



X-inactivation of HSD17B10 revealed by cDNA analysis in two females with 17 β -hydroxysteroid dehydrogenase 10 (HSD10) deficiency

Antonia Ribes, Judit García-Villoria, Laura Gort, Irene Madrigal, Carme Fons, Cristina Fernández-Sierra, Aleix Navarro-Sastre, Montserrat Mila, Paz Briones, M^a Angeles García-Cazorla, et al.

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1 X-inactivation of *HSD17B10* revealed by cDNA analysis in two females with 17 β -
2 hydroxysteroid dehydrogenase 10 (HSD10) deficiency.

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4 Judit García-Villoria ^{1,2}, Laura Gort ^{1,2}, Irene Madrigal^{2,3}, Carme Fons ^{4,2}, Cristina
5 Fernández ^{1,2}, Aleix Navarro-Sastre^{1,2}, Mila M ^{3,2}, Paz Briones ^{1,2,5}, M^a Angeles García-
6 Cazorla^{4,2}, Jaume Campistol ^{4,2}, Antonia Ribes ^{1,2}

7

8 ¹ Sección de Errores Congénitos del Metabolismo (IBC). Servicio de Bioquímica y
9 Genética Molecular. Hospital Clínic. IDIBAPS. Barcelona, Spain.

10 ² CIBER of Rare Diseases (CIBERER). Barcelona, Spain

11 ³ Servicio de Bioquímica y Genética Molecular. Hospital Clínic. IDIBAPS. Barcelona,
12 Spain.

13 ⁴ Servicio de Neurología. Hospital Sant Joan de Déu. Barcelona, Spain.

14 ⁵ CSIC. Barcelona, Spain

15

16 Corresponding author:

17 Dra. Antonia Ribes

18 Sección de Errores Congénitos del Metabolismo (IBC).

19 Servicio de Bioquímica y Genética Molecular.

20 CIBER de Enfermedades Raras (CIBERER).

21 Hospital Clínic.

22 C/ Mejía Lequerica, s/n, Edifici Helios III

23 08028 Barcelona, Spain

24 Tel. +34 93 227 9340

25 Fax. +34 93 227 5668

26 e-mail. aribes@clinic.ub.es

27

28 **Abstract**

29 17 β -hydroxysteroid dehydrogenase 10 (HSD10) is a mitochondrial enzyme involved in
30 the degradation pathway of isoleucine and branched-chain fatty acids. The gene
31 encoding HSD10, *HSD17B10*, has been reported as one of the few genes that escapes
32 X-inactivation. We previously studied two females with HSD10 deficiency, one of them
33 was severely affected and the other presented a mild phenotype. To elucidate why these
34 two carriers were so differently affected, cDNA analyses were performed. The
35 *HSD17B10* cDNA of eight control cell lines, two hemizygous patients and two carriers
36 was obtained from cultured fibroblasts, amplified by PCR and sequenced by standard
37 methods. All *HSD17B10* cDNAs were quantified by real-time PCR. In the fibroblasts of
38 the female who presented with the severe phenotype, only the mutant allele was
39 identified in the cDNA sequence which was further confirmed by Relative
40 Quantification (RQ) of *HSD17B10* cDNA. This is in agreement with an unfavourable
41 X-inactivation. The other female, with slight clinical affectation, showed the presence of
42 both mutant and wild-type alleles in the cDNA sequence, which was confirmed by RQ
43 of *HSD17B10* cDNA in fibroblasts. This is in line with normal X-inactivation and the
44 expression of both alleles in different cells (functional mosaicism). RQ results of
45 *HSD17B10* cDNA did not differ significantly between unaffected males and females,
46 which indicates that the genetic doses of mRNA of *HSD17B10* was the same in both
47 sexes. In conclusion, these results suggest that the *HSD17B10* gene does not escape X-
48 inactivation as has been previously reported.

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51 **Keywords:** HSD10 deficiency; HADH2; HSD17B10

52 **Introduction**

53 17 β -hydroxysteroid dehydrogenase 10 (HSD10) is a mitochondrial enzyme involved in
54 the degradation pathway of isoleucine and branched-chain fatty acids [1]. This enzyme
55 has also been found to be involved in the metabolism of sex steroid hormones,
56 neuroactive steroids and in the detoxification of cytotoxic aldehydes [2,3]. HSD10
57 deficiency (OMIM 300256) is an X-linked defect caused by mutations in the
58 *HSD17B10* gene. Clinically, the great majority of male patients show normal early
59 development followed by progressive loss of mental and motor skills [1, 4-11].
60 However, three patients were identified that presented symptoms in the first days of life
61 [1,11]. It has recently been demonstrated that symptoms of these patients are unrelated
62 to accumulation of metabolites in the isoleucine pathway and that the neurological
63 handicap can be associated with an imbalance in neurosteroid metabolism [12] or to
64 defects in general mitochondrial function [13]. In addition, the splice variant c.574C>A
65 of *HSD17B10* gene has been associated with a new syndromic form of X-linked mental
66 retardation, choreoathetosis and abnormal behaviour [14].

67 The *HSD17B10* gene has been mapped to chromosome Xp11.2 [15] and has been
68 reported as one of the few genes that escapes X-inactivation [16]. To date, 10 female
69 patients with HSD10 deficiency have been described presenting a variety of symptoms,
70 from borderline learning difficulties to psychomotor and speech delay [5,9,11]. We
71 previously studied two of these females. One of them was heterozygous for the p.N247S
72 mutation and was severely affected, whereas the other was heterozygous for the
73 p.P210S mutation and presented a slight clinical affectation [11]. To elucidate why
74 these two females were so differently affected, we performed *HSD17B10* cDNA

75 quantitative analysis in both females and in control fibroblasts, the results of which are
76 reported here.

77 **Materials and Methods**

78 *Material*

79 Skin biopsies from patients of two unrelated Spanish families with HSD10 deficiency
80 were obtained. Family 1: a male patient (1IIM) and his carrier sister (1IIF), both with a
81 severe phenotype (Figure 1A). Family 2: a male patient (2IIM), with a severe phenotype
82 and his heterozygous mother (2IF) with a slight clinical affection (Figure 1A). Both
83 families have been previously described [11]. Eight cell lines (four males and four
84 females) from our cell bank were used as controls.

85 All the samples were obtained according to the declaration of Helsinki and informed
86 consent was signed by all the patients or their parents.

87 *Molecular studies*

88 cDNAs were obtained from cultured fibroblasts, were amplified by PCR and sequenced
89 using standard protocols and oligonucleotides designed in-house (sequences available
90 upon request). All *HSD17B10* cDNAs were quantified by the StepOnePlus™ real-time
91 PCR System using the Comparative Ct ($\Delta\Delta C_t$) method from StepOne software v2.0
92 (Applied Biosystems, Foster City, CA, USA). The Primer Express 3.0 software
93 (Applied Biosystems) was used to design two sets of primers and probes to differentiate
94 wild-type (Wt) and mutant (Mut) alleles corresponding to mutations p.N247S
95 (c.740A>G) and p.P210S (c.628C>T) of the *HSD17B10* gene. We used two different
96 endogenous controls: Glyceraldehyde 3-phosphate dehydrogenase (GAPDH,
97 PN4310884E) and cyclophilin A (PPIA, PN4310883E) (Applied Biosystems). As
98 additional control, a mixed pool of four healthy male cDNAs was used in each analysis.

99 Gene nucleotide numbering was done according to sequence RefSeq NM_004493 with
100 +1 as A of the ATG start codon. The ATG codon represents +1 for the amino acid
101 numbering according to the HSD10 protein sequence NP_004484.

102 *X-inactivation studies*

103 The androgen-receptor locus (*AR*) methylation assay was performed in genomic DNA
104 of female carriers, as previously described [17]. If the *AR* locus was uninformative,
105 skewing was assessed at *FMRI* locus [18]. Briefly, genomic DNA (300ng) was digested
106 with 5U of *HpaII* (both for the *AR* and *FMRI* assays) in a total volume of 20μL. For
107 each sample, an undigested control was prepared. We define the pattern of X
108 chromosome inactivation as skewed when the inactivation percentage was over 80%.

109 *Statistical methods*

110 Statistical studies for the analyses of Relative Quantification (RQ) in male and female
111 controls were performed using the non parametric two-related sample test, Wilcoxon
112 test, with the SPSS[®] software (version 14.0 for Windows[®]).

113 **Results and discussion**

114 *HSD17B10* has been reported as one of the few genes that escapes X-inactivation [16],
115 which predicts that female carriers would not be affected. However, 10 females with
116 HSD10 deficiency have been described so far, presenting different degrees of clinical
117 affectation, which is in agreement with an X-linked inheritance with different degrees of
118 X-inactivation [11].

119 To elucidate if *HSD17B10* cDNA doses differed between males and females, we
120 performed RQ of wild-type (Wt) *HSD17B10* cDNA alleles in four female and four male
121 controls (Figure 2). Results of the Wilcoxon statistical test did not show any significant
122 difference between the doses in both sexes considering the two endogenous controls (p-

123 value=0.07). Therefore, these results are in favour of an X-linked disease that does not
124 escape X-inactivation.

125 We previously studied two unrelated females with different degrees of clinical
126 affection [11]. The female of Family 1 (1IIF), like her brother (1IIM), presented a
127 severe phenotype with psychomotor and speech delay, and a clear deficiency of HSD10
128 activity in fibroblasts [11]. When we sequenced her *HSD17B10* cDNA it seemed that
129 only the mutant allele was identified (Figure 1A). Results for the RQ of her *HSD17B10*
130 cDNA (Figure 3A) showed that amplification levels of the mutant (Mut) probe were
131 much higher than those of the Wt probe and very similar to those of her brother (1IIM),
132 independently of the endogenous control used (Figure 3A). To rule out a Turner
133 Syndrome chromosome analysis was performed, which resulted in a normal karyotype
134 (46,XX). Skewed X-inactivation was confirmed by methylation studies. Patient 1IIF
135 was homozygous for AR locus, consequently this study was uninformative, but *FMRI*
136 locus showed a skewed X-inactivation pattern (80/20). These results are in agreement
137 with an unfavourable X-inactivation effect of *HSD17B10* gene in the analysed tissue. In
138 addition, as the girl was severely affected, it could be expected a similar unfavourable
139 X-inactivation in other tissues.

140 The other female (2IF) showed a mild clinical affection, with learning disabilities and
141 HSD10 activity in fibroblasts within the control range [11]. *HSD17B10* cDNA
142 sequencing showed the presence of both mutant and wild-type alleles (Figure 1B). This
143 observation was in agreement with the results of the RQ studies showing similar
144 *HSD17B10* cDNA levels of both Wt and Mut probes, while we were only able to
145 amplify the Mut probe in her severely affected son (2IIM) (Figure 3B). X-inactivation
146 analysis showed a random X-inactivation pattern for AR locus in patient 2IF. These

147 results, suggest the presence of both *HSD17B10* alleles in this female, which is in line
148 with normal X-inactivation and the expression of both alleles in different cells
149 (functional mosaicism). In addition, the normal enzymatic activity found in this female
150 [11] might be due to lack of sensitivity of the enzymatic technique, or maybe there was
151 enough dose of wild type *HSD17B10* mRNA to produce enough HSD10 protein to
152 obtain normal activity.

153 However, we did observe that the amplification responses were different for each probe
154 when they were corrected by the two different endogenous controls (Figure 3). This
155 could be explained by the low specificity of the probes and by the variability of the
156 endogenous controls. However, in spite of it, the interpretation of the results did not
157 change.

158 To summarise, here we present the results of *HSD17B10* cDNA analysis in two female
159 carriers compared with affected males and controls. The hypothesis that *HSD17B10* is
160 inactivated in one of the X-chromosome is supported by the results in controls, which
161 showed that doses of *HSD17B10* cDNA were the same in both sexes (Figure 2). RQ
162 cDNA results for one of the females (1IIF), together with the enzymatic studies and the
163 severe clinical presentation were in agreement with an unfavourable X-inactivation
164 effect. In addition, RQ cDNA results for the other female (2IF) seem to reflect the
165 presence of a mosaicism in the studied tissue, which could explain the normal
166 enzymatic activity and her mild phenotype. Although, we cannot exclude that
167 differences in disease severity between both female carriers is at least partly due to
168 differences in the effect of the mutations, as the male patient with the p.N247S mutation
169 died at age two months whilst the patient with p.P210S mutation is alive at four years of
170 age.

171 In conclusion, our results suggest that *HSD17B10* gene does not escape X-inactivation
172 as previously reported [16]. Heterozygous females showed the classical biochemical
173 and clinical variability of X-linked diseases due to random X-chromosome inactivation
174 and the severity of the phenotype will depend on the total dose of mutant mRNA in
175 different tissues as well as on the severity of the mutation.

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290 **Titles and legends to figures**

291 Figure 1. Pedigree (1A) and cDNA sequence (1B) of Family 1 and Family 2.

292 Figure 2. RQ of *HSD17B10* Wt probes named as p.N247N and p.P210P, with two
293 distinct endogenous controls (GAPDH and PPIA) in four males and four females. The
294 bars represent the mean of four controls and the error bars the mean \pm SD.

295 Figure 3. RQ of *HSD17B10* Wt and Mut probes with two distinct endogenous controls
296 (GAPDH and PPIA) in patient and control cDNAs. The bars of controls represent the
297 mean of eight controls (4 males + 4 females), performed in triplicate. The bars of
298 patients represent the mean of triplicate measurements. The error bars represent the
299 mean \pm SD.

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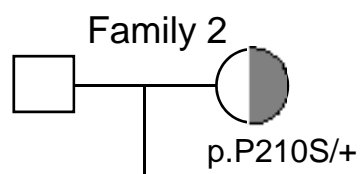
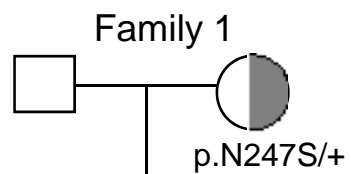
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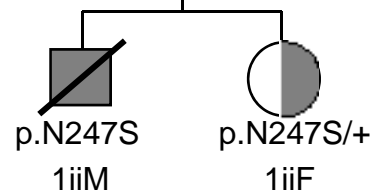
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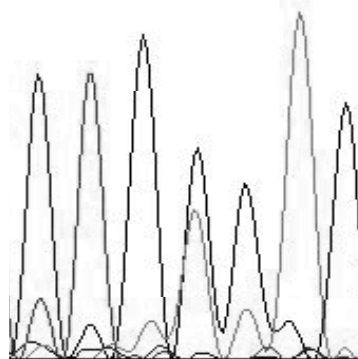
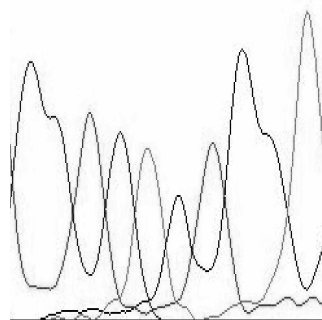
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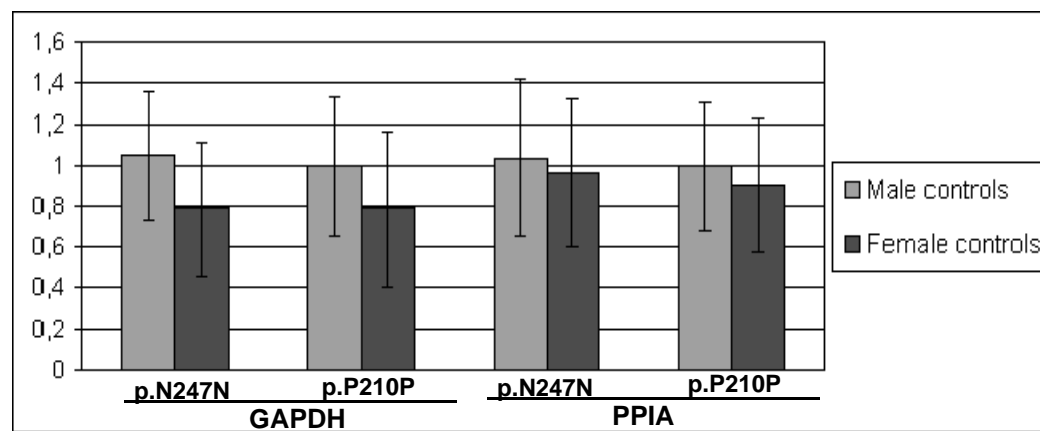
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1B)

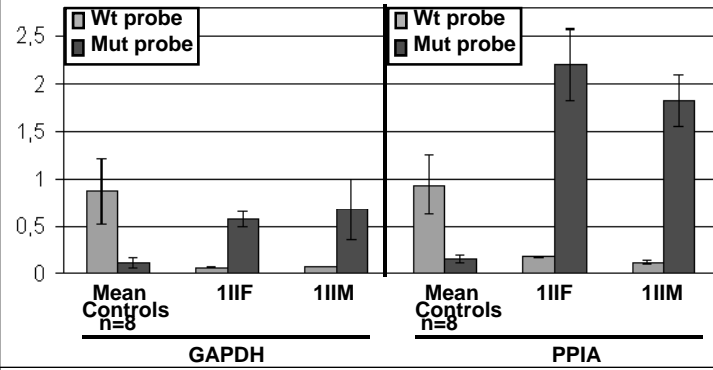
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C T G G G A G





A) Family 1 mutation p.N247S



B) Family 2 mutation p.210S

