Review

Genomics and genetics of gonadotropin beta-subunit genes: Unique FSHB and duplicated LHB/CGB loci

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The follicle stimulating hormone (FSH), luteinizing hormone (LH) and chorionic gonadotropin (HCG) play a critical role in human reproduction. Despite the common evolutionary ancestry and functional relatedness of the gonadotropin hormone beta (GtHB) genes, the single-copy FSHB (at 11p13) and the multi-copy LHB/CGB genes (at 19q13.32) exhibit locus-specific differences regarding their genomic context, evolution, genetic variation and expressional profile. FSHB represents a conservative vertebrate gene with a unique function and it is located in a structurally stable gene-poor region. In contrast, the primate-specific LHB/CGB gene cluster is located in a gene-rich genomic context and demonstrates an example of evolutionary young and unstable genomic region. The gene cluster is shaped by a constant balance between selection that acts on specific functions of the loci and frequent gene conversion events among duplicons. As the transcription of the GtHB genes is rate-limiting in the assembly of respective hormones, the genomic and genetic context of the FSHB and the LHB/CGB genes largely affects the profile of the hormone production.

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Contents

1. Introduction ............................................................................................................................................. 5
2. Comparative genomic context of gonadotropin beta genes ................................................................. 5
   2.1. FSHB genomic region ...................................................................................................................... 5
   2.2. LHB/CGB genomic region ............................................................................................................ 5
   2.2.1. LHB/CGB genes in human ...................................................................................................... 5
   2.2.2. LHB/CGB genes in primates .................................................................................................. 6
3. Forces affecting diversity and evolution of gonadotropin beta genes .................................................. 8
   3.1. Diversity patterns of gonadotropin beta genes .............................................................................. 8
   3.2. Role of gene conversion ............................................................................................................... 9
   3.3. Role of selective pressures .......................................................................................................... 9
   3.3.1. FSHB gene .......................................................................................................................... 9
   3.3.2. LHB/CGB genes .................................................................................................................. 9
4. Genetic variation of human gonadotropin beta genes ........................................................................... 10
   4.1. Gonadotropin beta gene mutations with known functional effects ............................................ 10
   4.2. Polymorphisms associated with phenotypic traits ....................................................................... 10
   4.2.1. FSHB gene ......................................................................................................................... 10
   4.2.2. LHB gene .......................................................................................................................... 10
   4.2.3. CGB genes ......................................................................................................................... 12
   4.3. Non-synonymous amino acid substitutions with unknown phenotypic consequence ................ 12

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1. Introduction

The follicle stimulating hormone (FSH), luteinizing hormone (LH) and chorionic gonadotropin (CG; HCG in human) are functionally and evolutionarily related hormones regulating reproductive function (Pierce and Parsons, 1981). FSH and LH are produced in the anterior lobe of pituitary gland in a pulsatile manner and their function in gonads is mediated over distinct FSH and LH receptors, respectively (Table 1) (Dalkin et al., 2001; Ascoli et al., 2002; Dias et al., 2002). FSH is required for follicle maturation and stimulation of ovarian estrogen production in women. In males, FSH promotes Sertoli cell proliferation and indirectly spermatogenesis (McGee and Hsueh, 2000; Plant and Marshall, 2001). LH stimulates female progesterone synthesis and ovulation and also theca cell androgen production. In males, LH stimulates testosterone production in Leydig cells (Moyle and Campbell, 1996). Apart from FSH and LH, the evolutionarily young primate-specific CG is synthesised in placenta (Table 1). Although CG and LH bind to the same receptor LH/CGR, their properties are different as CG is more stable (hormone half-life for LH <1 h compared to ~24 h for HCG), the production of hormone is continuous and pregnancy-specific (Wehmann and Nisula, 1981; Moyle and Campbell, 1996). The main function of CG is to delay apoptosis of the corpus luteum gravidium, prepare endometrium for the implantation of the fetus in early pregnancy and promote fetal testicular testosterone production.

FSH, LH and CG are all hetero-dimeric proteins that share a common alpha-subunit, but have a hormone-specific beta-subunit that mediates binding to the respective receptors (Morgan et al., 1975; Moyle et al., 1975; Rathnam and Saxena, 1975). The unique CGA gene coding for the alpha-subunit (116 aa) is located at chromosome 6q12–q21. It has a conservative nature in forming a functional hormone with all beta-subunits of gonadotropins as well as thyroid-stimulating hormone, and also contributes to the binding of the three receptors (FSHR, LHR, TSHR) (Pierce et al., 1971). So far no human patients have been described carrying CGA variants, although four non-synonymous changes have been predicted to exon 2 (dbSNP, build 131; http://www.ncbi.nlm.nih.gov/projects/SNP/). The genes coding for FSH, LH and CG beta-subunits have a common evolutionary ancestry and belong to gonadotropin hormone beta-subunit gene family (GtHB). Loci for FSH beta (denoted FSHB in primates, Fshb in mammals and GTH-I in fishes) and LH beta (denoted LHB in primates, Lhb in mammals and GTH-II in fishes) are conserved among vertebrates and functional genes have been cloned in fishes, amphibians, reptiles, birds and mammals (Gharib et al., 1989; Guzman et al., 1991; Kumar et al., 1995; Rosenfeld et al., 2001; Shen and Yu, 2002; Kawasaki et al., 2003; Watanabe et al., 2007). The beta-subunit of the derived CG gene compared to the ancestral LHB (Fiddes and Goodman, 1990; Talmadge et al., 1984). In Old World monkey and great ape lineages additional gene duplication events have occurred within the LHB/CGB gene cluster, further diversifying this genomic region among species (Maston and Ruvolo, 2002; Fortna et al., 2004; Dumas et al., 2007).

2. Comparative genomic context of gonadotropin beta genes

The evolution of single-copy FSHB and multi-copy LHB/CGB loci represent contrasting scenarios of functionally related GtHB gene family members, which are physically separated in the genome. The evolution, divergence and diversity patterns of the GtHB genes have been determined by the sequence properties and dynamics of the respective genomic regions.

2.1. FSHB genomic region

The beta-subunit of the human FSH (111 aa mature protein) is coded by the FSHB gene (4262 bp) at chromosome 11p13. FSHB is a single-copy gene located in a G/C-nucleotide deficient and gene-poor genomic region. The G/C content in FSHB exonic regions is 43–52% and 30–33% in introns. Only one more gene (C11orf46; ~100 kb downstream from FSHB) has been mapped within the flanking ±100 kb region (Fig. 1). The closest gene upstream of FSHB, KCNA4, is located ~200 kb away. Interestingly, another member of the same gene family, KCNA7, is located 11.7 kb downstream of the LHB/CGB gene cluster at chromosome 19 (Fig. 1). This is consistent with earlier studies showing the common origin of the gonadotropin beta genes (Li and Ford, 1998; Querat et al., 2000; Querat et al., 2001; Querat et al., 2004). The repeat-content of the flanking region of the human FSH gene (~44%) falls within the average range estimated for the human genome (40–50%) (Lander et al., 2001). The FSHB gene and its immediate flanking regions (~±100 kb) are characterized by low genome dynamics and high structural conservation since it has preserved very similar features not only in primates but also among mammals (Fig. 1).

2.2. LHB/CGB genomic region

2.2.1. LHB/CGB genes in human

The human genes coding for the beta-subunits of LH (121 aa mature protein) and HCG (145 aa mature protein) are located in tandem in a shared genomic region (45,165 bp) at 19q13.32 (Fig. 1; Table 1) (Policastro et al., 1986). The LHB/CGB gene cluster consists of one LHB gene (1111 bp), four HCG beta coding genes (CGB, CGB5, CGB8 and CGB7; 1467 bp) and two gene copies with unknown function (CGB1 and CGB2; 1366 bp) (Policastro et al., 1983, 1986; Fiddes and Talmadge, 1984). In contrast to the gene-poor genomic context of the FSHB locus, the LHB/CGB region is flanked by several genes. In total, 18 annotated genes are mapped within the ±100 kb proximate region of this gene cluster (Fig. 1). The LHB/CGB region has high G/C-nucleotide content (57% compared to 41% for the human genome average) and is rich in Alu-repeats (23.2% compared to 13.1%) (Lander et al., 2001; Hallast...
Table 1

| Characteristic features of gonadotropin specific beta-subunits. | FSH | LH | HCG |
|---------------------------------------------------------------|-----|----|-----|
| **Protein**                                                   |     |    |     |
| Mature beta-subunit protein                                   | 111 | 121| 145 |
| Time of production                                           | Postnatal | Postnatal | Prenatal |
| Production pattern                                           | Pulsatile | Pulsatile | Continuous |
| Biologic half-life                                           | 3–4 h | 20–30 min | ∼24 h |
| Main function                                                 | Stimulates and regulates spermatogenesis in men and follicular maturation in women | Stimulates steroidogenesis in testicular Leydig cells in men and promotes ovulation in ovarian luteal cells in women | Promotes implantation and placentation, and production of progesterone during pregnancy, stimulates sexual differentiation of the male fetus |
| Receptor                                                      | FSHR | LH/CGR | LH/CGR |
| Specific beta-subunit coding gene                             | FSHB | LHB | CGB, CGB5, CGB8, CGB7 |
| Chromosomal localisation                                      | 11p13 | 19q13.32 | 19q13.32 |
| Gene length                                                   | 4262 bp | 1111 bp | 1467 bp |
| Major site of expression                                     | Anterior lobe of pituitary gland | Anterior lobe of pituitary gland | Syncytiotrophoblastic cells in placenta |
| Alternative splice forms                                      | Yes | No | No |

et al., 2005, 2008). Alu-repeats represent primate-specific repeat elements, which have extensively spread by ‘copy-paste’ mechanism during the past 35–40 million years. These elements may trigger various genomic rearrangements and have been associated with recent abundant duplication events in primate genomes (Bailey et al., 2003).

The structure and sequence content of the human LHB/CGB gene cluster represents a typical young genomic region evolved by duplication events. There is a high DNA identity between the genes (85–99%) as well as between the inter-genic regions (81–97%) (Hallast et al., 2005). The amino acid sequence identity between the HCG beta-subunit is 98–100% and to LH beta 85% (Bo and Boime, 1992; Hollenberg et al., 1994). The DNA sequence identity between the HCG beta-subunit non-coding genes CGB1 and CGB2 and the four HCG beta genes is 85% (Supplementary Figure S1). However, the 132 aa protein predicted for CGB1 and CGB2 has no similarity to HCG beta-subunit or to any other known protein due to an alternative open reading frame (Supplementary Figure S2) (Bo and Boime, 1992; Hollenberg et al., 1994; Dirnhofer et al., 1996). Additionally, CGB1 and CGB2 have been shown to undergo extensive alternative splicing that potentially encode distinct protein isoforms (Rull et al., 2008a,b; Bo and Boime, 1992).

2.2.2. LHB/CGB genes in primates

Functionally divergent CGB probably emerged from a duplicate LHB gene copy in the common ancestor of anthropoid primates 55–35 million years ago and it demonstrates an excellent example of the power of evolution by gene duplication (Ohno, 1970). After the initial gene duplication event, the evolution of the LHB/CGB gene cluster in New World monkeys (Platyrrhini) compared to Old

![Fig. 1. Schematic representation of the genomic context of the FSHB and the LHB/CGB genes (±100 kb). The figure was drawn based on Ensembl database (http://www.ensembl.org/; Release 54). Boxes denote the genes and triangles above or below them point to the direction of transcription. The black boxes and arrowheads indicate CGB, white LHB and grey neighbouring genes. The CGB genes of rhesus macaque (Macaca mulatta) and common marmoset (Callithrix jacchus) are indicated as CGB A–C, since their ancestral status relative to the human CGB genes is unknown (reviewed in Henke and Gromoll, 2008; Hallast and Laan, 2009).](http://www.ensembl.org/)
World monkeys, apes and humans (Catarrhini) have followed different scenarios (reviewed in detail in Henke and Gromoll, 2008; Hallast and Laan, 2009). All the New World monkey species studied so far (e.g. common marmoset Callithrix jacchus) harbour one pseudogenized LHB gene and one CGB gene coding for dual functions of LH beta and CG beta (Fig. 1) (Simula et al., 1995; Maston and Ruvolo, 2002; Scammell et al., 2008). In Old World monkeys and apes additional duplication events have lead to variable numbers of gene copies among the species. The number of mapped CGB genes ranges from three in rhesus macaque (Macaca mulatta) to six...
Table 2

| Reference                                      | Nucleotide substitution | Amino acid substitution | Male phenotype | Female phenotype | Bioactivity/effect at protein level |
|------------------------------------------------|-------------------------|-------------------------|----------------|-----------------|------------------------------------|
| Matthews et al. (1993), Layman et al. (1997)   | fsbH: 818A>G, exon 3    | Gln54Arg                | Absent          | Absence of spontaneous puberty             | Abnormal sperm production          |
| Layman et al. (1997), Phillip et al. (1998)    | fsbH: 545G>C, intron 2  | Absent                  | Low FSH         | Impaired Leydig cells                        | Low reteintion level               |
| Lofrano-Porto et al. (2007)                    | fsbH: 265C>T, exon 3    | Glu89Lys                | Absent          | Absent, abnormal splicing of mRNA            | Absent binding to receptor         |
| Kottler et al. (2010)                          | fsbH: 535G>A, intron 2  | Val112Ile               | Absent          | Absent, abnormal splicing of mRNA            | Absent binding to receptor         |
| Berger et al. (2005)                           | fsbH: 475C>T, intron 2  | Val159Ile               | Absent          | Absent, abnormal splicing of mRNA            | Absent binding to receptor         |
| Valdes-Socin et al. (2004)                     | fsbH: 329 (2010)        | Val54Lys                | Absent          | Absent, abnormal splicing of mRNA            | Absent binding to receptor         |

a Nucleotide positions are defined relative to the transcription start site on the genomic DNA sequence; GenBank references: NC_000011.8 for FSHB, NC_000009.19 for LHB, and NC_000017.1 for CGB7 (Supplementary Figs. S1).

b Amino acid positions are defined based on the sequence of a mature protein.

c Consanguineous siblings.

d Intra- and interspecies diversification of a genomic segment is influenced by various forces such as selective pressures, mutation rate and DNA sequence rearrangements, but also by demographic history of the species (is not addressed in this review).

3. Forces affecting diversity and evolution of gonadotropin beta genes

FSHB has been a subject to evolutionary constraints due to its unique function essential for mammalian reproduction (Wallis, 2001). The FSHB gene is characterized by low genetic variation and excess of polymorphisms with intermediate allele frequencies (Lamminen et al., 2005; Grigorova et al., 2007). Resequencing study in three human populations (European Estonians, Chinese Han and African Mandenkalu) identified a density of polymorphisms of 3 SNPs/1 kb (Fig. 2) (Grigorova et al., 2007). Majority of these were common polymorphisms located in non-coding regions and were shared by three human populations (Fig. 3).

In contrast to FSHB, the LHB/CGB genes are among the top diverse genes in the human genome (Hallast et al., 2005). The highest density of polymorphisms was detected within the genes located at the edges of the gene cluster (12.6 SNPs/kb for LHB, 14.6 SNPs/kb for CGB and 15.6 SNPs/kb for CGB7) (Figs. 1–3). For the central genes CGB5 (8.1 SNPs/kb) and CGB8 (7.5 SNPs/kb) the density of polymorphic positions was lower, but still more than twofold higher as detected for the FSHB. Compared to FSHB, the LHB/CGB genes harbour a notably higher fraction of population-specific variants. For all LHB/CGB genes the most variable region was intron 1, whereas the polymorphisms in exons have been less tolerated due to their apparent functional consequences (Fig. 3; Table 2). The variation of two HCG beta non-coding genes, CGB2 (9.8 SNPs/kb) and CGB1 (6.7 SNPs/kb), is not as high as reported for the rest of the gene copies. On the one hand, this could be explained by relatively shorter period for accumulation of variants due to their evolutionary younger age compared to LHB and HCG beta genes (Hallast et al., 2007). On the other hand, CGB1 and CGB2 are located in the central part of the LHB/CGB region, which has been shown to be less active as a gene conversion acceptor and thus mostly unaffected by its diversifying effect (see following section).
3.2. Role of gene conversion

Gene conversion is a mechanism often found in duplicated genomic regions whereby DNA sequence information is transferred between a pair of highly identical sequences (Fig. 4A). On the one hand, it preserves the sequence similarity between duplicated gene copies of multigene families leading to concerted evolution of duplicons (Walsh, 1985; Ohta, 1991). On the other hand, it also spreads polymorphisms between homologous genes (Ohta, 1991; Huber et al., 1993; reviewed in Chen et al., 2007). Thus, when a polymorphism occurs in one gene copy, it can be transferred to another duplicated locus.

The high sequence similarity among the genes (up to 99%) as well as inter-genic regions (up to 97%) of the human LHB/CGB gene cluster provides an ideal surface for the gene conversion activity. Due to the homogenizing effect of gene conversion events, the intra-specific LHB/CGB genes have become more closely related to each other than to their counterparts in closely related species (Maston and Ruvolo, 2002; Hallast and Laan, 2005). The observed transferred sequence tracts between the LHB/CGB genes have been determined to range from a few bp to maximum 796 bp (Hallast et al., 2005). The entire gene cluster revealed the tendency of directional gene conversion: the donor loci are located in the centre and the acceptor genes at the edges of the cluster (CGB and CGB7). Consistently, the highest density of polymorphisms is mapped for the most active gene conversion acceptors, the CGB and CGB7 genes (Hallast et al., 2005). However, in addition to increasing diversity of entire LHB/CGB gene cluster, gene conversion could also be the source of mutations affecting the function of the acceptor gene (reviewed in Chen et al., 2007). The process may have a functional consequence if a DNA segment is transferred from HCG beta genes to the unique LHB gene or from more divergent CGB1-like genes to the protein-coding genes in the cluster (see examples in Section 4).

3.3. Role of selective pressures

3.3.1. FSHB gene

The major factor shaping the genomic context of FSHB is a stringent selective pressure. No mutations, which severely affect the function of this locus, are passed through to the next generation as the carriers fail to reproduce an offspring. A closer look at the diversity patterns across the FSHB gene reveals a strong allelic association between all common polymorphisms that form two major gene variants called haplotypes (Grigorova et al., 2007). A haplotype is defined as a combination of allelic variants of sequential polymorphisms, which are often inherited together. The two major FSHB haplotypes spread worldwide (58% and 26% of studied individuals, respectively) could be denoted as “yin-yang” gene variants since they have a contrasting composition of alleles in all common polymorphic positions (Fig. 4B). Minority of the individuals (in total 16% of subjects in three studied populations) carry rare haplotypes with distinguishing mutated positions (Fig. 5), thus the two major FSHB gene variants must have had a selective advantage in humans contributing to the reproductive success. Functional effects and biological consequence of the two major FSHB haplotypes are still to be addressed.

3.3.2. LHB/CGB genes

Compared to FSHB, the LHB/CGB genes are evolving by a more complex scenario. The maintenance of duplicated, highly homologous LHB and CGB genes must occur through the balance between inter-locus gene conversion activity and selection targeted to the specific functions of the loci. The unique functions of LH beta subunit are guaranteed mainly by the preservation of LHB-specific promoter and nucleotide positions that mediate the LH beta specificity (Henke and Gromoll, 2008). As the gene and its function are conserved in the entire vertebrate lineage, the LHB gene in Old world monkeys and apes must evolve under constant competition between gene conversion events and strong selective pressures (Hallast et al., 2008). In human and chimpanzee, there is evidence for Darwinian selection acting on LHB and the major HCG beta coding genes, CGB5 and CGB8 (Hallast et al., 2008). These genes provide 18–39% and 27–64% of the total HCG beta transcript pool, respectively (Bo and Boime, 1992; Miller-Lindholm et al., 1997; Ruil and Laan, 2005). Still, as HCG beta coding genes are represented in the human genome by four copies, the selective pressure acting on these genes is more relaxed and facilitates accumulation of polymorphisms either by de novo substitutions or gene conversion events. In three populations representing Africa, Asia and Europe, the CGB5 and LHB genes comprised of one (61% of individuals) or two (67% of individuals) major haplotypes, respectively (Fig. 5). The two major haplotypes in LHB differ from each other in seven out of 16 identified polymorphic positions. In contrast, FSHB has two completely different major haplotypes with no shared alleles. For all HCG beta genes 30–61% of studied individuals were carrying various minor gene variants.

![Fig. 4. Schematic description of gene conversion and definition of haplotype variants. (A) During gene conversion the genetic material is transferred between highly identical gene copies or alleles. One locus acts as a donor of a gene conversion tract and the other as an acceptor. In case the two gene copies differ in some sequence positions within the converted tract, the variant specific to the acceptor is replaced by copying the donor-specific variant. The donor DNA sequence remains unchanged. In the figure, the donor locus is displayed as a black line with circles indicating the gene-specific positions. The acceptor locus is drawn as a grey dashed line and the gene-specific positions as triangles. (B) Definition of gene haplotypes using the gene-specific positions. The acceptor locus is drawn as a grey dashed line and the other as an acceptor. In case the two gene copies differ in some sequence positions within the converted tract, the variant specific to the acceptor is replaced by copying the donor-specific variant. The donor DNA sequence remains unchanged.](http://www.ncbi.nlm.nih.gov/).
4. Genetic variation of human gonadotropin beta genes

A few mutations that affect hormonal function and metabolism have been characterised in the single-copy LHB and FSHB genes. As an analysis of the multi-copy CGB genes is technically challenging and the phenotypic effect of mutations is probably weaker due to redundancy, the mutational spectrum of these genes is not as extensively studied. In addition to mutations, a list of polymorphisms has been found to be associated with specific phenotypes.

4.1. Gonadotropin beta gene mutations with known functional effects

In total five mutations in FSHB have been reported in three male and six female patients, all having a severely impaired sexual development and infertility (Table 2; Fig. 3; Figure S1). Three mutations, Val61Δ2 bp/87X, Tyr76ΔX and Ala79Δ1 bp/Δ108X in exon 3 lead to a premature stop codon and truncation of the FSH beta protein (Matthews et al., 1993; Layman et al., 1997; Phillip et al., 1998; Kottler et al., 2010). Two other identified mutations in exon 3, Cys51Gly and Cys82Arg, alter a cystein knot structure of the FSH beta peptide. The cystein knot is crucial for hormone dimerization and bioactivity (Layman et al., 1997; Lindstedt et al., 1998).

Three mutations have been described in the LHB gene: Gly36Asp, Gln54Arg and a substitution G to C at position 545 from transcript start site has originally been derived from a male patient with azoospermia and isolated FSH deficiency (Mantovani et al., 2003). This SNP is located within a progesterone response element (PRE) conserved among numerous placental mammals and capable of enhancing the gene transcription up to 9-fold (Webster et al., 1995). A cohort of young European men (n = 554) showed an association between the minor allele T carrier status and reduced serum FSH level (Grigorova et al., 2008). Compared to the wild-type homozygotes (GG), the heterozygotes (GT) and the homozygotes (TT) for the minor allele had on average 15.7% and 40% lower levels of FSH in their bloodstream, respectively. The association with lower male serum FSH level was further confirmed in a cohort of men diagnosed with infertility (n = 1029) (Grigorova et al., 2010). Moreover, the minor allele of this polymorphism was shown to be overrepresented among infertility patients. FSHB –211G/T is the first described polymorphism shown to significantly affect the male serum FSH levels.

4.2. Polymorphisms associated with phenotypic traits

4.2.1. FSHB gene

Among the three common synonymous changes described in the coding region of FSHB, the 2623T>C (Tyr58Tyr) in exon 3 has been demonstrated to be more frequent among obese patients with polycystic ovary syndrome (PCOS) than in healthy females (Table 3; Fig. 3; Figure S1) (Tong et al., 2000). A substitution G>T located in FSHB promoter region at position −211 from mRNA transcription start site has originally been described in a male patient with azoosperma and isolated FSH deficiency (Mantovani et al., 2003). This SNP is located within a progesterone response element (PRE) conserved among numerous placental mammals and capable of enhancing the gene transcription up to 9-fold (Webster et al., 1995). A cohort of young European men (n = 554) showed an association between the minor allele T carrier status and reduced serum FSH level (Grigorova et al., 2008).

4.2.2. LHB gene

The most studied variation in LHB gene is a combination of two amino acid changes that are always found together as a haplotype on one chromosome: Trp8Arg/Ile15Thr (Table 3; Fig. 3; Figure S1) (Furui et al., 1994; Okuda et al., 1994). Trp8Arg substitution is mainly responsible for an altered immunoreactivity of the hormone and Ile15Thr substitution introduces an extra glycosylation site into the altered LH beta peptide. The carrier frequency of the variant allele (V-LH beta) differs widely between ethnic groups (0–50%) (Nilsson et al., 1997; Lamminen and Huhtaniemi, 2001). Compared to normal LH, the hormone formed by the V-LH beta possesses an increased in vitro biopotency and altered half-life in circulation, although the published data on the length of the half-life is contradictory (Manna et al., 2002; Wide et al., 2010). There are numerous published studies addressing the carrier status of the V-LH beta variant in relation to various clinical conditions (Table 3). The variant LH form has been suggested to suppress gonadal function and to be involved in the development of subfertility (Furui et
Table 3
Variants in the human gonadotropin beta-subunit genes associated with phenotypic traits.

| Nucleotide substitution\(^a\) | Amino acid change\(^b\) | Assessed phenotype | Number of carriers/total number of studied individuals | Association | Reference |
|-------------------------------|------------------------|--------------------|----------------------------------------------------|-------------|----------|
| **Variants in FSHB**          |                        |                    |                                                    |             |          |
| \(-211G>T\) upstream         |                        |                    |                                                    |             | Mantovani et al. (2003) |
|                               |                        |                    |                                                    |             |          |
| **2623T>C exon 3**            | Tyr58Tyr               | Polycystic ovary syndrome (PCOS) | 283/1029 male partners of infertile couples | Yes – minor allele carriers had lower FSH level | Grigorova et al. (2010) |
|                               |                        |                    | 89/135 female patients                             |             |          |
|                               |                        |                    | 54/105 normal control females                      |             |          |
| **Variants in LHB**           |                        |                    |                                                    |             |          |
| **443T>C/465T>C exon 2**      | Trp8Arg/Ile15Thr (v-LH) | PCOS               | 32/153 female patients                             | No          | Rajkhowa et al. (1995) |
|                               |                        |                    | 31/212 healthy females                              | No          | Elter et al. (1999) |
|                               |                        |                    | 5/30 healthy females                                | No          | Tapanainen et al. (1999) |
|                               |                        |                    | 46/328 female patients                             | No          |          |
|                               |                        |                    | 169/881 control females                            | No          |          |
|                               |                        |                    |                                                    |             |          |
| **Variants in CGB5**          |                        |                    |                                                    |             |          |
| \(-155G>C/\sim-147G>del/\)   | Gly102Ser              | Infertility, endometriosis, PCOS | 29/200 fertile males                              | No          | Liao et al. (1998) |
| \(-144T>C/\sim-142T>A\)      |                        |                    | 2/52 female patients                               | No          | Lee et al. (2000) |
| \(1038C>T\) intron 2         |                        | RM                 | 26–27/184 RM couples                               | Yes         | Rull et al. (2008b) |
|                               |                        |                    | 45–48/195 fertile females                           | Yes         | Rull et al. (2008b) |
|                               |                        |                    | 30/184 RM couples                                  |             |          |
|                               |                        |                    |                                                    |             |          |
| **Variants in CGB8**          | Arg8Trp                | RM                 | 1/184 RM couples                                   | na          | Rull et al. (2008b) |
| \(806C>T\) exon 2            |                        |                    |                                                    |             |          |
|                               | Pro73Arg               | RM                 | 1/184 RM couples                                   | na          | Rull et al. (2008b) |

\(^a\) Nucleotide positions are defined according to the location of the transcription start site in the genomic DNA sequence; GenBank references: NC_000011.8 for FSHB, NC_000019.8 for LHB/CGB/CGB5/CGB8/CGB7 (Supplementary material, Figure S1).

\(^b\) Amino acid positions are defined based on the sequence of a mature protein.

\(^c\) Originally described as 1502G>A; na – statistical tests were not applicable.
al., 1994; Haavisto et al., 1995; Lamminen and Huhtaniemi, 2001). The V-LH beta variant could have originated by an ancient gene conversion event where only one of the CGB genes has acted as a donor and LHB as an acceptor (see Section 3.2; Hallast et al., 2005). The concerted substitutions (443T>C and 465T>C) in V-LH exon 2 leading to Trp8Arg/Ile15Thr changes correspond to the nucleotide sequence of CGB8 (Rull et al., 2008b). Similarly, patients as possible variants increasing the risk of recurrent miscarriage (Rull et al., 2008b). A significant protective effect toward recurrent miscarriage was associated with two variants (−144T>C/−147G>del/−149G>del/−155G>C/−154C>T) and with four CGB promoter variants (−155G>C/−147G>del/−144T>C/−142T>A) (Table 3) (Rull et al., 2008b). These variants reduced the risk of recurrent miscarriage up to 1.8-fold. The haplotype-forming alternative signal transduction pathway apparently derives from the CGB8 promoter sequence as a donor.

4.2.3. CGB genes

A resequencing study of the CGB5 and CGB8 genes detected three rare non-synonymous substitutions (Val56Leu in CGB5 and Arg83Trp, Pro73Arg in CGB8) only among recurrent miscarriage patients as possible variants increasing the risk of recurrent pregnancy loss (Table 3; Fig. 3; Figure S1) (Rull et al., 2008b). Similarly, a rare T>A mutation in CGB8 located adjacent to an initiator element for HCG beta transcription (4 bp before transcription start site) was identified as a potential risk variant in developing a recurrent miscarriage (Rull et al., 2008b). A significant protective effect toward recurrent miscarriage was associated with two SNPs located at identical positions in intron 2 in both, CGB5 (1038C>T) and CGB8 (1045C>T), and with four CGB5 promoter variants (−155G>C/−147G>del/−144T>C/−142T>A) (Table 3) (Rull et al., 2008b). These variants reduced the risk of recurrent miscarriages up to 1.8-fold. The haplotype-forming alternative CGB5 promoter variant may have arisen by the transfer of a gene conversion tract derived from the CGB8 promoter sequence as a donor.

4.3. Non-synonymous amino acid substitutions with unknown phenotypic consequence

Several additional variants have been reported in the human FSHB, LHB and HCG beta coding genes that cause a non-synonymous substitution, but the data about the phenotypic consequences in the study participants is unavailable (Table 4; Figure S1) (Jiang et al., 2002; Hallast et al., 2005). The Ala-3Thr change in the signal peptide of LH beta has been shown to cause different in vitro signal transduction properties compared to a wild-type preprotein presumably due to altered conformation of secreted LH (Jiang et al., 2002). The alternative signal transduction pathway apparently prevents desensitisation of LH/HCG receptor and therefore supports the continuous function of corpus luteum during gestation ( Cameo et al., 2004). The polymorphism leading to Val79Met substitution in the exon 3 of CGB5 was shown in in vitro experiments to code for the altered protein unable to fold correctly and assemble with alpha-subunit (Miller-Lindholm et al., 1997). Individuals carrying this polymorphism could possibly have a subtle deficiency of bioactive HCG but its association with an impaired reproductive outcome has not been established.

Table 4

| Gene | Position relative to mRNAa | Position relative to ATGb | Amino acid changec | Reference |
|------|--------------------------|--------------------------|-------------------|-----------|
| FSHB | 946G>T                    | 950Arg                   | Ser2Ile           | Cargill et al. (1999) |
| LHB  | 406G>A                    | 409Thr                   | Met-6Ile          | Hallast et al. (2005) |
|      | 413G>A                    | 404Thr                   | Ala-3Thr          | Jiang et al. (2002) |
|      | 450A>G                    | 441Thr                   | His10Arg          | Hallast et al. (2005) |
| CGB  | 1363A>C                   | 1367Asp                  | Asp117Ala         | Hallast et al. (2005) |
| CGB5 | 794G>A                    | 798Arg                   | Arg6Gln           | Hallast et al. (2005) |
|      | 1247G>A                   | 1251Val                  | Val79Met          | Miller-Lindholm et al. (1997) |
| CGB8 | 869G>A                    | 873Asp                   | Asp299Le          | Rull et al. (2008b) |
| CGB7 | 782G>A                    | 786Arg                   | Arg2Lys           | Hallast et al. (2005) |
|      | 787/788AT>CC              | 792Met                   | Met4Pro           | |
|      | 1162G>A                   | 1166Asp                  | Ala51Thr          | |
|      | 1232C>T                   | 1238Arg                  | Arg74Cys          | |
|      | 1363C>A                   | 1367Asp                  | Ala117Lys         | |

a Nucleotide positions are defined according to the location of the transcription start site in the genomic DNA sequence; GenBank references: NC_000011.8 for FSHB, NC_000019.8 for LHB/CGB/CGB5/CGB7 (Supplementary material, Figure S1).

b Translation start site as in original reports; GenBank references: NC_000011.8 for FSHB, NC_000019.8 for LHB/CGB/CGB5/CGB7 (Supplementary material, Figure S1).

c Amino acid positions are defined based on the sequence of a mature protein.

5. Comparative expression profile of human gonadotropin beta genes

Pituitary FSH and LH are produced in a pulsatile manner with differential frequencies and amplitude regulated by gonadotropin releasing hormone (GnRH) (reviewed in Marshall et al., 1993; Miller et al., 2002). On the other hand, CGB genes are expressed and HCG produced continuously in placenta over two alternative signal transduction pathways in order to avoid receptor desensitisation (Cameo et al., 2004). Rate-limiting for the respective hormone production is the transcription of FSHB, LHB and HCG beta coding genes. Thus, the genetic and genomic context of the FSHB, LHB and HCG beta genes affects the profile of the hormone production.

For the FSHB gene at least four alternative transcripts have been described that encode an identical unmodified protein. Different transcripts arise due to combinations of alternate splicing of the first non-coding exon and at least two potential polyadenylation signals (Jameson et al., 1988). In case of LHB and HCG beta coding genes, no splice variants have been identified. Although alternative transcripts have been reported for the HCG beta non-coding genes CGB1 and CGB2, their functional effect is yet to be determined (Bo and Boime, 1992; Berger et al., 1994; Rull and Laan, 2005).

5.1. Profile of FSHB and LHB gene expression

Pituitary LH and FSH have been preserved during evolution as functionally critical hormones that guarantee successful reproduction and survival of a species. Thus, large-scale fluctuations in the transcriptional activity of the FSHB and the LHB genes might be fatal for the reproductive success. An optimal concentration of LHB and FSHB transcripts and the coded protein is kept strictly in narrow ranges in men as well as in women during follicular...
and luteal phases, but undergoes a rise during ovulation and post-menopausal period (Fig. 6A) (Owen, 1975; Sherman and Korenman, 1975; Zumoff et al., 1982; Baird, 1983). Overexpression of FSHβ could cause polycystic ovary syndrome in female FSH overexpressing mice, whereas severe reduction in FSHβ transcription and FSH production could cause infertility in females or poor sperm quality in males (Kumar et al., 1997, 1999; Plant and Marshall, 1995; Lin and Ge, 2009). The critical role of the FSHβ transcription in determining FSH levels was also confirmed by mapping of a polymorphism in a regulatory region −211 bp upstream of FSHβ. The alternative allele of this polymorphism reduces the transcription and FSHβ expression probably reflect high genetic variation in the genes (Huhtaniemi and Themmen, 2000).

Serum FSH levels depend on the selective regulation of FSHβ mRNA transcription by the action of pituitary gonadal factors such as activin and inhibin, while the expression of glycoprotein alpha-subunit (CGA) mRNA remains unaffected (Carroll et al., 1989; Farnworth, 1995; Lin and Ge, 2009). The critical role of the FSHβ transcription in determining FSH levels was also confirmed by mapping of a polymorphism in a regulatory region −211 bp upstream of FSHβ. The alternative allele of this polymorphism reduces the FSHβ transcription by 46–58% and leads to lower FSH production in men (Jiang et al., 1999).

Although wide ranges in HCG beta transcriptional activity are tolerated during the pregnancy, biparental expression of CGβ genes is required in order to provide a necessary amount of HCG beta-subunits and avoid complications in early pregnancy (Uusküla et al., unpublished data). Too low mRNA expression could lead to miscarriage and excessively high amount of HCG beta transcripts could be a marker for ectopic or molar pregnancy (Rull and Laan, 2005; Rull et al., 2008a).

5.3. Tissues of minor expression of gonadotropin beta genes

In addition to major sites of expression – pituitary and placenta – gonadotropin hormone production in minor concentrations has been reported in other tissues. An ectopic production of pituitary gonadotropins LH and FSH in normal non-malignant tissues is limited; minimal amount of LHβ transcripts have been detected in testis and placenta (Berger et al., 1994; Giovangrandi et al., 2001). Both hormones have been detected in tumours that arise from gonadotroph cells of the pituitary gland. Gonadotroph adenomas account for approximately 40% of all clinically recognized macroadenomas and approximately 80% of surgically excised clinically non-functioning adenomas (Valimaki et al., 1999). In these adenomas the glycoprotein hormone beta-subunits may be detected by molecular, immunological or immunohistochemical staining. The clinical manifestation is delayed due to inefficient secretion of the hormone and its subunits (Jaffe, 2006; Valimaki et al., 1999).

HCG β genes have been reported to be expressed in minimal amount in normal non-trophoblastic tissues, mostly in testis, prostate, thymus, skeletal muscle and pituitary gland (Bellet et al., 1997; Reimer et al., 2000). The role of HCG in normal tissues may be related to autoregulatory mechanisms, beta-subunit of the hormone has been shown to exert growth-promoting activity (Butler and Iles, 2004). HCG probably acts in an auto-/paracrine way, a transcrine pathway has been suggested for the testis, prostate and uterus (Berger et al., 2007).

Several non-trophoblastic tumors, like bladder, renal, prostate, lung, gastrointestinal, neuroendocrine, breast and gynecological cancers, have been shown to produce HCG beta-subunit and to a lesser extent intact HCG (Stenman et al., 2004). The role of HCG...
in the carcinogenesis could be associated with enhancement of invasion, angiogenesis, inhibition of apoptosis and escape from immune surveillance (Butler and Iles, 2004; Herr et al., 2007; Reisinger et al., 2007). The same biological means are used by trophoblasts to ensure successful implantation and placentation. At the genomic level, activation of transcription of CGB, CGBS and CGB8 genes has been associated with malignant transformation of non-trophoblastic cells (breast, bladder, prostate, thyroid, testis and renal cancer) (Bellet et al., 1997; Giovangrandi et al., 2001; Hotakainen et al., 2007).

6. Conclusive remarks

The gonadotropin beta-subunit genes are functionally related and have a common evolutionary ancestry. Nevertheless, the single-copy FSHB and the multi-copy LHB/CGB genes exhibit locus-specific differences in their genomic context and its evolution, genetic variation and expression profiles. The FSHB gene is conserved in vertebrates, maps to a gene-poor region and is evolving under strong selection due to its conserved function. Any non-synonymous change has an immediate phenotypic consequence impairing the reproductive potential of the carrier. In contrast, the primate-specific LHB/CGB gene cluster is located in a dynamic gene-rich region and its evolution is driven by the constant interplay between gene conversion and selective forces. Gene conversion is also the major force, which has raised the LHB/CGB genes among the most highly polymorphic genes in the human genome. Transcription of the pituitary FSHB and LHB genes is rate-limiting in the hormone production and kept in a narrow expression window. In contrast, placental specific differences in their genomic context and its evolution, activation of transcription of CGB8 genes in the CGB8 and CGB cluster is located in a dynamic gene-rich region and its evolution is driven by the constant interplay between gene conversion and selective forces. Gene conversion is also the major force, which has raised the LHB/CGB genes among the most highly polymorphic genes in the human genome. Transcription of the pituitary FSHB and LHB genes is rate-limiting in the hormone production and kept in a narrow expression window.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mce.2010.04.024.

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L. Nagirnaja et al. / Molecular and Cellular Endocrinology 329 (2010) 4–16
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