

## Human chorionic gonadotropin beta genes: expression, genetic variation and reproductive failure.

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**Background:** HCG is secreted by the syncytiotrophoblasts from early stages of pregnancy. Low level of hormone is related to miscarriage, ectopic pregnancy (EP) and failure of assisted reproduction procedures. Recurrent miscarriage (RM,  $\geq 3$  pregnancy losses) that affects 1-2% of fertile couples is a complex disease with a genetic contribution. Aberrations in the expression and genetic variation of the hCG genes may contribute to reproductive failure. Critical for hCG assembly and production rate is the beta-subunit of the hormone, which is coded by *CGB* genes. The four *hCG beta* genes share a common gene cluster with ancestral pituitary *LHB* and two beta-subunit non-coding *CGB* genes, *CGB1* and *CGB2*.

We present the data on (1) the expression profile of *CGB* genes during the normal and failed pregnancy as well in non-trophoblastic tissues; and (2) the genetic variants of the *hCG beta* genes associated with pregnancy success.

**Methodology:** (1) The expression of *CGB* genes in trophoblastic and non-trophoblastic tissues was assessed by real-time PCR. (2) Two most actively transcribed *hCG beta* genes – *CGB5* and *CGB8* were resequenced in Estonian and Finnish RM patients (n=184) and fertile women (n=195).

**Results:** We showed a wide transcriptional window of *CGB* genes in normal pregnancy, a significant reduction in RM, and a high expression in EP and molar pregnancies. As both RM and EP exhibit low hCG concentration in maternal serum, the result indicates that different mechanisms may dominate in the pathogenesis of RM and EP. Expression was several orders of magnitude lower in the non-placental tissues, with the highest *CGB* levels being seen in testis, prostate, thymus, skeletal muscle and lung samples.

We identified six SNPs associated with significant protective effect towards RM. The minor allele frequency of all these SNPs was 12.05%-14.36% in fertile compared to 7.10%-8.15% in RM group. The resequencing also revealed variants that potentially increase the risk for pregnancy loss. RM patients, but not fertile women, were carrying three rare amino acid changes and a rare SNP located in initiator element critical for transcription of *CGB8*.

**Conclusions:** RM is associated with low and EP with high transcriptional activity of all *CGB* genes. The promoter and intronic variants of *CGB5* and *CGB8* carrying the protective SNPs decrease the risk of RM ~1.7-fold and rare variants increase the risk for pregnancy loss.

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