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1           **Potential for pharmacogenetic use of FSH: a 2014-and-beyond view**

2

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8

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23 **Abstract**

24 **Objective:** To assess the potential pharmacogenetic use of FSH for infertility treatment.

25 **Design:** Review of the literature and genomic databases.

26 **Methods:** SNP assessed: rs6166 (c.2039A>G, p.N680S), rs6165 (c.919A>G, p.T307A), rs1394205  
27 (c.-29G>A) in *FSHR* and rs10835638 (c.-211G>T) in *FSHB*. Literature search via PubMed. Blast  
28 analysis of genomic information available in the NCBI nucleotide database. Comparison of allele  
29 frequency and haplotype distribution using the <http://spsmart.cesga.es> tool.

30 **Results:** All these SNPs appear first in *Homo*, result in reduced FSH action and are present with  
31 variable frequencies and combinations worldwide. Stringent clinical studies demonstrate that the  
32 *FSHR* genotype influences serum FSH levels and gonadal response in both sexes. Serum FSH levels  
33 depend on the -211G>T SNP, influencing transcriptional activity of the *FSHB* promoter. Genotypes  
34 reducing FSH action are overrepresented in infertile subjects.

35 **Conclusions:** While the clinical relevance of the *FSHR* polymorphisms alone is limited, the  
36 combination of *FSHR* and *FSHB* genotypes has a much stronger impact than either one alone in both  
37 sexes. About 20% of people are carrier of the alleles associated with lower serum FSH levels/reduced  
38 *FSHR* expression or activity, possibly less favorable for reproduction. Prospective studies need to  
39 investigate whether stratification of infertile patients according to their *FSHR-FSHB* genotypes  
40 improves clinical efficacy of FSH treatment compared to the current, naïve approach. A relative  
41 enrichment of less favorable *FSHR-FSHB* genotypes may be related to changes in human  
42 reproductive strategies and be a marker of some health-related advantage at the costs of reduced  
43 fertility.

44

45

46

47 **Introduction**

48 Follicle-stimulating hormone (FSH) is fundamental for gamete maturation and is widely used in the  
49 treatment of hypogonadotropic hypogonadism and infertility in both sexes (1, 2). Both urinary FSH  
50 (in form of human Menopausal Gonadotropins, hMG, and highly purified FSH) and recombinant (r)  
51 FSH (rFSH, and long acting rFSH) are commercially available and currently in use with very good  
52 results, especially in assisted reproduction. Since unwanted infertility (defined as lack of conception  
53 after one year of unprotected intercourse) affects around 30% of couples, fertility treatment is very  
54 popular and the gonadotropin market florid, especially among gynecologists performing assisted  
55 reproduction technologies (ART) as well as endocrinologists and andrologists treating hypogonadism  
56 and male infertility.

57

58 With a steadily increasing demand of infertility treatment, therapies involving the use of FSH  
59 continue to evolve rapidly, mostly on empirical bases, so that ART protocols are often adjusted based  
60 on availability of new drug preparations despite the paucity of scientifically sound data. Systematic  
61 reviews (e.g. from the Cochrane collaboration) are regularly issued in order to compare different  
62 protocols but often fail to demonstrate the purported superiority of the new approaches (3-6). On the  
63 other hand new protocols of infertility treatment and ART are highly needed, especially considering  
64 the constantly increasing age of women in couples undergoing ART (7), a factor which decreases the  
65 efficacy of the treatment by affecting both pregnancy and abortion rate. In such a scenario,  
66 pharmacogenetic approaches are appealing and have been proposed (8).

67

68 FSH works through binding to its specific receptor, the FSHR (*FSHR*, Gene ID: 2492, location: 2p21-  
69 p16, OMIM: 136435) (9). In physiological conditions serum FSH levels are under hypothalamic and  
70 gonadal control, depend on age and, in women, on the menstrual cyclicity. Research of the last two  
71 decades revealed the role of common genetic variants of *FSHR* and FSH beta subunit (*FSHB*, Gene  
72 ID: 14308, location: 11p13, OMIM: 136530) in determining individual serum hormone levels and  
73 target organ response (10, 11). However, controversies exist concerning the impact of genetic  
74 polymorphisms of these genes on gonadotropin treatment and some contradictory findings have been

75 published. Several good reviews appeared recently in the literature summarizing the current  
76 knowledge, covering various aspects of this topic (12-17) and showing clearly that, currently, there is  
77 not enough evidence to provide practical clinical recommendations for the pharmacogenetic use of  
78 FSH.

79

80 In this article we take a different approach and try to give a coherent interpretation to a rich literature  
81 containing partially contradictory and fragmented data: starting with the analysis of evolutionary  
82 aspects, considering the biological consequences of the genetic variants *in vitro* and *in vivo* under  
83 various pathophysiological conditions, and examining the possible explanations for the controversies  
84 in the literature, we propose that the true pharmacogenetic potential of FSH use in therapy becomes  
85 evident only when the genetic complexity of the FSH-FSHR system is considered in its whole. Since  
86 the most studied genetic variants of *FSHR* and *FSHB* are associated with an overall lower FSH  
87 activity and appear first in the species *Homo*, we are facing an apparent evolutionary paradox, which  
88 suggests the opportunity of a wider view of the FSH role in life-time reproductive success. We  
89 suggest that the genotypes of *FSHR* and *FSHB* both influence the final biological activity of FSH and  
90 should be considered together in future pharmacogenetic studies dealing with infertility.

91

## 92 **Genetic variants of *FSHR* and *FSHB*: Frequency, ethnic distribution and evolution**

93 The receptor for FSH belongs to the family of G protein-coupled receptors. Its three-dimensional  
94 structure was resolved recently, and the mode of FSH-FSHR interaction illustrated (18). Fig. 1 shows  
95 the genomic location of the *FSHB* and *FSHR* genes along with the position of the single nucleotide  
96 polymorphisms (SNP) considered in this article (Table 1), chosen because much information about  
97 their possible role in the biological response to FSH exist in the literature. As shown in Fig. 1, these  
98 SNPs are selected genetic markers among many others belonging to complex genomic regions: the  
99 HapMap database (<http://hapmap.ncbi.nlm.nih.gov>) currently shows about 900 SNPs for *FSHR* and  
100 24 for *FSHB*, respectively, organized in distinct linkage disequilibrium (LD) blocks, segments of the  
101 genome in which a given combination of alleles or genetic markers is inherited coordinately. The  
102 structure of *FSHR* and *FSHB* LD blocks in Caucasians is shown in Fig 1. LD blocks differ in their

103 structure between ethnic groups, resulting in various combinations of the different SNPs. This should  
104 be considered when assessing association studies obtained in different populations.

105

106 Progress in genomic research and studies on human diversity produce public databases, which can be  
107 consulted freely online. We interrogated these databases (HapMap, Release #28; Perlegen, complete  
108 data set; CEPH, U. Stanford HGDP; CEPH, NIH-U. Michigan HGDP; 1000 Genomes, Phase I May  
109 2011) to analyze, in different ethnic groups, frequency, distribution and combinations of the SNPs  
110 considered in this article. The analysis was performed using the <http://spsmart.cesga.es> tool, freely  
111 available online.

112

### 113 *FSHR*

114 Concerning the *FSHR*, the most studied, non-synonymous SNP is rs6166 (c.2039A>G, p.N680S),  
115 which shows a high degree of LD with rs6165 (c.919A>G, p.T307A) (10) in many ethnic groups  
116 (Table 2). Both SNPs are located in exon 10 and cause an amino acid exchange: rs6166 exchanges  
117 asparagine for serine in the intracellular domain of the receptor, introducing a potential  
118 phosphorylation site; rs6165 replaces threonine by alanine, i.e. it results in a change from a polar (T)  
119 to a non-polar, hydrophobic (A) amino acid and removes a potential O-linked glycosylation site.  
120 Another common, but less studied SNP (rs1394205) exchanges a nucleotide in the promoter region of  
121 the *FSHR* (-29G>A). The analysis of these SNPs using the <http://spsmart.cesga.es> tool, extracting the  
122 genomic data present in the available databases<sup>1</sup>, reveals differences in their frequency between  
123 human populations.

124

125 Looking at rs6166, its remarkable ethnic distribution is shown in Fig 2, freely available at  
126 <http://hgdp.uchicago.edu/cgi-bin/alfreqs.cgi?pos=49043425&chr=chr2&rs=rs6166&imp=false>, (19,

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<sup>1</sup> HapMap, Release #28; Perlegen, complete data set; CEPH, U. Stanford HGDP; CEPH, NIH-U.  
Michigan HGDP; 1000 Genomes, Phase I May 2011.

127 20) depicting the allelic frequency in 52 populations and demonstrating important inter-population  
128 differences.

129 The ancestral A allele is most predominant in South Eastern Asia, while the derived G allele is highly  
130 prevalent in other populations and reaches its higher frequencies in Kalash (North-Western Pakistan),  
131 Yakuts (Siberia), Suruì Paiter (Mato Grosso, Brazil) and Melanesians (Oceania). It is interesting to  
132 notice that these ethnicities are geographically isolated and genetically well distinct, and possess a  
133 number of other genetic peculiarities. For instance, Melanesians display genomic signs of direct  
134 introgression of Neanderthal genome in modern humans after migration from sub-Saharan Africa as a  
135 consequence of strong positive selection (21); Yakuts are characterized by very low genetic diversity  
136 (22); Kalash and Suruì Paiter are very small populations (only a few hundreds individuals left) at  
137 extinction risk. In these ethnic groups the enrichment of the rs6166 G allele of the *FSHR* may be a  
138 consequence of insulation or the result of genetic drift.

139

140 Considering all genomic data available in the public databases together (Fig. 3A summarizing  
141 genomic data of 3228 individuals), the ancestral A allele has a minor allele frequency (MAF) of 0.6 in  
142 sub-Saharan Africans, which may be considered closer to the ancient human population. The G allele  
143 is enriched in Europeans, Middle East, Central-South Asia and Oceania, while it shows the lowest  
144 frequency in Far East Asia and North America. The fixation index ( $F_{ST}$ ) value, a measure of the  
145 population differentiation due to genetic structure, calculated for rs6166, is high in East Asians  
146 (0.0525) compared to Europeans (0.0195) and Middle East (0.007) populations, a phenomenon seen  
147 with genetic variants in several other genes (the so-called East Asian sweep pattern) of uncertain  
148 meaning (23). In any case, these ethnic differences should be considered when we turn to genetic  
149 association- and clinical studies based on this SNP, as they might explain some heterogeneity in the  
150 results.

151

152 rs6166 is evolutionary recent. As shown in Supplementary Fig. 1, *FSHR* c.2039A>G (p.N680S) is not  
153 present in non-human primates and, in most animal genomes analyzed so far, the *Fshr* gene carries an  
154 Asn at the amino acid position corresponding to position 680 of the human *FSHR*. The analysis of the

155 Neanderthal genome (<http://neandertal.ensemblgenomes.org>) shows the presence of the G allele (Ser)  
156 in the three samples analyzed so far. This suggests that the new allele was already present in an  
157 extinct hominid branch very close to the modern human. Since, as it will be discussed below, the  
158 *FSHR* allele carrying a Ser at amino acid position 680 is functionally “resistant” to FSH both in  
159 women and in men, the evolutionary advantage of this allele is still unclear.

160

161 Population data about rs6165 are less abundant and show some difference compared to rs6166 (Fig  
162 3B, summarizing genomic data of 2287 individuals). In particular the c.919A>G ancestral G allele is  
163 predominant in sub-Saharan African populations, with a MAF of 0.274, while the MAF is grossly  
164 similar to that of rs6166 in the other ethnic groups. This suggests that the two SNPs are not in LD in  
165 Africans and, to a minor extent, in other ethnicities as well (Table 2). In practically all other species  
166 sequenced so far the G allele is the rule and Ala is the amino acid occupying the position  
167 corresponding to 307 of the human *FSHR* (Supplementary Fig. 2). Therefore, both amino acids 307  
168 and 680 are highly conserved across species but, interestingly, the non-human *FSHR* haplotype is  
169 Ala307-Asn680, which is rarely found in modern humans, with the notable exception of Africans. We  
170 consider this as that the ancestral haplotype and assume that it changed through two independent  
171 mutational events, one introducing rs6166, (c.2039A>G; p.N680S), with the ancient allele encoding  
172 Asn still predominating in most populations (Fig. 3); the other event, rs6165 (c.919A>G; p.T307A)  
173 now results in a predominance of the derived allele (encoding Thr) in all ethnic groups except  
174 Africans. As a result, these changes are now in LD in most people and form the two major exon 10  
175 haplotypes, Thr307-Asn680 and Ala307-Ser680.

176

177 The third SNP in the *FSHR* considered in this article is rs1394205 (c.-29G>A), located in a separated  
178 LD block (Fig. 1) and found with different frequencies and independently of the exon 10 haplotype  
179 (24). Ethnic differences in the distribution of this SNP are known already for some time (25) and are  
180 confirmed by the current database collections (Fig. 4A, summarizing genomic data of 2288  
181 individuals). Comparative alignment analysis suggests that the dominant G allele is the ancestral  
182 allele (Supplementary Fig. 3).



183

184 *FSHB*

185 As far as *FSHB* is concerned, a detailed populations genetics study identified two major haplotypes  
186 possibly influencing conception (26). Most clinical studies performed so far consider only the SNP  
187 rs10835638 (c.-211G>T) and genomic databases contain information about 1093 individuals (Fig.  
188 4B). The MAF of the -211G>T varies between 0.028 (in Africans) and 0.145 (in Europeans). It  
189 appears that this SNP increased in frequency after migration out of Africa and is epidemiologically  
190 relevant only in Europe and in America, with 20-25% of people carrying at least one T allele.  
191 Although rare, this SNP has a significant functional importance (11), and it is located in an element of  
192 the *FSHB* promoter, which binds the LHX3 homeodomain transcription factor influencing gene  
193 transcription (27). Alignment of the corresponding genomic region in several species demonstrates a  
194 large predominance of G (Supplementary Fig. 4), which therefore represents the ancestral allele.

195

#### 196 **Genetic variants of *FSHR* and *FSHB*: *in vitro* effects**

197 In clinical studies the variants of *FSHR* described above have been associated with changes in the  
198 sensitivity to FSH and the -211G>T SNP of the *FSHB* gene was associated to reduced FSH serum  
199 levels, as will be considered in the next chapter. Some *in vitro* studies were devoted to ascertain the  
200 molecular mechanism causing such changes in the levels/activity of FSH.

201

202 *FSHR*

203 Since a number of clinical studies suggest a different sensitivity to FSH of the two *FSHR* exon 10  
204 haplotypes *in vivo* (see next chapter), some experiments were dedicated to clarify the possible  
205 mechanism at the molecular level. The molecular consequences of the combination of p.T307A and  
206 p.N680S (Ala307-Ser680) have been studied in transiently transfected cell lines and in human  
207 granulosa-lutein cells (hGLC) naturally expressing one of the two receptor variants (28-31).  
208 Intriguingly, no difference between the receptor activation depending on the *FSHR* haplotype could  
209 be demonstrated when very early events of cellular response, such as cAMP and IP3 production, were  
210 studied in various transiently transfected cell lines (28-31). hGLC homozygous for each *FSHR*

211 haplotype responded identically in terms of cAMP, progesterone and estradiol production, measured  
212 at saturation (31). Another experiment suggested that hGLC homozygous for Asn at amino acid  
213 position 680 only “tended” towards higher FSH-induced *FSHR* expression, whereas the expression of  
214 other, well-known *FSHR* dependent genes, such as *LHCGR* and *CYP19A1* was not affected (32).

215

216 We recently analyzed the effects of *FSHR* exon 10 SNPs *in vitro* more in depth, looking at the  
217 kinetics of response and at different signal transduction pathways. Our data show that the two *FSHR*  
218 variants, studied in hGLC, respond by activating the different signal transduction pathways with  
219 different kinetics, suggesting that the final biologic response involves different mechanisms. As an  
220 example of these novel experiments we show in Fig. 5 that ERK1/2 activation is blunted in hGLC  
221 naturally expressing the combination of p.T307A A and p.N680S S allele (Ala307-Ser680). These  
222 data, repeatedly confirmed in our lab (Casarini et al., manuscript in preparation), suggest for the first  
223 time that the Ala307-Ser680 *FSHR* is indeed less “active” *in vitro*, providing a molecular explanation  
224 for the clinical data.

225

226 Concerning the *FSHR* -29G>A, this SNP is located in the promoter region, in a consensus sequence  
227 for the cellular homolog to the viral E26 transformation specific sequence (cETS-1). Our early  
228 experiments did not show statistically significant difference in the activity of the promoter in the two  
229 different cell lines COS7 and SK11 (25). However, others could demonstrate, using CHO cells, that  
230 this single base exchange resulted in a significant, 56% decrease of the transcriptional promoter  
231 activity of the A allele (33). It has been shown that promoter activity by reporter assay can vary  
232 consistently depending from the cell line used (34), possibly explaining this discrepancy. The reduced  
233 promoter activity *in vitro*, as shown by Nakayama et al. (33), fits well with the clinical findings.

234

235 *FSHB*

236 The SNP rs10835638 (-211G>T) in the *FSHB* promoter falls in a binding element for the LHX3  
237 homeodomain transcription factor, capable of influencing gene transcription. The -211G>T T allele  
238 decreased transcriptional promoter activity *in vitro* of about 50% in the LβT2 gonadotrope cell line

239 (27). This confirmed earlier studies *in vitro* showing a reduction of promoter activity varying from  
240 46% (in JEG3 cells) to 58% (in TE671 cells) and 86% (in HEK293T cells) (34). The *FSHB* promoter  
241 region containing SNP rs10835638 is located in a putative hormone responsive element but recent  
242 experiments revealed that, unlike the murine *Fshb*, progestins and androgens are unable to induce  
243 *FSHB* transcription (27). Therefore, in the human, circulating progesterone levels are not expected to  
244 modulate serum FSH directly at the pituitary level *via* this mechanism.

245

246 In summary, the evolutionary more recent SNPs, both in the *FSHR* and in the *FSHB* gene, are  
247 associated *in vitro* with changes either in signal transduction (*FSHR* exon 10) or in transcriptional  
248 activity (*FSHR* and *FSHB* promoter) resulting in an overall reduced FSH action.

249

250 ***FSHR* and *FSHB* polymorphisms influence serum FSH levels and reproductive parameters:**

251 **Studies in women**

252 *FSHR*

253 The most popular model to study whether *FSHR* polymorphisms have any effect on FSH levels/action  
254 is represented by women with seemingly normal ovarian function undergoing ART for couple  
255 infertility due to male- or tubal factor. These women are treated with FSH to induce multiple follicle  
256 development. Classically they receive between 2000-5000 IU of FSH over 7-15 days of stimulation  
257 with remarkable inter-individual variability in ovarian response. In these women, the measurement of  
258 basal serum FSH levels, the amount of exogenous FSH needed to reach multi-follicular development  
259 and the levels of serum estradiol at the time of hCG administration for final follicular maturation can  
260 be taken as parameters of *FSHR* sensitivity.

261

262 The first observation that the *FSHR* haplotype consisting of the two SNP in exon 10 could be a  
263 determinant of serum FSH levels and ovarian response to FSH dates back to 2000 (10). In  
264 normoovulatory women undergoing ART we showed that the *FSHR* exon 10 haplotype p.T307A A  
265 and p.N680S S allele (Ala307-Ser680) was less sensitive to FSH, since these women had significantly  
266 higher basal serum FSH levels and required significantly more FSH to achieve multiple follicular

267 maturation (10). This observation immediately opened the questions whether *FSHR* genotype might  
268 be relevant for pharmacogenetic FSH therapy and for menstrual cycle physiology. A very stringent  
269 menstrual cycle monitoring study in young (mean age: 25 yrs), normally cycling, ovulatory women of  
270 European origin, indeed demonstrated the *FSHR* genotype to be instrumental in the determination of  
271 serum levels of FSH, menstrual cycle length and dynamics of reproductive hormones (35).

272

273 Following our original observation, a large number of studies have been published, mostly in women  
274 undergoing ART and reporting partially conflicting results. The reasons for these discrepancies will  
275 be analyzed here. In Suppl. Table 1 we summarize the main data of the studies published so far  
276 concerning *FSHR* genotype frequency distribution (rs6166), basal FSH levels and FSH dosage  
277 subdivided by genotype. In addition, from each study, we extracted the age of the study subjects and  
278 whether or not a cutoff of basal serum FSH levels was an inclusion criterion, two parameters crucial  
279 to interpret the results. In fact, age per se affects serum FSH levels in women and results obtained in  
280 young ovulatory women may well be different for (usually) older, infertile patients undergoing ART.

281

282 The data reported in Suppl. Table 1 show that some studies confirmed our original results (29, 36-40)  
283 but others did not, especially those conducted in women of advanced age (41-44). Serum FSH levels  
284 was not significantly different between *FSHR* genotypes in older women (41-44), and in conditions of  
285 very high FSH concentrations, such as in postmenopausal women (45) and in women with premature  
286 ovarian failure (46). Patient inclusion criteria are important as well, because studies excluding  
287 (young) women with basal FSH levels > 10 IU/L, potentially excluding p.N680S S carriers, did not  
288 reveal any genotype-related difference (37, 47, 48). Some studies included women with ovarian  
289 and/or unexplained infertility (e.g. 49). Finally most studies analyzed only rs6166 (p.N680S),  
290 assuming perfect LD with rs6165 (p.T307A) but, as shown in Fig. 3 this may not always be the case  
291 and represents another reason for inconsistency.

292

293 Many studies in women undergoing ART, tried to assess whether the *FSHR* genotype is useful to  
294 predict response and/or decide the FSH starting dose. This would result in personalized ovarian

295 stimulation with lower incidence of side effects (e.g. ovarian hyperstimulation syndrome) and saving  
296 of resources. These studies, reviewed recently in detail (12-17, 50) are extremely heterogeneous  
297 (Supplementary Table 1) and provided partly conflicting results. For instance, several studies were  
298 unable to confirm that women carriers of the *FSHR* c.2039A>G G allele (Ser680) have higher basal  
299 serum FSH levels compared to Asn carriers (42-44). Nevertheless, a recent meta-analysis of seven  
300 studies including 1421 patients undergoing ART confirmed a significant difference in basal FSH  
301 levels depending on the *FSHR* genotype: carriers of one or two c.2039A>G A alleles (Asn 680)  
302 showed significantly lower FSH levels, with a weighted mean difference of - 1.57 IU/L (C.L. -2.51/  
303 0.64 IU/L) (51), therefore quantitatively small. Again, failure to detect increased basal FSH levels in  
304 c.2039A>G G allele carriers in individual studies involving women undergoing ART is likely to  
305 depend both on age (practically all ART patients are over 25 and several over 40, with a mean age  
306 varying in the individual studies between 30 and 38 yrs) and heterogeneity of patients included  
307 (Supplementary Table 1).

308

309 The importance of the woman's age in assessing the impact of the *FSHR* c.2039A>G on serum FSH  
310 levels has been recently proven by a very elegant study involving only fertile, young women (mean  
311 age of about 25 years) undergoing COH within an oocyte donation program (52). In this study,  
312 including 355 CHO cycles in 145 well characterized, healthy and homogenous oocyte donors, basal  
313 FSH levels, total FSH dose, antral follicle count and number of eggs retrieved were significantly  
314 different between the genotypes, with the homozygous c.2039A>G G allele confirmed to be less  
315 sensitive to FSH stimulation. This study demonstrated once more that, in young, ovulatory women,  
316 the *FSHR* polymorphism c.2039A>G is indeed a major determinant of ovarian sensitivity to FSH.

317

318 A conservative but reasonable conclusion from the studies performed so far is that the *FSHR*  
319 genotype is a physiological determinant of basal FSH levels evident in young, normo-ovulatory  
320 women. This is not necessarily the case in older women of infertile couples especially in the presence  
321 of non-optimal ovarian function and/or reduced reserve, which *per se* result in increase of serum FSH.  
322 Finally, lack of consideration of the *FSHR* -29G>A and/or *FSHB* -211G>T effect might also be one

323 reason why not all studies have been able to demonstrate *FSHR*-dependent differences in FSH levels  
324 in women.

325

326 Concerning the *FSHR* -29G>A, associated with reduced transcriptional activity *in vitro* (33) this SNP  
327 received less attention so far. This SNP was not associated with serum FSH levels in women (25, 41),  
328 but Indian carriers of the *FSHR* -29G>A A allele needed significantly more FSH for multiple follicle  
329 maturation in ART compared to carriers of the *FSHR* -29G>A G allele (41). This might be due to  
330 reduced *FSHR* expression: the less active -29G>A A allele was reported to result in significantly  
331 lower levels of *FSHR* mRNA and protein in granulosa cells obtained from women undergoing ART  
332 (53). The first attempt to assess the combined effect of *FSHR* c.2039A>G and *FSHR* -29G>A  
333 genotypes revealed that the amount of exogenous FSH required for ovarian stimulation and the  
334 frequency of poor responders was the highest in double homozygous carriers of the *FSHR* -29G>A A  
335 and *FSHR* c.2039A>G G (Asn) combination (54). This is intriguing because, while the *FSHR* -  
336 29G>A A allele is transcriptionally less active, the *FSHR* c.2039A>G G (Asn) is expected to be more  
337 sensitive to FSH. Most probably, a much higher number of subjects is needed to reach conclusive  
338 evidence when combined genotypes are considered. Therefore, these observations require  
339 independent confirmation in different ethnic groups, but the concept of genotype-dependent *FSHR*  
340 expression levels is interesting and increases the complexity of the *FSHR* genotype influence on FSH  
341 action.

342

### 343 *FSHB*

344 After the demonstration of a strong *FSHB* -211G>T effect on serum FSH levels in men (11, see  
345 below) the role of this polymorphism was studied in women. An intriguing retrospective study  
346 involving 365 normally cycling women undergoing ART suggested that the presence of T allele, i.e.  
347 that showing lower promoter activity *in vitro* and associated with the lowest FSH levels in men, was  
348 unexpectedly associated with significantly higher serum FSH concentrations in the follicular phase  
349 and lower progesterone levels in the luteal phase (55). Unable to explain this finding, the authors  
350 concluded for a gender-specific difference in the control of gonadotropin secretion. Granted that

351 gonadotropin regulation is different in the two sexes, some factors could have affected the results,  
352 such as luteal insufficiency in 20.5% of the patients, inclusion criteria (FSH levels comprised between  
353 3-15 IU/L; no age limit), and, perhaps more importantly, the lack of stratification by the *FSHR*  
354 genotype. In fact, considering both *FSHR* and *FSHB* polymorphisms together, we observed in women  
355 the opposite results. In a prospective study involving a homogeneous group of 193 women with  
356 regular cycles, age < 40 yrs and normal antral follicle count and serum AMH levels, day 3 FSH  
357 concentrations were significantly lower in carriers of T allele, when stratified by the *FSHR* genotype.  
358 Interestingly, women carriers of the T allele did not show the age-related increase of serum FSH  
359 levels observed in GG homozygotes. This shows the importance of *FSHB* genotype in women,  
360 something deserving attention especially when FSH levels are evaluated as a marker of ovarian  
361 reserve (56). This study suggested that, in women, not considering the *FSHR* genotype might mask  
362 the *FSHB* polymorphism effect (56). In support to this concept, a very recent study in a longitudinal  
363 cohort of peripubertal girls confirmed that the combined effect of *FSHB* -211G>T G allele and *FSHR*  
364 c.2039A>G A allele was associated with a more effective FSH action, with a tendency to anticipate  
365 puberty entry and a significant reduction of serum AMH (57). More studies, however, are necessary  
366 in order to confirm the interaction between the two genotypes in women.

367

368 So far, no data are available about the possible role of the *FSHB* -211G>T polymorphism, alone or in  
369 combination with *FSHR*, on ART outcome.

370

### 371 Pharmacogenetic studies

372 Are these polymorphisms in *FSHR/FSHB* relevant for ART? Can we improve ART outcome using a  
373 pharmacogenetic approach based on their genotype? To date only one prospective, randomized,  
374 controlled study was conducted, in which women homozygous for the c.2039A>G of the *FSHR* were  
375 treated with fixed doses of FSH to assess ovarian response, measured as serum estradiol levels on the  
376 day of hCG triggering (47). This study demonstrated that homozygous *FSHR* p.N680S S women  
377 produced less estradiol compared to homozygous p.N680S N women treated with the same dose of  
378 FSH and this difference could be overcome by increasing the FSH dose. While demonstrating, in a

379 clean way, that the *FSHR* genotype effectively impacts ovarian response in an interventional,  
380 prospective, controlled design, the study did not answer the question whether this effect is clinically  
381 relevant for the success of an ART program. In fact, the study was not powered to assess possible  
382 effects on pregnancy rate and live births, i.e. the real, clinically relevant end points of each ART  
383 intervention. In addition, it completely disregarded heterozygous women, i.e. the majority of patients,  
384 and did not consider the other SNPs in the promoter of *FSHB* and of *FSHR*, the relevance of which  
385 was never studied in prospective, interventional studies. Answering the question whether a  
386 pharmacogenetic approach based on the *FSHR* and/or *FSHB* genotype may improve ART outcome in  
387 terms of live births and/or reduced side effects would require a very large, multicenter effort,  
388 involving thousands of well-selected women. Given the socio-economical aspects currently involved  
389 (pressure of the infertile couple to achieve a pregnancy, financial aspects) and pathophysiological  
390 variables (woman's age, male factor, ovarian reserve, unexplained infertility), it is unlikely that such a  
391 study will ever be performed and the question is whether it would be worth the trouble. There is,  
392 however, a plethora of studies analyzing retrospectively the relevance of *FSHR* and other gene  
393 polymorphisms for ovarian response (variably defined) in ART programs based on different types of  
394 patients. To mention only the most recent ones, some studies confirm the impact of the *FSHR*  
395 c.2039A>G SNP (40), some do not (49) and this reflects faithfully the results of over a decade of  
396 literature summarized in recent reviews (12-17, 50). The heterogeneity of the study designs, patient  
397 characteristics and primary end points, often in the absence of power analysis, together with relatively  
398 advanced age of women in ART programs are the possible reasons for this inconsistency  
399 (Supplementary Table 1) In addition, other genes (e.g the *ESR2*, *AMH*, *AMHR2*, *MTHFR*, etc.) have  
400 been variably shown to be associated with ovarian response, to which they could contribute (15, 17).

401

#### 402 ***FSHR* and *FSHB* polymorphisms influence serum FSH levels and reproductive parameters:**

##### 403 **Studies in men**

##### 404 *FSHR*

405 Several studies assessed the association of *FSHR* exon 10 polymorphisms and reproductive  
406 parameters in men. However, for long time, no effect of *FSHR* genotype on serum FSH levels could



407 be demonstrated (reviewed in 69). This was surprising because, meanwhile, many papers were  
408 published supporting the role of *FSHR* genotype in determining serum FSH levels women (12-14, 10,  
409 35, 40, 59, 60) (Supplementary Table 1). As possible explanation, gender-specific differences in the  
410 feed-back regulation of FSH secretion were assumed.

411

412 The significant role of the *FSHR* p.N680S polymorphism in the male was very recently demonstrated  
413 by a study involving a very large number of Baltic men (61). Thanks to the large dimension of the  
414 study, performed on 1790 men, and to the meta-analytical approach it was possible to demonstrate for  
415 the first time the effects of the *FSHR* pN680S polymorphism alone on testis volume, serum FSH,  
416 inhibin B and testosterone levels. The effect was of small entity (effect of the Ser allele on testis  
417 volume: -1.40 mL) but significant. One reason why it was not demonstrated earlier could be that,  
418 apart from the sample size effect, it might have confounded by the *FSHB* -211G>T SNP effect in the  
419 previous studies based on much smaller subject numbers (62).

420

421 No data concerning the *FSHR* -29G>A SNP in normal males are available so far.

422

#### 423 *FSHB*

424 The -211G>T SNP in the *FSHB* promoter was demonstrated to have a strong effect *in vitro* on  
425 transcription by luciferase reporter assay, with the T allele showing a relative activity which was only  
426 half of that of the G allele (34). Together with the evidence that the haplotype structure of the *FSHB*  
427 gene might be subjected to balancing selection (26) and, thereby, influence reproductive parameters,  
428 and the high evolutionary conservation across species of the promoter region including rs10835638,  
429 this induced the analysis of whether *FSHB* -211G>T polymorphisms could be associated with serum  
430 FSH levels. A seminal study in young male volunteers of Baltic origin demonstrated that this SNP  
431 indeed influenced serum FSH levels and other reproductive parameters. In particular FSH levels in  
432 homozygous *FSHB* -211G>T T carriers were significantly reduced compared to both heterozygous  
433 and homozygous G carriers (11). This was then amply confirmed in German and Italian men (63, 64).  
434 In accordance with the epidemiological and *in vitro* data reported above, homozygous carriers of the

435 minor allele represent only 1.5% of men with normal semen parameters but have serum FSH levels  
436 which are about 25% lower than homozygous, major allele carriers (11, 63, 65). In addition, this  
437 polymorphism is associated with lower testicular volume, lower sperm count, lower testosterone and  
438 higher LH serum levels (11, 63-66).

439

440 The association of *FSHB* -211G>T polymorphism with serum FSH and other reproductive parameters  
441 in men prompted to a reassessment of the (mild) effects of exon 10 *FSHR* polymorphisms in the male  
442 In an elegant study Tüttelmann et al (62) showed that when men are stratified by the *FSHB* genotype,  
443 significant differences in serum FSH levels and testicular volume between carriers of the different  
444 *FSHR* genotype become evident. This study demonstrated for the first time that the *FSHR*  
445 polymorphism effect is indeed present also in the male, although it can be masked by the *FSHB*  
446 polymorphism, suggesting, in addition, that the interplay between polymorphisms in hormone and  
447 receptor is of relevance under physiological conditions. Taking into account both *FSHR* and *FSHB*  
448 and considering their allelic frequencies, the authors suggested that carriers of the potentially  
449 “unfavorable” allele combination (i.e. those associated with lower testis volume and serum FSH  
450 levels) represent about 45% of all males, have smaller testes and could be at risk for reduced  
451 reproductive fitness (62).

452

453 In summary, the current evidence shows that both *FSHR* and *FSHB* genotypes are physiologically  
454 very relevant and interact with each other to determine gonadotropin levels in both sexes. So far only  
455 few studies assessed the effects of the combination of rs6166 in *FSHR* and rs10835638 in *FSHB* (62,  
456 56, 57). This combination results in nine different genotype combinations, which have been  
457 demonstrated to be associated with significantly different serum FSH levels (56, 62). The matter is  
458 further complicated by the possible effects of the *FSHR*, -29G>A SNP, influencing levels of *FSHR*  
459 expression: only one study considered the combination of rs6166 and rs1394205 in women (54). The  
460 combination of these three SNPs would result in 27 combinations (Table 3). Among them, those  
461 predicted to be associated with the “less favorable” *FSHB/FSHR* genotype combinations (in terms of  
462 serum FSH levels and *FSHR* expression/sensitivity) are expected in about 20% of Caucasians. Very

463 large number of subjects will be necessary to address systematically the pathophysiological relevance  
464 of these allele combinations and to attempt a meaningful pharmacogenetic approach.

465

466 **The *FSHR* and *FSHB* genotypes associated with lower FSH levels and/or less sensitive *FSHR*  
467 **are enriched in human infertility****

468 *Ovulation disorders*

469 Polycystic ovary syndrome (PCOS) represents an intriguing and frequent form of infertility and  
470 anovulation, object of intensive research. Given the role of gonadotropins in ovarian physiology, the  
471 association with the *FSHR* gene SNP c.2039A>G has been repeatedly investigated in several  
472 populations. Overall, there seems to be a selective enrichment of the c.2039A>G G allele in PCOS  
473 and a meta-analysis including 1028 PCOS and 3587 controls demonstrated a mild protective effect of  
474 the homozygous c.2039A>G A allele (67). There is evidence that the c.2039A>G G genotype is more  
475 frequent in ovulation disorders, infertility and poor or exaggerated FSH response in ART (36, 42, 59,  
476 60, 68-74) (Supplementary Table 1). Interestingly, two genome-wide association studies in Chinese  
477 women identified, among other interesting candidates, the *FSHR* and the *LHCGR* as risk loci for  
478 PCOS (75, 76). Another, very carefully conducted, genetic association study based on selected,  
479 haplotype-tagging SNPs, confirmed these findings in women of European origin (77). In the case of  
480 the *FSHR* both a SNP 5.3 kb upstream of the gene, and rs6165 (p.T307A) were nominally strongly  
481 associated with PCOS. Therefore, evidence is accumulating that the genomic region of chromosome 2  
482 encompassing *FSHR*, *LHCGR* and *GTF2AIL* (general transcription factor IIA, 1-like, involved in  
483 gametogenesis) confers susceptibility to PCOS and deserves further intensive, functional and genetic  
484 research. Experimentally, none of the existing mouse models recapitulate the whole spectrum of the  
485 human PCOS but, remarkably, overexpression of LH/hCG activity, androgen excess and reduced  
486 aromatization/estrogen action reproduce the ovarian cystic phenotype in mice (78). Together, these  
487 evidences identify (maybe not surprisingly) altered gonadotropin action as the most plausible  
488 determinant of the ovarian phenotype of PCOS. Women with PCOS may be treated with clomiphene  
489 or gonadotropins for ovulation induction. However, no prospective, pharmacogenetic trial on  
490 ovulation induction in PCOS based on the *FSHR* genotype has been performed so far.

491

492 Concerning the *FSHR* c.-29G>A an association was reported in Indian patients with poor ovarian  
493 response (41) as well as with primary and secondary amenorrhea (79). No data are available about the  
494 possible role of the *FSHB* c.-211G>T in female infertility. Understanding the molecular events related  
495 to the genetic *FSHR* (and *LHCGR*) variants will be essential for a pharmacogenetic approach to PCOS  
496 treatment.

497

#### 498 Male infertility

499 Most studies failed to find any correlation between *FSHR* genotype and male infertility phenotype  
500 (reviewed in 58 and 80). Conversely, the rare, *FSHB* -211G>T homozygous T genotype was reported  
501 to be significantly overrepresented in infertile men (66). Whether the *FSHR* -29G>A SNP is of any  
502 relevance in the male remains controversial. A small effect of *FSHR* -29G>A A allele on testis  
503 volume was reported in a small group of infertile men (81) but not replicated so far. The original  
504 description of an association of *FSHR* haplotypes including -29G>A, 919A>G and 2039A>G with  
505 male infertility (82) has not been confirmed by the subsequent literature (80) and, for the time being,  
506 there is no evidence that the study of the -29G>A SNP adds any useful information to the *FSHB* -  
507 211G>T - *FSHR* c.2019A>G combination in the male.

508

#### 509 Pharmacogenetic studies in men

510 Male idiopathic infertility remains a pathophysiological dilemma and a therapeutic challenge. In the  
511 ART era, most forms of male infertility are empirically resolved by intracytoplasmic sperm injection  
512 (ICSI) but, given the burden (not only economical) of the procedure, the question is whether sperm  
513 quality can be improved by a less invasive, medical intervention. Owing to the role of FSH in  
514 spermatogenesis, FSH treatment has always been tempting and is actively prescribed by many doctors  
515 in spite of the lack of evidence of its superiority compared to placebo. Several studies suggest that  
516 FSH treatment might be useful in a subgroup of normogonadotropic infertile men (83), purportedly  
517 those without spermatogenic blockade, but these men are impossible to identify beforehand. Could  
518 knowledge of the *FSHB* and/or *FSHR* genotype be helpful here for?

519

520 So far only two studies addressed the question whether a pharmacogenetic approach would be helpful  
521 in identifying responders to FSH treatment. One study showed that patients with at least one *FSHR*  
522 c.2039A>G G allele had a significant increase of total sperm count after 3 months of treatment with  
523 rFSH (150 IU/thrice per week) (84). Another study considered the *FSHB* -211G>T genotype and  
524 showed that TT homozygotes, representing 25% of men with oligozoospermia and low FSH levels,  
525 could significantly benefit from FSH treatment (65). No study addressed the combination of the two  
526 genotypes so far. Since both *FSHB* and *FSHR* genotypes affect testis volume and serum FSH levels  
527 (62) it would be interesting to investigate whether normogonadotropic, oligozoospermic men carrier  
528 of the less favorable SNP combinations could improve fertility upon FSH treatment. However, sperm  
529 parameters are naturally quite variable within the same individual and the problem of the correct (and  
530 robust) primary end point in such a study remains unresolved. Studies are ongoing assessing whether  
531 sperm DNA fragmentation, believed to be ameliorated by FSH, changes upon FSH treatment in  
532 dependence of the genotype (EudraCT 2010-020240-35).

533

#### 534 **Conclusions and outlook**

535 In the light of the actual knowledge, is there any potential for the pharmacogenetic use of FSH in  
536 infertility treatment (male and female)? Overall, the available *in vitro* and *in vivo* data support the  
537 physiological relevance of the considered SNPs for FSH action. The genetic complexity thereof,  
538 illustrated by the four SNPs considered here, is probably much higher than supposed so far and only  
539 studies considering the *FSHB/FSHR* genotype combinations with numbers of subjects large enough in  
540 dependence of the frequency of each genotype in a given ethnic group, will be able to assess this  
541 issue. Efforts should be dedicated to identify subjects with the “less favorable” variant combination,  
542 expected to be overrepresented among infertile patients, and assess whether they would be candidates  
543 for FSH treatment and/or usage of higher FSH doses in case of ART or ovulation induction. This,  
544 however, needs to be proven in large, prospective studies, objectively difficult to conduct in the ICSI  
545 era. Nevertheless, FSH therapy might become an interesting medical option, especially for treatment  
546 of selected cases of male infertility, in which the reduction of sperm parameters is combined with a

547 “sloppy” *FSHB/FSHR* genotype combination and the female partner has normal ovarian function and  
548 a good reserve. Interventional trials should be started with such couples, because successful medical  
549 treatment of the man would relieve the woman of carrying the burden of male infertility.

550

551 There is another interesting and intriguing aspect to consider. When epidemiological, functional and  
552 clinical data are considered together with the evolutionary aspects illustrated above, it appears that an  
553 overall trend towards a less efficient FSH-FSHR system emerges in the human species. This poses an  
554 interesting evolutionary biology question: which environmental conditions select the evolutionary  
555 more recent *FSHB* and *FSHR* genotypes and are there unrecognized advantages for life-time  
556 reproductive success for such genotypes which, counter intuitively, reduce fertility/fecundity?

557 Another example of evolutionary paradox is PCOS, a common polygenic condition linked to both  
558 infertility and metabolic disturbances, which is steadily increasing in epidemiological relevance in  
559 spite of reduced fertility (85). Since evolution maximizes reproduction other aspects may come into  
560 play.

561

562 In a recent evolutionary study, the *FSHR* gene was identified as a determinant of human birth timing  
563 (86) suggesting its association in as yet unclear processes involved in shortening gestation time and  
564 accelerating parturition in the human species. Although a driving evolutionary role of the less  
565 “sensitive” *FSHR* c.2039A>G G allele was postulated (35), there is no evidence that specific  
566 polymorphisms the *FSHR* gene influence duration of fertile life. The possible role in pregnancy  
567 duration remains intriguing. Other studies described association of the *FSHR* and its variants with an  
568 increasing number of non canonical and non obviously FSH-related pathophysiological events, such  
569 as osteoporosis (45), vasculogenesis (87), hypertension (33), ovarian (88) and testis cancer (89). All  
570 these suggestions would question the dogma of the unique action of FSH at the gonadal level. The  
571 expression of the *FSHR* in extragonadal tissues is currently matter of vivid debate and the possible  
572 role of FSH in non canonical target organ remains enigmatic. Should perhaps the *FSHR* gene be  
573 viewed as a marker of health at large and these apparently weird associations reconsidered with a  
574 wider, evolutionary vision?

575

576 *FSHR* exon 10 homozygous Ser680 genotype was significantly associated with lower risk of  
577 developing sporadic Alzheimer disease (90) and with surviving over the age of 90 years in women but  
578 not in men (91), independently of previous fertility, age of menopause or other known risk factors,  
579 suggesting a role for the *FSHR* genotype as determinant of longevity. In our study in normal  
580 menstrual cycle (35) we observed differences in menstrual cycle length between carriers of the two  
581 *FSHR* haplotypes, resulting in about one menstrual cycle less per year in homozygous p.N680S S  
582 compared to homozygous p.N680S N carriers. Since no difference in the age of menopause was  
583 demonstrated so far, women with the homozygous p.N680S S genotype would experience 30–40  
584 cycles less than women with p.N680S N genotype during their reproductive life and would be  
585 exposed to a lower incidence of pregnancies and related risks. In the absence of medical assistance,  
586 maternity-related lifetime risk, still relevant in underdeveloped countries, must have been very high in  
587 the earlier times of human evolution, possibly making pregnancy-related death an important factor in  
588 determining a evolutionary selection of this genotype. We speculated that fewer menstrual cycles  
589 during the reproductive life span might represent an evolutionary advantage, provided that the fertility  
590 of the species is maintained (35). Medical progress, better nutrition, contraception, and the recent  
591 dramatic changes in women' role in modern society are modifying the current human reproductive  
592 strategies, so that the less fertile genotype becomes epidemiologically relevant, especially in couples  
593 attempting to conceive in their late thirties.

594

595 In conclusion, the current evidence suggests that the combination of *FSHR* and *FSHB* genotypes is  
596 predicted to have a much stronger impact than either one alone, both on male and female gonadal  
597 function, since about 20% of people are carrier of the allele combinations associated with lower serum  
598 FSH levels and lower *FSHR* expression/activity (Table 3). The advent of powerful, sensitive and  
599 inexpensive techniques assessing several SNPs simultaneously will be very helpful in the  
600 identification of the patients at infertility risk. Interventional, prospective studies are eagerly needed  
601 to investigate whether stratification of patients according to their *FSHR-FSHB* genotype combination  
602 is of any advantage in the treatment of male and female infertility compared to the current, naïve

603 approach. In the absence of this, every pharmacogenetic approach to the clinical use of FSH is  
604 empirical and there is no hard evidence that it would be superior in terms of better outcome, reduced  
605 side effects, and/or pharmaco-economic impact.

606

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610

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615 **Author contribution statement:**

616 Both authors selected and reviewed the literature and prepared the manuscript. LC performed the *in*  
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618

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922 **Legend to Figures**

923

924 **Figure 1**

925 Intron-exon structure, SNP positions and LD blocks in the human *FSHR* and *FSHB* genes.

926 The LD blocks (dark triangles) were calculated by the software Haploview

927 (<http://www.broadinstitute.org>) in the selection of 121 samples obtained from Utah residents

928 with Northern and Western European ancestry from the CEPH collection (CEU). The

929 darkness of the squares indicates the strength of LD. The SNPs are indicated by black lines

930 above the LD blocks (arrows). Gene name, chromosome number and coordinates of the

931 shown region, specified in the white boxes within each panel, were obtained from the

932 HapMap database (<http://hapmap.ncbi.nlm.nih.gov>). Panel A) 200 Kb genomic region of

933 chromosome 2 including the human *FSHR* gene. Exons are represented by boxes and the

934 positions of SNPs rs6166 (c.2039A>G), rs6165 (c.919A>G) and rs1394205 (-29G>A) are

935 indicated. B) 200 Kb genomic region of chromosome 11 including the human *FSHB* gene.

936 The genomic region in the boxed area is enlarged in panel C. C) 5 Kb genomic region of

937 chromosome 11 including the human *FSHB* gene and the position of SNP rs10835638 (-

938 211G>T).

939

940 **Figure 2**

941 Geographic distribution of the rs6166 (*FSHR* c.2039A>G) allele frequencies. The world map

942 with geographic coordinates show the distribution of the alleles A and G in different

943 countries by pie charts, calculated by the HGDP Selection Browser

944 (<http://hgdp.uchicago.edu>). The panel on the right side shows the allele frequencies by bar

945 charts for each population sample from different regions of the earth. The data on which the

946 analysis is based are from the Stanford University SNPs selection from Human Genome  
947 Diversity Project (<http://www.hagsc.org/hgdp/files.html>).

948

949 **Figure 3**

950 Allele and genotype frequencies of the *FSHR* exon 10 SNPs rs6166 (c.2039A>G; panel A)  
951 and rs6165 (c.919A>G; panel B) in different geographic regions. The sample size is indicated  
952 above each bar; data were analyzed by SPSmart (<http://spsmart.cesga.es>) using the databases  
953 HapMap, 1000 Genomes, HGDP CEPH Stanford selection and Perlegen as data sources;  
954 n.a.=data not available.

955

956 **Figure 4**

957 Allele and genotype frequencies of the *FSHR* and *FSHB* promoter SNPs rs1394205 (-  
958 29G>A; panel A) and rs10835638 (-211G>T; panel B) in different geographic regions. The  
959 sample size is indicated above each bar; data were analyzed by SPSmart  
960 (<http://spsmart.cesga.es>) using the databases HapMap, 1000 Genomes, HGDP CEPH  
961 Stanford selection and Perlegen as data sources; n.a.=data not available.

962

963 **Figure 5**

964 Kinetics of phospho-ERK1/2 activation in the absence (left panels) and in the presence (right  
965 panels) of 50 nM r-FSH (Gonal-F, Merck-Serono S.p.A., Rome, Italy) in p.N680S  
966 homozygous -N or -S human primary lutein granulosa cells (hGLC), detected by Western  
967 blotting. Cell preparation was performed as previously described (31) and specified in  
968 supplementary methods. One representative of three independent experiments is shown.  
969 hGLC carrying the p.N680S N homozygous genotype shown potent and rapid ERK1/2 FSH-  
970 dependent phosphorylation. hGLC homozygous for the p.N680S S genotype response to FSH

971 was much lower and delayed in terms of ERK1/2 activation. PMA indicates the ERK1/2  
972 activator phorbol myristate acetate (positive control); U0126 is a MEK (MAPK kinase)  
973 inhibitor (negative control).  
974

Table 1: Nomenclature of genes and polymorphisms discussed in this article (<http://www.ncbi.nlm.nih.gov/pubmed>).

<b>Gene</b>	<b>refSNP</b>	<b>DNA Nucleotide</b>	<b>Single nucleotide variation</b>	<b>NCBI nucleotide ref. sequence</b>	<b>Protein</b>	<b>NCBI protein ref. sequence</b>
<i>FSHR</i>	rs1394205	-29G>A	g.49381585C>T	NT_022184.15		
<i>FSHR</i>	rs6165	c.919G>A	g.49191041C>T	NT_022184.15	p.A307T	P23945.3
<i>FSHR</i>	rs6166	c.2039A>G	g.49189921T>C	NT_022184.15	p.N680S	P23945.3
<i>FSHB</i>	rs10835638	-211G>T	g.30252352G>T	NT_009237.18		

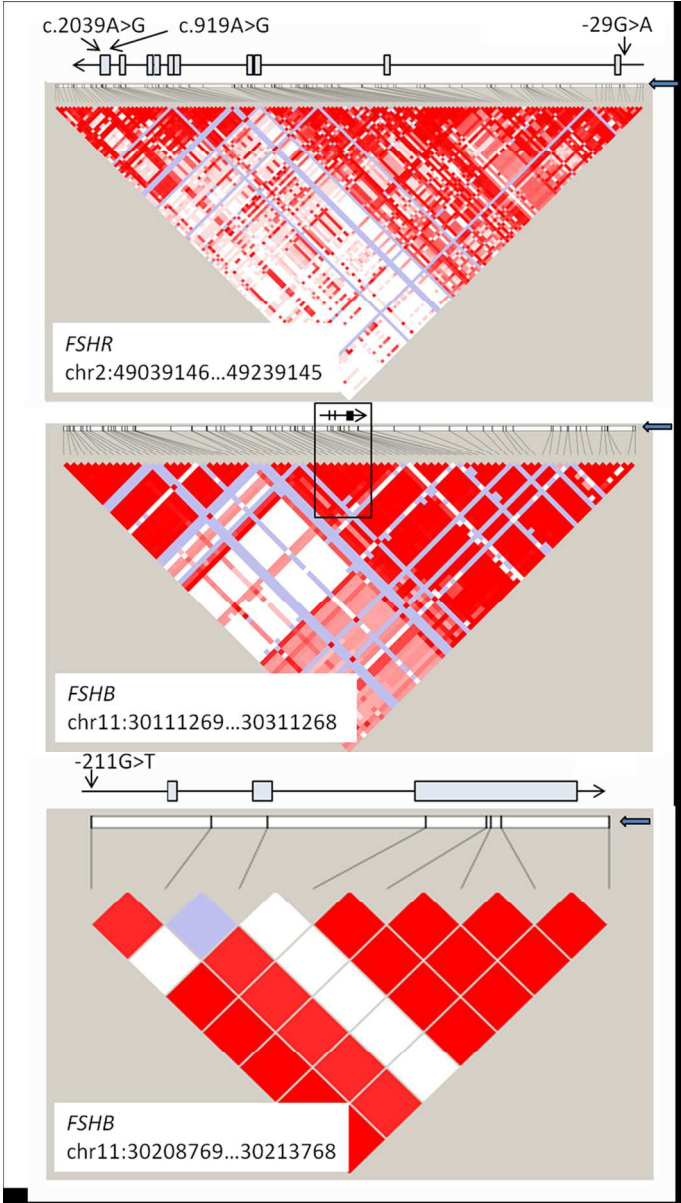
Table 2: Pairwise linkage disequilibrium (LD) parameters between the polymorphisms rs6165 and rs6166 in HapMap populations calculated by the software Haploview (<http://www.broadinstitute.org>). Population names and their abbreviation (in brackets), sample size and minor allele frequencies (MAF) of rs6166 (*FSHR* c.2039A>G) and rs6165 (*FSHR* c.919A>G) were taken from the HapMap database (<http://hapmap.ncbi.nlm.nih.gov>). The reference allele is placed before each MAF value. The two LD values  $D'$  and  $r^2$  are shown.  $D'$  is calculated by Haploview as  $D'=D/D_{\max}$ , where D is the deviation of the observed from the expected.  $r^2$  is the correlation coefficient between pairs of loci. The maximum values of  $D'$  and  $r^2$  is 1.000, which indicates complete LD or pairwise correlation between the loci, respectively.  $D'$  and  $r^2 = 0.000$  indicates random coupling of the SNPs.

HapMap populations	Sample size	rs6166 MAF	rs6165 MAF	$D'$	$r^2$
African ancestry in Southwest USA (ASW)	53	G 0.411	A 0.325	1.000	0.362
Luhya in Webuye, Kenya (LWK)	110	G 0.350	A 0.209	1.000	0.143
Maasai in Kinyawa, Kenya (MKK)	156	G 0.374	A 0.436	0.979	0.445
Yoruban in Ibadan, Nigeria (YRI)	153	G 0.493	A 0.224	0.961	0.251
Utah residents with European ancestry (CEU)	121	G 0.403	G 0.403	1.000	1.000
Tuscan in Italy (TSI)	102	G 0.475	G 0.470	1.000	0.977
Gujarati Indians in Houston, Texas (GIH)	101	A 0.490	A 0.490	1.000	0.978
Han Chinese in Beijing, China (CHB)	139	G 0.281	G 0.312	1.000	0.865
Chinese in Metropolitan Denver, Colorado (CHD)	109	G 0.303	G 0.321	1.000	0.924
Japanese in Tokyo, Japan (JPT)	116	G 0.348	G 0.363	0.974	0.902
Mexican ancestry in Los Angeles, California (MEX)	58	G 0.328	G 0.316	0.956	0.876

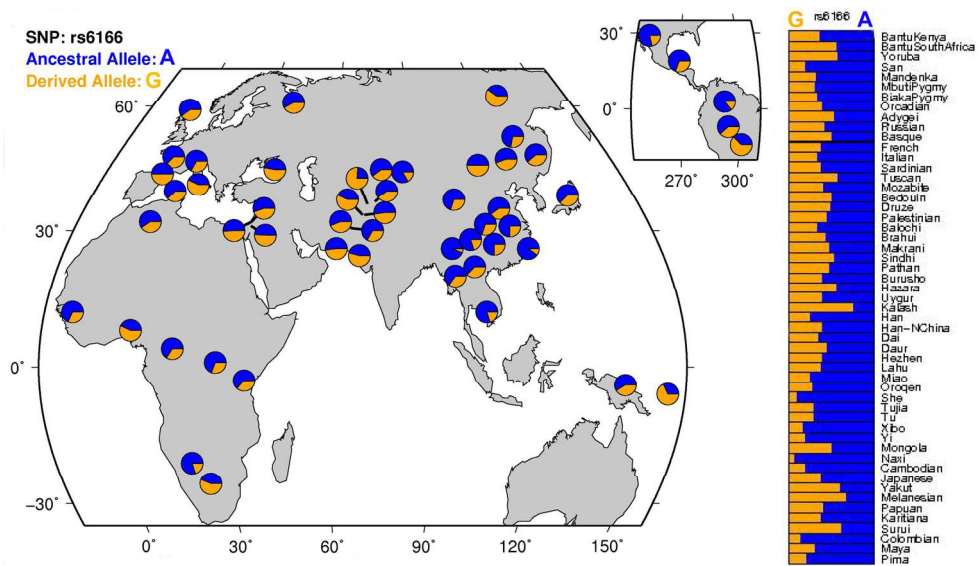


Table 3: Expected genotype frequencies in Caucasians of the 27 allele combinations resulting from rs6166 (FSHR p.N680S), rs1394205 (FSHR -29G>A) and rs1083563 (FSHB -211G>T) frequencies collected from the HapMap database (<http://hapmap.ncbi.nlm.nih.gov>) based on 121 CEU subjects. Shadowed areas indicate the *FSHB/FSHR* genotype combinations associated with lower serum FSH levels and lower FSHR expression and activity.

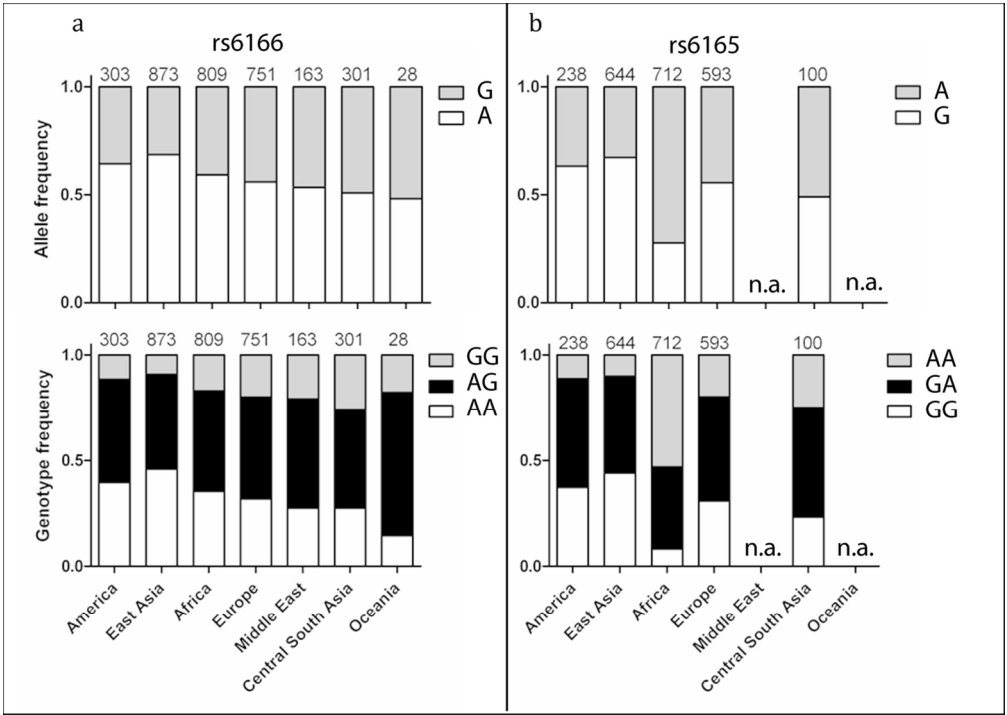
rs6166		AA			GA			GG		
rs1394205		GG	GA	AA	GG	GA	AA	GG	GA	AA
rs10835638	GG	13,82	10,26	2,85	19,01	14,12	3,93	6,21	4,61	1,28
	GT	4,15	3,08	0,86	5,71	4,24	1,18	1,86	1,38	0,38
	TT	0,20	0,15	0,04	0,27	0,20	0,06	0,09	0,07	0,02



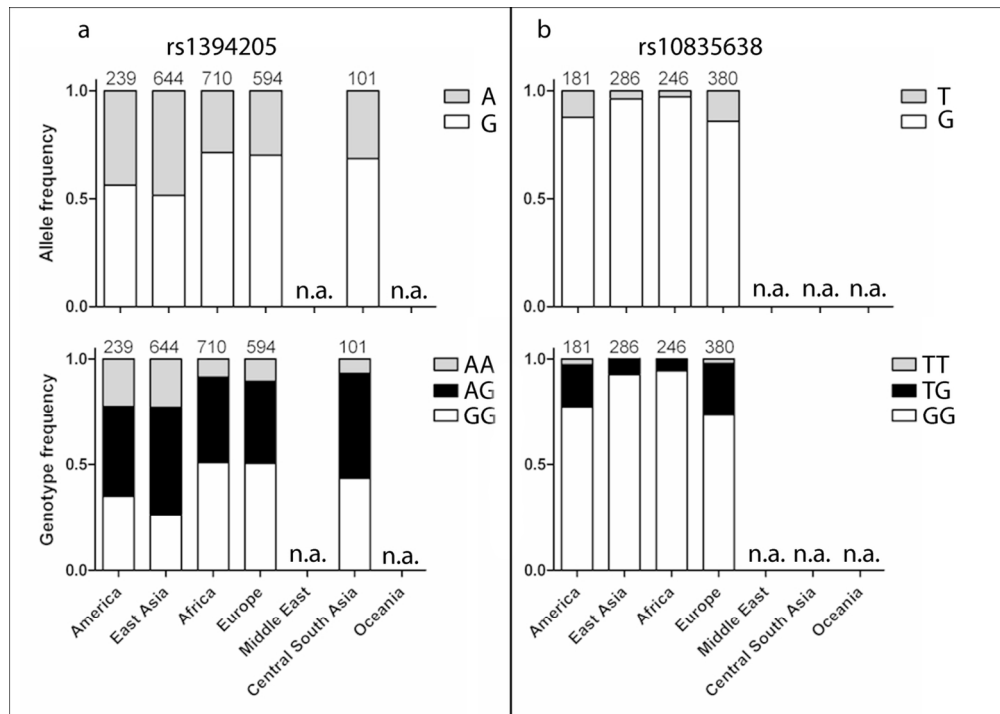
82x145mm (300 x 300 DPI)



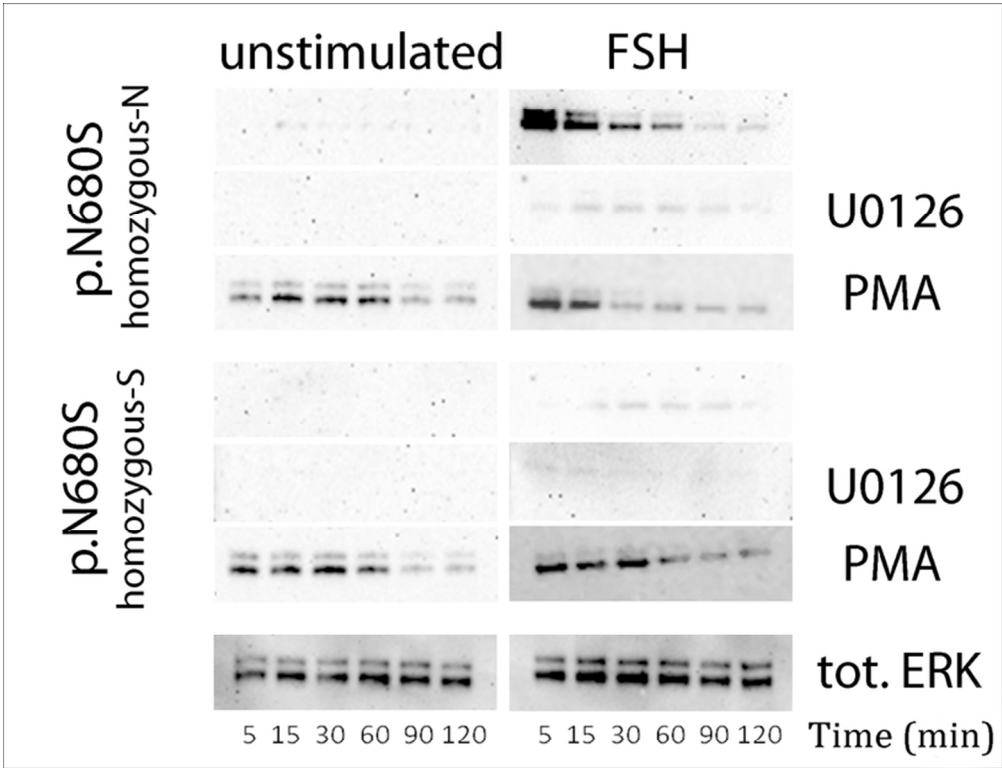
163x92mm (300 x 300 DPI)



116x82mm (300 x 300 DPI)



116x82mm (300 x 300 DPI)



68x57mm (300 x 300 DPI)