University of Groningen

Interspecies comparison of gene structure and computational analysis of gene regulation of 17beta-hydroxy steroid dehydrogenase type 1

Keller, B.; Ohnesorg, T.; Mindnich, R.; Gloeckner, C.J.; Breitling, R.; Scharfe, M.; Moeller, G.; Blocker, H.; Adamski, J.; Blöcker, H.

Published in:
Molecular and Cellular Endocrinology

DOI:
[10.1016/j.mce.2005.10.014](https://doi.org/10.1016/j.mce.2005.10.014)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2006

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Keller, B., Ohnesorg, T., Mindnich, R., Gloeckner, C. J., Breitling, R., Scharfe, M., Moeller, G., Blocker, H., Adamski, J., & Blöcker, H. (2006). Interspecies comparison of gene structure and computational analysis of gene regulation of 17beta-hydroxy steroid dehydrogenase type 1. *Molecular and Cellular Endocrinology*, 248(1-2), 168 - 171. <https://doi.org/10.1016/j.mce.2005.10.014>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Interspecies comparison of gene structure and computational analysis of gene regulation of 17beta-hydroxysteroid dehydrogenase type 1

B. Keller^a, T. Ohnesorg^a, R. Mindnich^a, C.J. Gloeckner^b, R. Breitling^c,
M. Scharfe^d, G. Moeller^a, H. Blöcker^d, J. Adamski^{a,*}

^a GSF-National Research Center for Environment and Health, Institute of Experimental Genetics,
Genome Analysis Center, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany

^b GSF-National Research Center for Environment and Health, Institute of Human Genetics, Germany

^c University of Groningen, Groningen Bioinformatics Centre, 9751 NN Haren, The Netherlands

^d GBF-German Research Centre for Biotechnology, Mascheroder Weg 1, D-38124 Braunschweig, Germany

Abstract

17beta-Hydroxysteroid dehydrogenase type 1 (HSD17B1) is a key enzyme of 17beta-estradiol biosynthesis, and in rodents is additionally involved in testosterone biosynthesis. The human *HSD17B1* gene, located on chromosome 17q12-21, is duplicated in tandem, with the 3'-copy being the functional gene. Here we show by sequencing the gene from a diverse set of related species that this duplication is of very recent evolutionary origin, having occurred in the common ancestor of Hominoidea (apes and humans) while being absent in the closely related Old World monkeys (*Macaca*) and the outgroup species *Tupaia belangeri* and *Mus musculus*. By computational analysis of the conserved regulatory elements in the 5'-untranslated (5'-UTR) and putative promoter region of the *HSD17B1* gene and, where present, pseudogene, across our broad sample of species we can show significant differences that might point to the origin of the divergent substrate specificity of human and rodent HSD17B1 and highlight potential functionally relevant differences in regulatory patterns in different evolutionary lineages.

© 2005 Elsevier Ireland Ltd. All rights reserved.

Keywords: 17beta-Hydroxysteroid dehydrogenases; 17beta-Hydroxysteroid dehydrogenase type 1; *HSD17B1*; Regulation; Duplication

1. Introduction

17beta-Hydroxysteroid dehydrogenases are a large group of steroid hormone activating and inactivating enzymes. All but one of them belong to the short-chain dehydrogenase/reductase family (SDR family; Mindnich et al., 2004). 17beta-Hydroxysteroid dehydrogenase type 1 (HSD17B1) is a key enzyme of steroid hormone biosynthesis. The rodent enzyme can convert both androstenedione to testosterone and estrone to 17beta-estradiol, while in humans only the latter reaction is catalyzed (Peltoketo et al., 1999). Since the murine enzyme is expressed mainly in the ovaries there is an obvious relation to estradiol biosynthesis in vivo. Elevated estradiol levels due to over-active 17beta-HSD1 have been shown to support the development of breast cancer in humans (Oduwole et al., 2004).

The human *HSD17B1* gene, located on chromosome 17q12-21, is duplicated in tandem, with the 3'-copy being the functional gene. To elucidate the origin of this duplication, we determined the gene structure of the homologous genes in *Tupaia belangeri* (tree shrew), *Macaca mulatta* (rhesus macaque), *Hylobates klossi* (Kloss' gibbon), *Pongo pygmaeus* (orang-utan) and *Pan troglodytes* (chimpanzee).

Considering the known and diverse substrate specificity between human and rodent HSD17B1 we also analyzed regulatory elements in the 5'-untranslated region (5'-UTR) of the gene bioinformatically.

2. Material and methods

2.1. Identification of genomic *HSD17B1* clones

Genomic clones carrying the *HSD17B1* gene were identified from RZPD libraries of primate species and *Tupaia belangeri* by screening with radioactively labelled human HSD17B1-cDNA (labelling: RT labelling kit, Stratagene, LaJolla, USA; d*CTP: Amersham Biosciences) according to the distributor's instructions. RZPD library numbers and clone IDs are: *Tupaia belangeri* library number 161, clone MPMGc161H0137Q2; *Macaca mulatta* library number

* Corresponding author. Tel.: +49 89 3187 3155; fax: +49 89 3187 3225.
E-mail address: adamski@gsf.de (J. Adamski).

159, clones MPMGc159O1081Q2 and MPMGc159F6106Q2; *Hylobates klossi* library number 140, clones MPMGc140J2213Q2 and MPMGc140D9233Q2; *Pongo pygmaeus* library number 141, clone MPMGc252B526Q2; *Pan troglodytes* library number 733, clones RCPIB733D0666Q2 and RCPIB733-K23180Q2.

2.2. DNA isolation, sequencing and fragment assembly

BAC DNA was isolated by a manual procedure involving alkaline lysis, centrifugation, isopropanol precipitation and phenol/chloroform extraction. After shearing of the BAC DNA 1 and 5 kb fragments were cloned into pTZ18R. Plasmid DNA was routinely amplified using a TempliPhi kit (GE Healthcare, Freiburg, Germany) according to manufacturer's instructions. Sequencing of the isolated DNA was carried out according to the dideoxy sequencing method of Sanger et al. (1977) using the ET terminator technology according to the manufacturers instructions (GE Healthcare, Freiburg, Germany). Reads obtained for every BAC insert were assembled using GAP4 (<http://staden.sourceforge.net/>).

2.3. Annotation

Annotation of the *HSD17B1* genes was carried out by pairwise alignment of the human genomic *HSD17B1* sequence (GenBank accession number M84472) with the newly acquired sequence using Blast2seq. Exon–intron boundaries were annotated manually based on this alignment and the in-frame translation into protein. Sequences were submitted to GenBank and have the following accession numbers: *Tupaia belangeri* DQ026303, *Macaca mulatta* DQ023263, *Hylobates klossi* DQ023265, *Pongo pygmaeus* DQ023264 and *Pan troglodytes* DQ023266.

2.4. Promoter analysis

Conserved patterns of transcription factor binding sites were identified using the Genomatix suite of promoter analysis tools (MatInspector, FrameWorker; Genomatix software GmbH, Munich, Germany).

3. Results

3.1. Genomic Structure of *HSD17B1* genes

Genomic *HSD17B1* carrying clones of various species were sequenced and the received sequence annotated. *HSD17B1* gene structure was already known to be conserved between rodents and humans. As expected, we found the gene structure of the *HSD17B1* genes in all mammals highly conserved, consisting of six exons and five introns (Fig. 1). Exon sizes appear to be well conserved at least for exons 1–5; exon 6 varies slightly in size between the species. The situation is different for intron sizes with introns 3 and 4 varying significantly in length.

3.2. Origin of *HSD17B1* duplication

While the existence of a 5'-located pseudogene for human *HSD17B1* was described already some time ago (Luu-The et

Fig. 1. Comparison of the genomic structure of *HSD17B1* genes of indicated species. *HSD17BP1* pseudogene of *HSD17B1*, *NAGLU*: *N*-acetylglucosaminidase; primate genes are indicated in capital letters. Length of sequences is indicated in bps; (?) indicates an unknown number of bps.

al., 1990), according to the published genomic sequences, the rodent *HSD17B1* genes are not duplicated (GenBank accession numbers: AF363242 (mouse), NM.012851 (rat)). *HSD17BP1*, the 5'-located pseudogene of *HSD17B1*, arises first in gibbon and is conserved throughout Hominoidea, while it is absent in old world monkeys (*Macaca*) and the outgroup species *Tupaia* and *Mus*. Interestingly, the 3'-copy of the *HSD17B1* tandem duplication appears to be the functional copy since the putative coding sequence in all presumable pseudogenes is disrupted by in-frame stop codons in all primates examined (data not shown).

3.3. Bioinformatical promoter analysis

All examined primate *HSD17B1* promoters including the 5'-UTRs of the pseudogenes reveal a high degree of conservation. For the murine and primates genes, the transcription start site has not been shown experimentally. Therefore, we analyzed 1 kb upstream of the start codon and could find matching patterns of putative transcription factor binding sites (Fig. 2). Transcription factor binding sites previously not reported to be connected to *HSD17B1* regulation such as steroidogenic factor 1 (SF1), nerve growth factor-induced clone B (NGF1B) and estrogen related receptor (ERE) were predicted by Genomatix

software and point to a regulation in the context of steroidogenesis (for initial description of *HSD17B1* promoter, cf. Piao et al., 1997). However, mouse and *Tupaia* promoter regions display significant differences compared to the pattern of transcription factor binding sites in primate promoters. For example, a NKX 3.1 binding site is found at position –320 relative to the start codon in *Tupaia* and at position –500 in mouse. Besides, *Tupaia* also displays a well-conserved FXR/RXR site at position –460, which is not predicted in the other species examined.

4. Discussion

4.1. Genomic structure and duplication

The genomic structure of the *HSD17B1* gene is highly conserved between all species analyzed in this study. We were able to show that *Macaca mulatta* carries no *HSD17B1* tandem duplication while the gene is present in tandem duplication from *Hylobates klossi* to *Homo sapiens*. Therefore, we conclude that the duplication event happened early in the evolution of Hominoidea (apes and humans). Furthermore, the inactivation of the 5'-copy seems to have taken place soon after the duplication since all 5'-copies are inactivated. However, each of the

Fig. 2. Comparison of bioinformatically identified transcription factor binding sites in the 5'-UTR and putative promoter region of *HSD17B1* genes and pseudogenes (*HSD17BP1*) in indicated species. Previously described regulatory regions are boxed (Piao et al., 1997; Leivonen et al., 1999). Number of bases relative to start codon.

pseudogenes displays stop codons at different positions. This is probably due to the missing evolutionary pressure after inactivation. The physiological relevance of this duplication, though, remains unclear.

4.2. Promoter analysis

In a comparative examination of primate *HSD17B1* promoters we find a well-conserved pattern of transcription factor binding sites that strongly points to regulation in a steroidogenic context. This corresponds well to the known substrate specificity of the 17 β -hydroxysteroid dehydrogenase type 1. We could confirm the enhancer region described by Leivonen et al. (1999), but our comparative genomics data refine the reported pattern of transcription factor binding sites.

In addition, we find that the previously described retinoic acid responsiveness (Zhu et al., 2002) of the human *HSD17B1* promoter is unlikely to be due to a FXR/RXR binding site, since only the *Tupaia* promoter displays this binding site well conserved, while a matching motif is absent in all other species in the data set.

Within primates, including *Macaca*, the regulatory motifs are highly conserved. In contrast, murine and *Tupaia* promoters display a significantly different pattern of transcription factor binding sites. Since dual substrate specificity requires a correspondingly more elaborate regulation, and differences in the transcription factor binding motifs would be expected as the basis of this divergent regulation, we hypothesize that the loss of dual substrate specificity took place before primate evolution. Considering the expression pattern of the murine gene, mainly in the ovaries, the pattern of putative transcription factor binding sites could also be due to differences in regulatory processes in mouse compared to primates and not to differences in substrate specificity. More detailed examinations could help to light these problems. It is, however, interesting to note that the gene duplication event is preceded by the emergence of a new

highly conserved SF1/ERE regulatory element. It will therefore be interesting to examine the functional biology of *HSD17B1* in Old World monkeys in more detail.

Acknowledgements

This work was in part supported by a DFG grant to J.A. and a BMBF grant to H.B. We also thank Dr. Christian Roos, Genebank of Primates for his support.

References

- Leivonen, S., Piao, Y.S., Peltoketo, H., Numchaisrika, P., Vihko, R., Vihko, P., 1999. Identification of essential subelements in the hHSD17B1 enhancer: difference in function of the enhancer and that of the hHSD17BP1 is due to –480C and –486G. *Endocrinology* 140 (8), 3478–3487.
- Luu-The, V., Labrie, C., Simard, J., Lachance, Y., Zhao, H.F., Couet, J., Leblanc, G., Labrie, F., 1990. Structure of two in tandem human 17 β -hydroxysteroid dehydrogenase genes. *Mol. Endocrinol.* 4 (2), 268–275.
- Mindnich, R., Moeller, G., Adamski, J., 2004. The role of 17 β -hydroxysteroid dehydrogenases. *Mol. Cell. Endocrinol.* 218 (1–2), 7–20.
- Oduwale, O.O., Li, Y., Isomaa, V.V., Mantyniemi, A., Pulkka, A.E., Soini, Y., Vihko, P.T., 2004. 17 β -Hydroxysteroid dehydrogenase type 1 is an independent prognostic marker in breast cancer. *Cancer Res.* 64 (20), 7604–7609.
- Piao, Y.S., Peltoketo, H., Vihko, P., Vihko, R., 1997. The proximal promoter region of the gene encoding human 17 β -hydroxysteroid dehydrogenase type 1 contains GATA, AP-2, and Sp1 response elements: analysis of promoter function in choriocarcinoma cells. *Endocrinology* 138 (8), 3417–3425.
- Peltoketo, H., Nokelainen, P., Piao, Y.S., Vihko, R., Vihko, P., 1999. Two 17 β -hydroxysteroid dehydrogenases (17HSDs) of estradiol biosynthesis: 17HSD type 1 and type 7. *J. Steroid Biochem. Mol. Biol.* 69, 431–439.
- Sanger, F., Nicklen, S., Coulson, A.R., 1977. DNA sequencing with chain-terminating inhibitors. *PNAS* 74 (12), 5463–5467.
- Zhu, S.J., Li, Y., Li, H., Wang, Y.L., Xiao, Z.J., Vihko, P., Piao, Y.S., 2002. Retinoic acids promote the action of aromatase and 17 β -hydroxysteroid dehydrogenase type 1 on the biosynthesis of 17 β -estradiol in placental cells. *J. Endocrinol.* 172 (1), 31–43.