THEMATIC REVIEW

The diversity of sex steroid action: novel functions of hydroxysteroid (17 β) dehydrogenases as revealed by genetically modified mouse models

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Abstract

Disturbed action of sex steroid hormones, i.e. androgens and estrogens, is involved in the pathogenesis of various severe diseases in humans. Interestingly, recent studies have provided data further supporting the hypothesis that the circulating hormone concentrations do not explain all physiological and pathological processes observed in hormone-dependent tissues, while the intratissue sex steroid concentrations are determined by the expression of steroid metabolising enzymes in the neighbouring cells (paracrine action) and/or by target cells themselves (intracrine action). This local sex steroid production is also a valuable treatment option for developing novel therapies against hormonal diseases. Hydroxysteroid (17 β) dehydrogenases (HSD17Bs) compose a family of 14 enzymes that catalyse the conversion between the low-active 17-keto steroids and the highly active 17 β -hydroxy steroids. The enzymes frequently expressed in

sex steroid target tissues are, thus, potential drug targets in order to lower the local sex steroid concentrations. The present review summarises the recent data obtained for the role of HSD17B1, HSD17B2, HSD17B7 and HSD17B12 enzymes in various metabolic pathways and their physiological and pathophysiological roles as revealed by the recently generated genetically modified mouse models. Our data, together with that provided by others, show that, in addition to having a role in sex steroid metabolism, several of these HSD17B enzymes possess key roles in other metabolic processes: for example, HSD17B7 is essential for cholesterol biosynthesis and HSD17B12 is involved in elongation of fatty acids. Additional studies *in vitro* and *in vivo* are to be carried out in order to fully define the metabolic role of the HSD17B enzymes and to evaluate their value as drug targets.

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Introduction

Imbalanced action of sex steroid hormones, i.e. androgens and estrogens, is involved in the pathogenesis of various severe diseases in humans. Hormone-dependent cancers are commonly lethal both in women and in men, with breast cancer being the most prevalent cancer in women and prostate cancer in men in several Western countries (<http://info.cancerresearchuk.org/cancerstats/>). In addition, there are various other common hormone-dependent diseases, such as polycystic ovary syndrome (PCOS) and endometriosis, having poorly understood aetiology and

lacking efficient pharmacological treatments (Giudice 2010, Goodarzi *et al.* 2011). The ovaries and testes are the main sources of the circulating sex steroids. However, changes in circulating hormone concentrations do not explain all physiological and pathological processes observed in hormone-dependent tissues. A more inclusive explanation is provided by paracrine and intracrine action of the sex steroids, namely the regulation of intratissue hormone concentrations by the expression of steroidogenic enzymes in the neighbouring cells and/or by target cells themselves, respectively. Interfering with the local sex steroid production using pharmaceutical compounds is also a valuable treatment option for developing novel therapies against hormonal diseases (Labrie 2007). Among the steroid metabolic enzymes, hydroxysteroid (17 β) dehydrogenases (HSD17Bs) compose a family of enzymes that catalyse the conversion between the low-active 17-keto steroids and the highly active

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17β-hydroxy steroids. The enzymes, thus, catalyse the reactions between oestrone (E₁) and estradiol (E₂), androstenedione (A-dione) and testosterone, and 5α-androstenedione (5αA-dione) to dihydrotestosterone (DHT). However, in addition to their HSD17B activity, certain HSD17B enzymes have also shown to be involved in other metabolic pathways including the synthesis of lipids and cholesterol, for example. At present, 14 mammalian HSD17Bs have been identified. These enzymes possess variable sequence homology, expression pattern (Table 1), cofactor preference, substrate specificity and subcellular localisation (Peltoketo *et al.* 1999a, Lukacik *et al.* 2006, Möeller & Adamski 2006, Wu *et al.* 2007, Meier *et al.* 2009, Mindnich & Adamski 2009, Möeller & Adamski 2009, Persson *et al.* 2009). All except one of the HSD17Bs belong to the short-chain dehydrogenase/reductase family, while HSD17B5 is a member of the aldoketo reductase family. Numerous studies have indicated the expression of various HSD17Bs in sex steroid target tissues. Several of these enzymes are also considered as promising drug targets, and inhibitors are under development, for example for HSD17B1, HSD17B2, HSD17B3, HSD17B5, HSD17B7, HSD17B10 and HSD17B12 (Bagi *et al.* 2008, Chen *et al.* 2008, Day *et al.* 2008a,b, Hsieh & Ryan 2008, Fournier & Poirier 2009, Laplante *et al.* 2009, Sasano *et al.* 2009). Genetically modified (GM) mice provide an elegant approach to identify novel and unknown functions for genes, and the approach is also successfully used to validate novel drug targets. In this review, we summarise the recent data obtained for the role of HSD17B1, HSD17B2, HSD17B7 and HSD17B12 enzymes in various metabolic pathways and their physiological and pathophysiological roles as revealed by the recently generated GM mouse models.

Enzymatic activities

HSD17B1

Based on the *in vitro* studies, human (h) HSD17B1 has been considered to be highly estrogen specific, with markedly lower catalytic efficacy towards androgenic substrates (Poutanen *et al.* 1993, Miettinen *et al.* 1996a, Peltoketo *et al.* 1999b). Structural studies have also revealed that the substrate-binding site in hHSD17B1 is highly complementary to estrogenic substrates (Azzi *et al.* 1996, Lin *et al.* 2006). The enzyme catalyses both oxidative (17-hydroxy to 17-keto) and reductive (17-keto to 17-hydroxy) 17β-hydroxysteroid dehydrogenase activity with a proper cofactor added *in vitro*. However, in cultured cells, the hHSD17B1 has been shown to catalyse predominantly the reductive reaction (Poutanen *et al.* 1993, Miettinen *et al.* 1996a,b, Puranen *et al.* 1997, Day *et al.* 2008b), and recent studies have further provided evidence for reductive HSD17B activity for hHSD17B1 *in vivo*. For example, MCF-7 cells stably transfected with hHSD17B1 presented with increased estrogen-dependent tumour growth in immunodeficient mice in the presence of

Table 1 Predicted expression of human hydroxysteroid (17β) dehydrogenase (HSD17B) enzymes analysed by the data available at <https://www.genevestigator.com/gv/biomed.jsp>, a search engine for gene expression, data not available for HSD17B13

Tissues	HSD17B1	HSD17B2	HSD17B3	HSD17B4	HSD17B5	HSD17B6	HSD17B7	HSD17B8	HSD17B10	HSD17B11	HSD17B12	HSD17B14
Adrenal	+	+		+			+	+	+	+	+	+
Adipose	+	+		+			+	+	+	+	+	+
Bone	+	+		+			+	+	+	+	+	+
Brain	+	+		+			+	+	+	+	+	+
Heart	+	+		+			+	+	+	+	+	+
Intestine	+	+		+			+	+	+	+	+	+
Kidney	+	+		+			+	+	+	+	+	+
Liver	+	+		+			+	+	+	+	+	+
Lung	+	+		+			+	+	+	+	+	+
Mammary	+	+		+			+	+	+	+	+	+
Muscle	+	+		+			+	+	+	+	+	+
Ovary	+	+		+			+	+	+	+	+	+
Pancreas	+	+		+			+	+	+	+	+	+
Placenta	+	+		+			+	+	+	+	+	+
Prostate	+	+		+			+	+	+	+	+	+
Skin	+	+		+			+	+	+	+	+	+
Spleen	+	+		+			+	+	+	+	+	+
Thyroid	+	+		+			+	+	+	+	+	+
Testis	+	+		+			+	+	+	+	+	+
Uterus	+	+		+			+	+	+	+	+	+

E₁, under condition where no such effect was detected with the parental MCF-7 cells lacking significant HSD17B activity (Husen *et al.* 2006). Furthermore, hHSD17B1-dependent tumour growth was significantly reduced by HSD17B1 inhibitor treatment (Husen *et al.* 2006). In addition to HSD17B1, several other hHSD17Bs are capable of converting E₁ to E₂, including HSD17B7 (Torn *et al.* 2003) and HSD17B12 (Luu-The *et al.* 2006), but the role of these enzymes in the extra-gonadal E₂ formation still remains unclear. Although hHSD17B1 expression in various peripheral tissues is low, its catalytic efficacy is markedly higher than those measured for HSD17B7 and HSD17B12 (Luu-The *et al.* 2006), suggesting an important role for HSD17B1 in peripheral E₂ formation. We have recently analysed the role of hHSD17B1 in estrogen metabolism *in vivo* using transgenic (TG) mice ubiquitously over-expressing the hHSD17B1 under the chicken β -actin promoter (HSD17B1TG mice). In these mice, a small dose of E₁ treatment induced a markedly increased uterine weight under a condition where no such effect was observed in wild-type (WT) mice (Saloniemi *et al.* 2010). Furthermore, an increased E₂ concentration, in response to E₁ treatment, was apparent in all TG tissues with the exception of the ovaries (Saloniemi *et al.* 2010) that presented a high endogenous Hsd17b1 expression (Nokelainen *et al.* 1996). These data further demonstrated the ability of hHSD17B1 to enhance estrogen action in target tissues in the presence of low-active E₁. Moreover, over-expression of hHsd17b1 in TG mice under a MMTV promoter resulted in significantly increased reductive estrogenic activity *in vivo*, and the activity was significantly decreased after treating the mice with HSD17B1 inhibitors (Lamminen *et al.* 2009). These data suggest that HSD17B1 plays a major role in determining the gradient between the E₂ concentrations in serum and peripheral tissues. This type of gradient has been reported, e.g. for breast cancer tissue (van Landeghem *et al.* 1985, Lønning *et al.* 2009), where the

tissue presents with high HSD17B1 expression (Poutanen *et al.* 1993, Miyoshi *et al.* 2001, Shibuya *et al.* 2008).

There is a clear difference in the substrate specificity between the human and the rodent HSD17B1 enzymes; the catalytic efficacy for the rodent enzyme *in vitro* is similar for both androgens and estrogens (Nokelainen *et al.* 1996). However, the human enzyme has also been shown to catalyse conversion of A-dione to testosterone (Puranen *et al.* 1997), and the formation of androstenediol from dehydroepiandrosterone (DHEA) and 3 β -androstenediol from DHT (Lin *et al.* 2006), but the significance of these activities *in vivo* has been unclear. Interestingly, despite the high K_m value measured *in vitro* for hHSD17B1 towards A-dione (Puranen *et al.* 1997, Lin *et al.* 2006), in cultured cells, the activity obtained for A-dione to testosterone conversion was 20% of that observed for E₁ to E₂ conversion (Poutanen *et al.* 1993). This suggested that the hHSD17B1 possesses considerable androgenic activity with the preference for estrogenic substrates. Accordingly, a significantly increased conversion of A-dione to testosterone was observed in both female and male HSD17B1TG mice, together with significantly increased testosterone concentration in foetal TG females (Saloniemi *et al.* 2007). In summary, the data observed indicate that hHSD17B1 is not fully estrogen-specific but presents with significant androgenic activity.

HSD17B2

Both human and mouse HSD17B2 enzymes possess oxidative activity and are capable of catalysing *in vitro* the conversion of the 17 β -hydroxy forms of estrogens and androgens, such as E₂, testosterone and DHT to their less active 17-keto forms, E₁, A-dione and 5 α A-dione respectively. The enzyme also possesses 20 α -HSD activity, thereby activating 20 α -hydroxyprogesterone to progesterone (Wu *et al.* 1993, Labrie *et al.* 1995). Therefore, the HSD17B2 potentially regulates the ligand availability for several nuclear receptors (Fig. 1).

Figure 1 The HSD17B2 potentially regulates the ligand availability for several nuclear receptors (ERs, estrogen receptors, α and β); AR, androgen receptor; PR, progesterone receptor; RAR, retinoid acid receptor; RXR, retinoid X receptor) simultaneously. The enzyme has shown to inactivate estradiol (E₂) to oestrone (E₁) and testosterone (T) to androstenedione (A), to activate the 20 α -hydroxyprogesterone 1 (20-OH-Prog) to progesterone (prog), and is potentially involved in the metabolism of retinoids, such as 9-*cis*-retinal (9-*cis*-RA). The other HSD17Bs and the 20 α -hydroxysteroid dehydrogenase (20HSD) potentially catalyse the opposite reactions, and thus, the balance between the oxidative and reductive enzyme activities determines the ligand concentrations at the target cells.

HSD17B2 is localised in the endoplasmic reticulum, and it is widely expressed in various estrogen and androgen target tissues both in humans and in rodents including breast (Haynes *et al.* 2010), endometrium (Casey *et al.* 1994), placenta (Mustonen *et al.* 1998b) and prostate (Elo *et al.* 1996). The data, thus, suggest a role for the enzyme in the hormonal regulation of these tissues. However, the role of HSD17B2 in various metabolic pathways is only superficially known, and further studies have indicated that the enzyme is also expressed throughout the gastrointestinal tract and in the liver both in mice and in humans (Miettinen *et al.* 1996a, Mustonen *et al.* 1997a,b, 1998a, Sano *et al.* 2001). Phylogenetic analyses have indicated that HSD17B2 is a close homologue of retinoid-converting enzymes and presents with a high sequence similarity to retinol dehydrogenase type 1 (Chai *et al.* 1995a,b, Simon *et al.* 1995). In addition, studies have shown that retinoic acid (RA) induces expression of *HSD17B2* in a dose- and time-dependent manner in human endometrial epithelial (Cheng *et al.* 2008) and placental cells (Su *et al.* 2007). In order to test the activity of hHSD17B2 *in vivo*, we generated TG mice ubiquitously expressing the enzyme under the chicken β -actin promoter (Zhongyi *et al.* 2007). In these mice, the conversion of E_2 to E_1 was followed *in vivo* by injecting tritium-labelled E_2 into the mice, and a significantly increased oxidative HSD17B activity was detected in the TG mice compared with WT mice. The results, thus, confirmed that the HSD17B2 present with oxidative HSD17B activity *in vivo*. However, the enzymatic activity in the generated TG lines was relatively low. Owing to the possibility of inducing embryonic lethality in TG mice with high expression of HSD17B2, we also dissected a set of TG founder mice at the end of embryonic life. In these fetuses, a high E_2 to E_1 conversion was detected, further confirming the E_2 to E_1 reaction catalysed by HSD17B2. However, in the generated TG mouse lines, the male mice presented with severe phenotypic alterations (see below) without significantly altered serum LH, FSH or testosterone. Furthermore, no significant alterations were observed in intratissue testosterone concentrations between the TG and the WT males measured in several peripheral tissues. However, there was a trend to lower testosterone concentration in the TG tissues compared with the WT tissues. Only a limited suppression of androgen action was confirmed by analysing the expression of the androgen-dependent genes in the prostate and epididymis. Similar to that observed for certain androgen-dependent genes in the males, the expression of several estrogen-dependent genes in the mammary glands were significantly down-regulated by the local expression of HSD17B2 in the HSD17B2TG mammary glands transplanted in WT hosts. Thus, the data suggest a modulatory role for HSD17B2 in the expression of androgen and estrogen target genes, while sex steroid-independent mechanisms are partially responsible for the phenotypic alterations observed in the HSD17B2TG mice.

HSD17B7

The HSD17B7 enzyme was first characterised as a prolactin receptor-associated protein in the rat *corpus luteum* (Duan *et al.* 1996), although its role in prolactin signalling has remained unknown. The rodent (Nokelainen *et al.* 1998) and human (Torn *et al.* 2003) HSD17B7 enzymes catalyse the conversion of E_1 to E_2 *in vitro*, and based on the high expression of *HSD17B7* in mouse ovary, the enzyme is considered to be important in E_2 production, especially during pregnancy (Nokelainen *et al.* 1998, 2000). Also a shorter splice variant of hHSD17B7 has been identified (Liu *et al.* 2005), which has also been observed to catalyse the conversion of E_1 to E_2 in cultured cells. However, opposite results have been reported, as in some studies the truncated HSD17B7 protein was found to be inactive (Marijanovic *et al.* 2003). Owing to its amino acid sequence similarity with the yeast ERG27 protein, the HSD17B7 enzyme was also suggested to possess a 3-ketosteroid reductase activity (Breitling *et al.* 2001a,b, Marijanovic *et al.* 2003). Accordingly, *in vitro*, both the human and the mouse HSD17B7 enzymes have been shown to catalyse the conversion of zymosterone to zymosterol (Marijanovic *et al.* 2003), an essential reaction in the cholesterol biosynthesis. A role for mouse HSD17B7 in cholesterol biosynthesis was also suggested by the studies showing a similar expression pattern for HSD17B7 and the known cholesterologenic enzymes during mouse embryonic development (Laubner *et al.* 2003, Marijanovic *et al.* 2003). However, there are certain differences between the *HSD17B7* expression compared with the gene expression of the other cholesterol biosynthetic enzymes, suggesting additional roles for HSD17B7. Analysis of the concentration of cholesterol and various cholesterol biosynthesis intermediates in the WT, heterozygous and HSD17B7KO mouse embryos evidently showed that HSD17B7 is essential for cholesterol biosynthesis also in mice *in vivo* (Jokela *et al.* 2010). The data indicated that HSD17B7 catalyses the conversion of zymosterone to zymosterol, as in the knockout (KO) mouse embryos, the earlier intermediates such as lanosterol and squalene accumulated, and cholesterol, lathosterol and desmosterol (representing the later steps of cholesterol biosynthesis) were markedly reduced. Furthermore, the data show that lack of HSD17B7 was not efficiently compensated by other enzyme activities. The present data, thus, unquestionably show the *in vivo* role of HSD17B7 in cholesterol biosynthesis in mice, while its role in E_2 production *in vivo* remains to be further clarified (Fig. 2).

HSD17B12

The mammalian HSD17B12 was initially characterised as a 3-ketoacyl-CoA reductase, involved in the long-chain fatty acid synthesis (Moon & Horton 2003). Both the human and the mouse HSD17B12 share a 40% sequence similarity with HSD17B3, and the data indicate that *HSD17B12* is an ancestor of *HSD17B3* (Mindnich *et al.* 2004). In humans, the

Figure 2 Cholesterol synthesis pathway: the enzymes involved in the synthesis and the syndromes associated with the enzyme defects. The data obtained *in vitro* and in the HSD17B7KO mice *in vivo* indicate a role for HSD17B7 in zymosterol synthesis, and thus, *Hsd17b7* is a new candidate gene for inborn errors of cholesterol metabolism. The cholesterol synthesis with intermediates is shown in blue, the enzymes involved are indicated in green and the characterised inborn errors of cholesterol synthesis are indicated in red.

highest expression of *HSD17B12* is detected in the tissues involved in lipid metabolism, including the liver, kidney and muscle (Sakurai *et al.* 2006). In mice, the expression has also been detected in the brown and white adipose tissue (Moon & Horton 2003, Sakurai *et al.* 2006, Blanchard & Luu-The 2007). Studies on zebra fish have, furthermore, supported the role of HSD17B12 in fatty acid synthesis (Mindnich *et al.* 2004), and studies with LET-767, the *Caenorhabditis elegans* ortholog of HSD17B12, have shown that the enzyme is required for the production of branched-chain and long-chain fatty acids *in vivo* (Entchev *et al.* 2008). *HSD17B12* expression is also regulated by sterol regulatory element-binding proteins, identically to that shown for several genes involved in fatty acid and cholesterol biosynthesis (Nagasaki *et al.* 2009). Interestingly, a reduced expression of *HSD17B12* in cultured breast cancer cells results in significant inhibition of cell proliferation that is fully recovered by supplementation of arachidonic acid (AA; Nagasaki *et al.* 2009). Our studies in embryonic stem (ES) cells with reduced expression of *Hsd17b12* (Rantakari *et al.* 2010) further indicated an imbalanced fatty acid synthesis. In these cells, the relative amount of linoleic acid was unchanged while the amount of AA was significantly decreased compared with the WT ES cells, suggesting that HSD17B12 deficiency caused insufficient synthesis of AA. In addition to its putative role in fatty acid synthesis, human HSD17B12 has been shown to catalyse the conversion of E₁ to E₂ in cultured cells (Fig. 3), and the enzyme was suggested to be a major enzyme converting E₁ to E₂ in postmenopausal women (Luu-The *et al.* 2006). However, neither over-expressed nor reduced expression of *Hsd17b12* affects the E₂ production in cultured breast cancer cells (Day *et al.* 2008b).

Phenotypic alterations in GM mice for HSD17B enzymes

TG mice

HSD17B1TG mice Several studies (Stinnakre 1975, Wolf *et al.* 2002, 2004, Welsh *et al.* 2008) have shown that excess of androgens during foetal life disturbs normal development, resulting, for example, in masculinisation of the female reproductive tract. This is also suggested by the data obtained from human patients with congenital adrenal hyperplasia. In these patients, the female foetuses have increased adrenal testosterone production, resulting in masculinisation of the external genitalia (Merke & Bornstein 2005). Along with the significant androgenic activity of hHSD17B1 and with the increased foetal testosterone concentration, the TG female mice expressing hHSD17B1 ubiquitously presented with androgen-dependent phenotypic alterations, such as increased anogenital distance, suppressed nipple development, lack of vaginal opening and the combination of vagina with the urethra (Table 2). These alterations observed in the HSD17B1TG females were effectively rescued by prenatal anti-androgen (flutamide) treatment, further confirming the dependence of these phenotypes on androgens (Saloniemi *et al.* 2007, 2009). Androgen receptor (AR) is expressed in both female and male reproductive tissues during development, and consequently, the female reproductive tract responds also to androgens (Bentvelsen *et al.* 1995). Nipple development in mice has also been shown to be androgen dependent. Male rodents normally lack nipples but, for example in rats, those are formed in under-masculinised males, e.g. after prenatal flutamide treatment (Miyata *et al.* 2002, Foster & Harris 2005), or in the TG mice having

Figure 3 HSD17B12 has shown to possess both 3-ketoacyl-CoA reductase activity involved in elongation of free fatty acids, and HSD17B activity converting low-activity sex steroid oestrone to highly active estradiol.

an increased E₂/testosterone ratio due to the expression of P450 aromatase (CYP19A1; Li *et al.* 2002). Accordingly, prenatal flutamide treatment retained nipple development and rescued vaginal morphology in masculinised HSD17B1TG female mice (Saloniemi *et al.* 2007, 2009). Interestingly, around the urethra of HSD17B1TG females, we identified an enlarged Skene’s paraurethral gland, also referred to as the female prostate. The female prostate has been reported to be present in several species and, similarly to the males, it has been shown to respond to androgens (Santos *et al.* 2006). In HSD17B1TG females, flutamide treatment also suppressed the gland development (Saloniemi *et al.* 2007). The fact that placental testosterone concentration was not increased indicated that elevated androgen exposure was not of maternal origin. These data unequivocally show that over-expression of hHSD17B1 leads to increased androgen exposure during embryonic development. However, the source of androgen could not be determined.

Interestingly, the androgen exposure during the second half of pregnancy in the HSD17B1TG mice resulted in benign ovarian serous cystadenomas in adulthood (Saloniemi *et al.* 2007). These are common, slowly proliferating, benign lesions that can be precursors of ovarian serous borderline tumours, which can, in turn, progress to low-grade carcinomas (Cheng *et al.* 2004). However, no progression for cancer was observed in the TG females, suggesting that additional factors are required for malignant transformation of the ovarian surface epithelium. The development of the cystadenomas in HSD17B1TG females was prevented by treating the mice prenatally with an anti-androgen (flutamide) or by transplanting WT ovaries to HSD17B1TG females. Based on the data, it is evident that there is a connection between the foetal androgen production in the HSD17B1TG females and the development of benign serous cystadenomas in the TG mice (Saloniemi *et al.* 2007). Foetal masculinisation has not been directly linked to ovarian surface epithelial pathologies in humans, but ovarian surface epithelium-lined surface invaginations and epithelial inclusion cysts, which are considered as an initiation site for ovarian carcinogenesis, are more frequent in PCOS patients than in healthy individuals (Auersperg *et al.* 2001). It has also been shown that ovarian serous borderline tumours are positively associated with a history of PCOS, thus with a history of (foetal) hyperandrogenism (Olsen *et al.* 2008). HSD17B1 expression has been linked to ovarian epithelial cancers and was shown to positively correlate with an increasing malignancy of ovarian surface epithelial tumours (Sasano *et al.* 1996, Blomquist *et al.* 2002, Chura *et al.* 2009). Both estrogens and androgens have been associated with ovarian epithelial carcinogenesis (Syed *et al.* 2001, Ho 2003). Therefore, HSD17B1 may promote ovarian carcinogenesis via increasing estrogen concentration, but also via enhanced androgen production.

The data have shown that over-expression of hHSD17B1 in mice enhances estrogen action in the uterus and, in combination with persistent anovulation, causes endometrial hyperplasia, ranging from simple to complex hyperplasia with atypia (Saloniemi *et al.* 2010). However, endometrial carcinomas were not observed in HSD17B1TG mice,

Table 2 Sex steroid-dependent phenotypic alterations in various transgenic mice ubiquitously expressing human hydroxysteroid (17β) dehydrogenase 1

Transgenic mouse lines	Severity of the phenotypic alteration				
	016	020	012	050	013
Level of transgene expression	+	++	++	+++	++++
Phenotypic alterations in female mice					
Anovulation	ND	+	+	++	++++
Lack of nipples	–	–	–	+++	++++
Male-like vaginal/urethral morphology	–	–	–	+++	++++
Longer anogenital distance	ND	ND	ND	+++	++++
Hyperplastic endometrium	ND	ND	ND	++	++++
Ovarian cysts	ND	ND	+++	+++	++++
Hyperplastic pituitaries	ND	ND	ND	++++	++++

–, no change; +, mild; ++, moderate; +++, strong; ND, not determined

indicating that other mechanisms, such as phosphatase and tensin homologue inactivation, loss of forkhead box O subclass transcription factor 1 and hyperactivity of the PI3K pathway, are important pathways in endometrial carcinogenesis (Goto *et al.* 2008). Endometrial hyperplasia in HSD17B1TG mice closely resembled human disease and was efficiently reversed upon normalisation of the estrogen/progestin ratio in response to either ovulation induction or exogenous progestins, and in addition, they responded to HSD17B1 inhibitor treatment (Saloniemi *et al.* 2010). The data concerning the expression of HSD17B1 in the human normal and diseased endometrium are not fully conclusive. However, in most of the studies, the HSD17B1 expression is detected in normal endometrium, endometriosis specimens and endometriotic cancer (Mäntäusta *et al.* 1991, 1992, Casey *et al.* 1994, Zeitoun *et al.* 1998, Smuc *et al.* 2006, Dassen *et al.* 2007, Fechner *et al.* 2007). Various other HSD17B enzymes including HSD17B2, HSD17B5, HSD17B7 and HSD17B12 have also been detected in the endometrium under different pathological conditions (Rizner *et al.* 2006, Smuc *et al.* 2006), endometriosis (Dassen *et al.* 2007, Delvoux *et al.* 2009) and PCOS (Bacallao *et al.* 2008, Leon *et al.* 2008). Studies have indicated that in the endometrium, the oxidative HSD17B activity is higher than the reductive activity (Delvoux *et al.* 2007, 2009), while significant reductive activity has also been detected in the human endometrium (Delvoux *et al.* 2007, 2009). Recent studies in endometrial and cervical cancer cell lines indicate that reductive activity can be inhibited by using the inhibitors of HSD17B1, HSD17B5, HSD17B7 and HSD17B12, indicating that all four enzymes contribute to estrogen synthesis in the cancer cell lines. However, full inhibition was not obtained even when using a combination of the inhibitors, indicating that there are still other HSD17B(s) participating in endometrial estrogen synthesis (Fournier & Poirier 2009). Thus, combination of the activities of these enzymes ultimately determines the hormonal status of the endometrium and the drug combination to be used. Collectively, the data suggest that HSD17B1 inhibition is one of the several possible approaches to reduce estrogen production both in eutopic and in ectopic endometrial tissue.

HSD17B2TG mice In HSD17B2TG male mice, there were certain phenotypes that prompted us to seek for phenotypes that are not directly known to be related to altered sex steroid action, including a delayed eye opening and growth retardation (Zhongyi *et al.* 2007). In addition, we have previously found a lack of correlation between HSD17B2 expression and the hormonal status in female rat (Akinola *et al.* 1997). These observations have also encouraged us to seek other pathways putatively affected by HSD17B2 expression. Thus, it has been fascinating to note that certain observed phenotypes in HSD17B2TG mice expressing the enzyme under a ubiquitous promoter, such as the growth retardation, resemble the phenotypes observed in a variety of RA receptor knockout mice (Lohnes *et al.* 1993, Lufkin *et al.*

1993, Kastner *et al.* 1994, 1996, Ghyselinck *et al.* 1997). Vitamin A exposure induces disruption of spermatogenesis, while only the undifferentiated spermatogonia remain in the seminiferous tubuli. Accordingly, providing proper amount of RA to vitamin A-deficient animals restores and synchronises spermatogenesis in mice (Gaemers *et al.* 1996, 1998a, Schrans-Stassen *et al.* 1999). In order to analyse whether HSD17B2 participates in the retinoid metabolism, retinoid acid receptor agonist (TTNPB) has been provided for the HSD17B2TG males, and interestingly, the TTNPB treatment efficiently reduces the seminiferous tubule damage observed in the HSD17B2TG males.

In addition to males, the HSD17B2TG female mice displayed delayed eye opening, and some mice were present with squint appearance of the eyes long after the normal age of eye opening. The eyes are known to be the most sensitive organ to retinol deficiency, and it is considered to be the first place of malformations in vitamin A-deficient fetuses (Lohnes *et al.* 1994, Grondona *et al.* 1996). Moreover, various retinoid receptor mutant mice (Kastner *et al.* 1994, Lohnes *et al.* 1994) have implicated the role of retinoid signalling at most steps of prenatal eye morphogenesis and eye development.

HSD17B1 and HSD17B2 bi-TG mice To study whether the phenotypic alterations observed in the HSD17B2TG mice could be rescued by the expression of HSD17B1 (and *vice versa*), the bi-TG (HSD17B1–HSD17B2TG) mice were generated by utilising mice expressing the transgenes under the same promoter (Shen *et al.* 2009). The bi-TG mice showed features of both HSD17B1TG and HSD17B2TG mice, while the phenotypes observed in the single TG mice were not efficiently rescued in the bi-TG. The female bi-TG mice were masculinised similar to the HSD17B1TG mice (Saloniemi *et al.* 2007), but were growth retarded at prepubertal and young adult age, and had extensive lobulo-alveolar development similar to the HSD17B2TG mice (Zhongyi *et al.* 2007, Shen *et al.* 2009). Furthermore, the bi-TG females did not present with vaginal opening, while they showed improper separation of vagina and urethra, suppressed nipple development and ovarian benign serous cystadenomas, similar to that previously reported for HSD17B1TG females. However, androgen-dependent increase in the anogenital distance and reduced uterus weight of HSD17B1TG females were partially rescued in bi-TG mice. Like HSD17B2TG males, bi-TG males also showed growth retardation and severely disrupted spermatogenesis. Histological analysis showed that at the age of 6 months, seminiferous tubules were severely atrophic and contained mostly only Sertoli cells. The data suggest that certain phenotypic characteristics in the HSD17B1TG and HSD17B2TG lines are caused by different signalling pathways (Shen *et al.* 2009). The most notable changes in the HSD17B1TG mice are well explained by alterations in sex steroid action, whereas in the HSD17B2TG mice the connection to sex steroids is weaker.

Knockout mice

As described above, the recent characterisation of the activities of some HSD17B family members, together with the data obtained in TG mice, have provided evidence for the fact that the substrate specificity of HSD17B enzymes is broader than initially expected. In line with these observations, the embryonic lethal phenotypes observed in the HSD17BKO2, 7 and 12 mice do not mimic the phenotype alterations observed in mice deficient in estrogen receptor- α (*Esr1*), estrogen receptor- β (*Esr2*) and *AR* (Matsumoto *et al.* 2003, Hewitt *et al.* 2005, Zhao *et al.* 2008, Kerkhofs *et al.* 2009), or those over-exposed to sex steroids. This further points towards other enzymatic activities associated with these HSD17B enzymes.

Hsd17b2KO mice In addition to several other tissues, *Hsd17b2* is expressed in the placenta, and there is also a marked expression of the enzyme during embryogenesis. During the placental development, the mouse *Hsd17b2* expression presents with differential distribution among the various placental compartments. The data have revealed that during the mid-gestation, the HSD17B2 enzyme is present in the trophoblast giant cells (Mustonen *et al.* 1997b), while at the embryonic day (E) 12.5, the expression emerges at the labyrinth region. Also at the end of pregnancy, the expression is at its strongest in the labyrinth region (Mustonen *et al.* 1997b). Furthermore, the *Hsd17b2* expression appears simultaneously both in the placenta and in the foetal liver and intestine. This, together with the observed oxidative HSD17B activity of the enzyme, has been the basis for the hypothesis, suggesting a role for the enzyme in lowering the sex steroid exposure of the foetus (Tong *et al.* 2005). The importance of the regulation of foetal estrogen exposure is shown by the results indicating that estrogen excess in *Sult1e1* KO mice results in placental thrombosis and spontaneous loss of the foetus (Mahendroo *et al.* 1997). However, the placentas of HSD17B2KO mice displayed histological malformations, while no signs of thrombosis were observed. Furthermore, the treatment of pregnant female mice with an anti-estrogen or with progesterone did not prevent the foetal loss of the HSD17B2KO mice (Rantakari *et al.* 2008). Thus, the cause of embryonic deaths in the HSD17B2KO mice is likely not due to the lack of progesterone or due to an increased action of estrogens. Interestingly, the embryonic deaths of the HSD17B2KO mice occur at the time when there is a shift in the *Hsd17b2* expression from the maternal to the foetal part of placenta and manifestation of the *Hsd17b2* expression in the foetus. Similar to those identified in the TG mice expressing hHSD17B2 (Zhongyi *et al.* 2007), certain phenotypic alterations in the HSD17B2KO mice closely resembled those identified in mice with altered RA metabolism (Cohlan 1953, Lohnes *et al.* 1994, Gaemers *et al.* 1998a,b). Thus, the defects in the placenta development in the HSD17B2KO mice might be due to simultaneously altered actions of RA and sex steroids. *In vitro* studies have, furthermore, shown that

retinoids induce *Hsd17b2* expression and enzyme activity in a dose- and time-dependent manner (Ito *et al.* 2001).

HSD17B7KO mice As shown above, in addition to catalysing the reactions involved in sex steroid metabolism, HSD17B7 catalyses the conversion of zymosterone to zymosterol both *in vitro* and in mice *in vivo*. The concentrations of the cholesterol biosynthesis intermediates measured in HSD17B7KO embryos were altered (Jokela *et al.* 2010) in the manner expected by the activity for the enzyme based on the data obtained *in vitro* (Marijanovic *et al.* 2003). The lack of HSD17B7 resulted in a marked blockage in foetal *de novo* cholesterol synthesis, while the amount of total cholesterol was normal in the HSD17B7KO embryos, as measured at E10.5. This, together with other studies, suggests that the pregnant female supplies most of the cholesterol present in the embryo for the first 10–12 days of pregnancy (Tint *et al.* 2006). However, the data indicated that the maternal cholesterol supply is not sufficient to support the neuroectodermal survival and cardiovascular differentiation in the absence of foetal HSD17B7 activity (Jokela *et al.* 2010). In addition to HSD17B7, 7-dehydrocholesterol reductase (*Dhcr7*) is one of the enzymes in the final steps of cholesterol synthesis. Interestingly, mice deficient in *Dhcr7* survive until birth (Fitzky *et al.* 2001), while the HSD17B7KO mice present with embryonic lethality (Shehu *et al.* 2008, Jokela *et al.* 2010). This data suggest that, in contrast to 7-dehydrocholesterol, zymosterone is not able to compensate for the lack of cholesterol in embryonic development or that zymosterol has other vital functions apart from its role as a cholesterol biosynthesis intermediate. In connection to this, it has been shown that zymosterol is one of the liver X receptor (LXR) ligands (Yang *et al.* 2006). The *Lxr*-deficient mice, however, do not present with defects in embryonic development (Alberti *et al.* 2001). In addition, it has to be considered that the accumulation of cholesterol precursors may possess toxic effects on the embryos, as postulated, for example, for Smith-Lemli-Opitz syndrome (Gaoua *et al.* 1999). However, the mechanisms of such toxic effects have not been presented and the evidence for toxicity *in vivo* is lacking.

Histological analysis revealed that the *Hsd17b7* deficiency results in defects in the development of front brain hemispheres, related to an increased apoptosis in the neuronal tissues (Shehu *et al.* 2008, Jokela *et al.* 2010). The need of *de novo* cholesterol synthesis in the foetal brain development is supported by the data showing that the blood-brain barrier in mice forms at around E10 (Tint *et al.* 2006). Furthermore, the cardiovascular system was also affected at E9.5 onwards, as no organised vessels were observed in the HSD17B7KO yolk sac, and the complexity of the vasculature was also reduced in the brain of the KO embryos. Furthermore, heart development was affected, and we observed pericardial effusion, reduced number of myocardial cells and thin pericardium (Jokela *et al.* 2010). Similar phenotypic alterations, such as malformation of the nervous system, have also been observed in other

mutant mice with a defect in cholesterol biosynthesis (Liu *et al.* 1999, Tozawa *et al.* 1999, Fitzky *et al.* 2001). Interestingly, the mutant mice deficient in *Ebp*, *Sc5d*, *Dhcr24* and *Dhcr7* survive until birth (Derry *et al.* 1999, Fitzky *et al.* 2001, Krakowiak *et al.* 2003, Wechsler *et al.* 2003, Mirza *et al.* 2006), while the HSD17B7KO and mice deficient in the steps prior to the HSD17B7 (Liu *et al.* 1999, Tozawa *et al.* 1999, Caldas *et al.* 2005) present with embryonic lethal phenotypes.

HSD17B12KO mice HSD17B12 has been shown to present with activities involved in fatty acid elongation, particularly in the elongation of essential fatty acids such as AA (Moon & Horton 2003) and to catalyse the conversion of E₁ to E₂ (Luu-The *et al.* 2006). AA is derived from one of the essential fatty acid compounds, linoleic acid, through a series of metabolic steps involving desaturation and subsequent chain elongation (Cook *et al.* 1991). AA, in turn, gives rise to a whole group of biologically important 20-carbon substances known as the eicosanoids, including prostaglandins, leukotrienes and thromboxanes. These compounds are important for the regulation of a variety of metabolic and physiological processes (Chatzipanteli *et al.* 1996). The expression pattern of *Hsd17b12* further supports its role in lipid metabolism. Both the mouse and the human *Hsd17b12* are expressed universally (Sakurai *et al.* 2006, Blanchard & Luu-The 2007) but show highest levels in the tissues related to lipid metabolism, including liver, kidney, heart and skeletal muscle (Sakurai *et al.* 2006). *Hsd17b12* is also strongly expressed during mouse development. It is observable in the embryonic ectoderm at E7.5, and high expression is present in the neural tissue from E8.5 onwards (Rantakari *et al.* 2010). The expression pattern of *Hsd17b12* refers to a role in neuronal development. In addition, *Hsd17b12* expression was observed in the yolk sac and in the ectoplacental cone. Analysis of the HSD17B12KO embryos indicated that the embryos initiated gastrulation but further organogenesis was severely disrupted. The HSD17B12KO embryos showed shortened anterior–posterior axis and presented with severe defects in the neuronal development. In addition, they failed to grow several mesoderm-derived structures (Rantakari *et al.* 2010). Therefore, the embryos at the age of E8.5–E9.5 were void of all normal embryonic structures. Furthermore, the proliferation capacity of the inner cell mass obtained from the HSD17B12KO blastocysts was strongly decreased as analysed *in vitro* (Rantakari *et al.* 2010). In line with the suggested function of HSD17B12 in the fatty acid elongation, these phenotypic features observed were similar to those previously reported for the acetyl-CoA carboxylase- α (*ACACA*, *Acat1*) null mice (Abu-Elheiga *et al.* 2005). The brain is rich in structural lipids, and AA and docosahexaenoic acid are the predominant essential fatty acids in the mammalian brain (Crawford *et al.* 1976, Crawford 1993). Studies have demonstrated that the deficiency of AA leads to neurodevelopmental disorders accompanied by impaired foetal growth and behavioural retardation (Sinclair & Crawford 1973, Galli

& Spagnuolo 1976, Leaf *et al.* 1992, Wainwright *et al.* 1997, Birch *et al.* 1998, 2000). Studies with tissue-specific KO models are to be carried out to further investigate the role of HSD17B12 in the brain development and to understand its putative role in eicosanoid production.

Declaration of interest

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