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Short Communication

A 5-methylcytosine hotspot responsible for the prevalent *HSD17B10* mutation

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ABSTRACT

Approximately half of the cases of hydroxysteroid (17β) dehydrogenase X (HSD10) deficiency are due to a missense C>T mutation in exon 4 of the *HSD17B10* gene. The resulting HSD10 (p.R130C) loses most or all catalytic functions, and the males with this mutation have a much more severe clinical phenotype than those carrying p.V65A, p.L122V, or p.E249Q mutations. We found that the mutated cytosine which is +2259 nucleotide from the ATG of the gene, is >90% methylated in both the active and inactive X chromosomes in two normal females as well as in the X chromosome of a normal male. Since 5-methylcytosine is prone to conversion to thymine by deamination, the methylation of this cytosine in normal X chromosomes provides an explanation for the prevalence of the p.R130C mutation among patients with HSD10 deficiency. The substitution of arginine for cysteine eliminates several hydrogen bonds and reduces the *van der Waals* interaction between HSD10 subunits. The resulting disruption of protein structure impairs some if not all of the catalytic and non-enzymatic functions of HSD10. A meta-analysis of residual HSD10 activity in eight patients with the p.R130C mutation showed an average 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) activity of only 6 (±5) % of the normal control level. This is significantly lower than in cells of patients with other, clinically milder mutations and suggests that the loss of HSD10/MHBD activity is a marker for the disorder.

1. Introduction

Hydroxysteroid (17β) dehydrogenase X deficiency also known as 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency, is a relatively rare X linked neurological syndrome resulting from a missense mutation in the *HSD17B10* gene (Ofman et al., 2003; Seaver et al., 2011; Yang et al., 2009). The urine organic acid profile of affected individuals is characterized by high levels of isoleucine metabolites, namely 2-methyl-3-hydroxy butyrate and tiglylglycine, which indicate a blockade of the isoleucine and branched-chain fatty acid degradation pathway (Cazorla et al., 2007; Ensenauer et al., 2002; García-Villoria et al., 2009, 2010; He and Yang, 2006; Olpin et al., 2002; Perez-Cerda et al., 2005; Poll-The et al., 2004; Sass et al., 2004; Sutton et al., 2003; Zschocke et al., 2000). Although the accumulation of these isoleucine and branched-chain fatty acid metabolites (He and Yang, 2006) may not be completely benign (Rossa et al., 2005), it does not cause neurological symptoms in patients with a different blockade of such pathways, β-KT thiolase deficiency (Fukao et al., 2001) that also shows an accumulation of these metabolites. Thus, the pathophysiology of HSD10 deficiency must lie in the disruption of other catalytic or non-catalytic HSD10 activities, e.g., an imbalance of neurosteroid metabolism (Yang et al., 2009, 2011).

The diagnosis of this disease is sometime difficult because of subtle or intermittent metabolite secretion (especially in female) unless the HSD10/MHBD activity in patients' cells is determined to be lower than the normal level (García-Villoria et al., 2009). There are at least nine reported and clinically related missense mutations in the *HSD17B10* but about half of the patients with HSD10 deficiency carry a potent c.388C>T transition. Male patients with this mutation have a severe clinical phenotype. This mutated gene generates a mutant protein HSD10(p.R130C), whose residual activity appears to be negligible (Ofman et al., 2003; Yang et al., 2009). One group, however, reported (Rauschenberger et al., 2010) that the recombinant mutant protein, HSD10(p.R130C), exhibits up to 64% of residual MHBD activity of the wild type enzyme, and proposed a non-enzymatic theory of HSD10 deficiency. They also suggested that the prevalence of the c.388C>T mutation among HSD10 deficiency patients is due to the lethality of other HSD10 mutations (Rauschenberger et al., 2010).

Here we report that a 5-methylcytosine is present in both active and inactive X chromosomes at +2259 nucleotide from the initiation ATG of the *HSD17B10* gene. The presence of this hypermutable nucleotide at this position explains the prevalence of the p.R130C mutation among HSD10 deficiency patients. Our analysis of results from different laboratories suggests that cells of HSD10 deficiency patients carrying the c.388C>T transition at the *HSD17B10* gene, have minimal

Abbreviations: HSD10, hydroxysteroid (17beta) dehydrogenase X; MHBD, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase.

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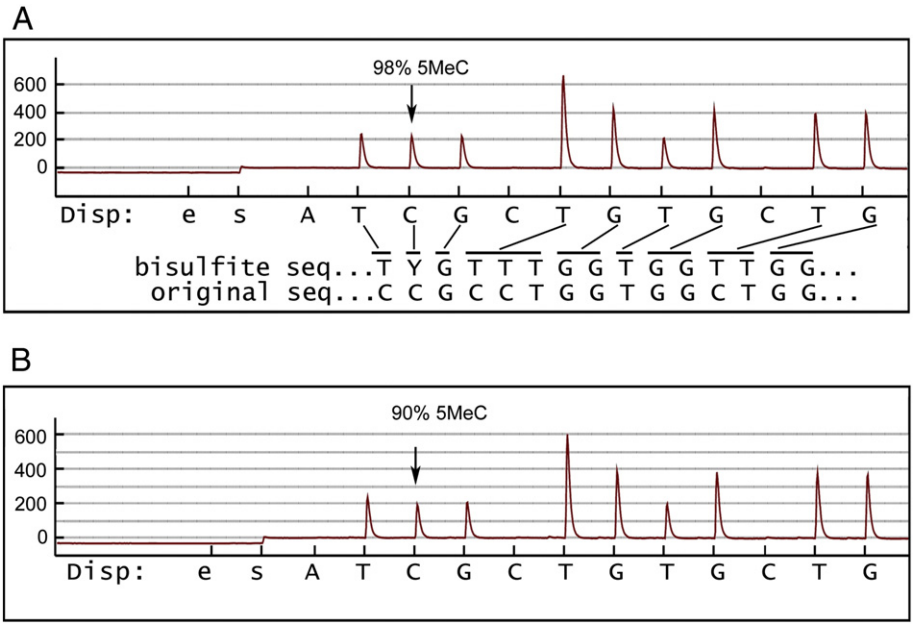

Fig. 1. Methylation analysis of the exon 4 of the *HSD17B10* gene. The bisulfite sequencing of the chromosome DNA from a normal male is displayed in (A); and that from a normal female in (B). The ordinate shows the relative light unit detected after dispensation of each nucleotide substrate. The abscissa shows the dispensation order ("e" and "s" are controls). The "bisulfite" line shows the predicted sequence after modification. "Y" represents C or T. Lines connecting the dispensed nucleotide to the bisulfite sequence indicate how the light signal represents the modified sequence. The height of the signal is proportional to the number of nucleotides at that position.

residual HSD10 activity which is consistent with the highly disruptive nature of the amino acid substitution in the p.R130C mutant protein.

2. Materials and methods

2.1. Chromosomal DNA

Chromosomal DNA was isolated from blood samples of normal individuals (one male and two females) with the FlexiGene kit (Qiagen, Volencia) and used as the template for the *HSD17B10* gene-specific methylation analysis. This study was approved by the Institutional Review Board of NYS Institute for Basic Research in Developmental Disabilities and the human DNA samples were obtained in conformance with their guidelines and included the acquisition of written informed consent for genetic testing.

2.2. Bisulfite sequencing

Bisulfite modification of human chromosomal DNA and pyrosequencing analysis (Sheridan et al., 2011; Tost et al., 2003) of a 118 bp segment of the exon 4 of the *HSD17B10* gene (AF037438) was done by the EpigenDx, Inc. (http://www.epigendx.com) using a pair of primers designed by EpigenDx, Inc., ADS2501FP and biotin-labeled ADS2501RPB, and the PSQ™ 96HS system. Pyrosequencing dispensation order was ATCGCTGTGCTG.[1]

2.3. Enzymatic data analysis

Enzymatic activity data were gathered and analyzed by reference to kinetic monographs (Segel, 1993). The statistic evaluation was accomplished by use of the Wilcoxon rank-sum test (Conover and Iman, 1981).

[1] For experimental details the ASSAY DESIGN REPORT of the EpigenDx Inc. will be provided upon request.

2.4. Tertiary structural model of HSD10(p.R130C)

Structural differences between the HSD10(p.R130C) mutant and the wild-type HSD10 were ascertained by bioinformatics analysis. The published crystal structure of human HSD10 was employed as the template structure (Kissinger et al., 2004). Data were extracted from a pdb file (1U7T) of the X-ray coordinates available from the RCSB Protein Data bank (www.resb.org) using DeepView/Swiss-pdb Viewer 4.04 (Guex and Peitsch, 1997). Substitution of the mutant amino acid was made with the "Mutating Amino-Acids" function in DeepView/Swiss-pdb Viewer 4.04.

3. Results

3.1. 5-Methylcytosine at nucleotide +2259 from ATG

Because the cytosine (C) at nucleotide +2259 from the initiation ATG of the *HSD17B10*gene (He et al., 1998) has a 3′ flanking guanine (G), it is a potential target for methylation. Surprisingly we found that it is virtually completely (93 ± 5%) methylated not only on the inactive X chromosome of the two female DNAs, but also on the active X chromosome as well. DNA from a normal male also showed >90% methylation of this cytosine (Fig. 1).

3.2. Structural changes due to the substitution of cysteine for arginine at residue 130

Residue 130 is located in the α-helix E2 of HSD10 (Kissinger et al., 2004). Since arginine 130 was present at the interface of subunits A and B as well as that of subunits C and D, its side chain takes part in the stabilization of the dimer (Fig. 2 upper panel). Its guanidinium group forms hydrogen bonds with the side chain of asparagine 127 in the same α-helix. This positively charged group also forms a hydrogen-bonded ion-pair to the side chain of glutamate 68 in α-helix D and a hydrogen bond with the carbonyl oxygen of histidine 109 of the neighboring subunit. The p.R130C mutation eliminates three hydrogen bonds and the volume of the substituted cysteine side chain is much smaller than that of arginine (Fig. 2 lower panel).