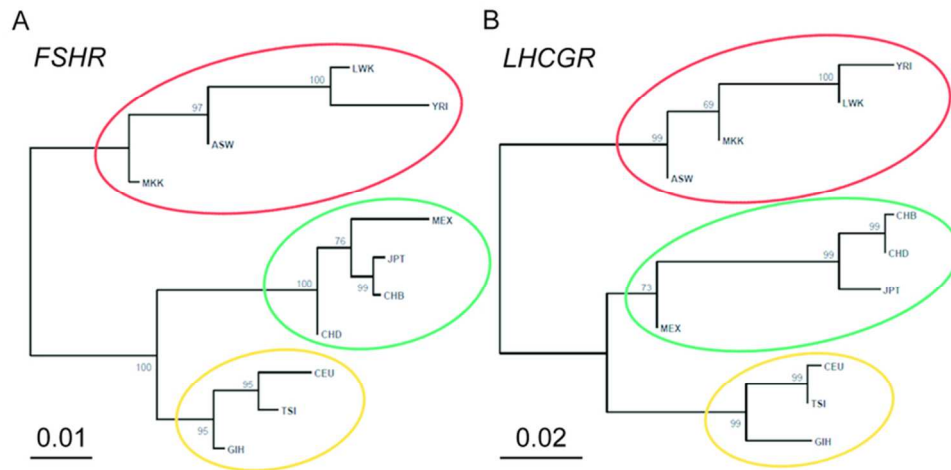
SNPs ID are shown above each column and grouped by gene. Pink panels above the bars indicate when mean SNP frequencies of African are significantly different versus that of all other continents (Kruskal-Wallis and Dunn's post-test; $p < 0.001$); non-significant differences are indicated by green panels (exceptions: Africa versus America for SNPs rs753306 and rs3752210, $p \geq 0.001$). Data were obtained using the Human Genome Diversity Project (HGDP) selection browser (<http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP>).

107x72mm (300 x 300 DPI)



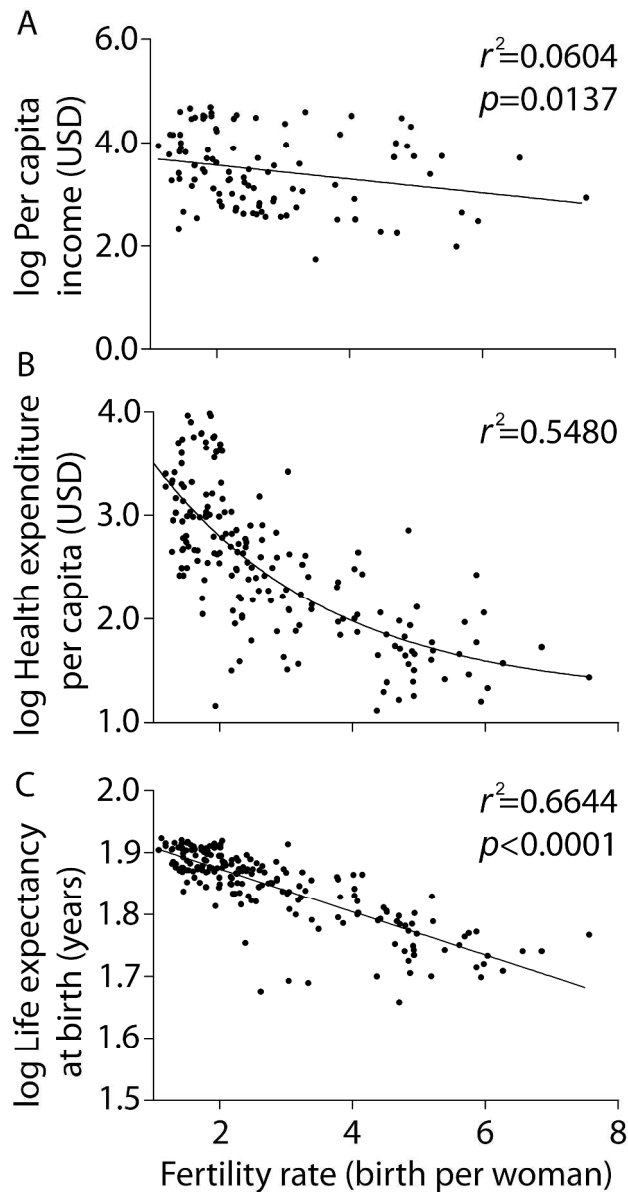
Analysis of the natural selection pressure sustained by the LHB/CGB gene cluster. The measure of natural selection was inferred from the gene cluster sequences of several human populations using the XP-EHH index (Sabeti et al., 2007) and represented on the Y-axis (relative units). The name, ID and exon 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.

99x62mm (300 x 300 DPI)



Phylogenetic 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 F_{st} 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 $\geq 70-75$ (relative units) (Takezaki et al., 2010). POPTREE2 software was used with these default settings: Fixation index (F_{st}) Uncorrected, NJ, Bootstrap 100000.

76x36mm (300 x 300 DPI)



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)

1 Figure Legends

2

3 Figure 1: Allele frequencies of SNPs within LHB/CGB gene cluster in human populations.
 4 Orange/blue bars indicate the proportion in percentage of the two alleles in the different
 5 human groups, which are represented by the colored lines in each column (please refer to the
 6 web browser for the populations order and name). The populations belonging the same
 7 geographical area were grouped as indicated on the right side of the panel. SNPs ID are
 8 shown above each column and grouped by gene. Pink panels above the bars indicate when
 9 mean SNP frequencies of African are significantly different versus that of all other continents
 10 (Kruskal-Wallis and Dunn's post-test; $p < 0.001$); non-significant differences are indicated by
 11 green panels (exceptions: Africa versus America for SNPs rs753306 and rs3752210,
 12 $p \geq 0.001$). Data were obtained using the Human Genome Diversity Project (HGDP) selection
 13 browser (<http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP>).

14

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

27

28 Figure 3: Phylogenic analysis of the FSHR (A) and LHCGR (B) genes. SNPs frequencies
 29 were extracted from HapMap populations (<http://hapmap.ncbi.nlm.nih.gov>) and analyzed by
 30 the POPTREE2 software (Takezaki et al., 2010). The population belonging the same
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 32 Green=East Asian/American; Yellow=European Caucasian/Central Asian), resulting in
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 37 Europe despite they are from USA residents. The measure of genetic distance F_{st} is indicated
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 39 article for references about genetic distance); the numbers throughout the trees are percentage
 40 values representing an index of reliability of the analysis, which is assumed significantly
 41 reliable when $\geq 70-75$ (relative units) (Takezaki et al., 2010). POPTREE2 software was used
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43

44 Figure 4: Relationship between fertility rate and socio-economical current indexes in World
45 countries. Fertility rate is represented as “birth per woman” (X-axis) and plotted against
46 measures of socio-economic status, i.e. per capita income (A), health expenditure per capita
47 (B) and life expectancy at birth (C) (logarithmic Y-axis). Fertility rate is inversely related to
48 all these indexes, demonstrating that the countries in which people has high standard of living
49 are featured by low number of births, and vice versa (linear or non-linear regression were
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