

Research Letter

Is the Disruption of an N-Myristoyltransferase (*NMT2*) Associated With Hypoplastic Testes?

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To the Editor:

To uncover genes critical in human development, patients with congenital anomalies and apparently de novo chromosomal rearrangements are being studied through the Developmental Genome Anatomy Project (DGAP, <http://:dgap.harvard.edu>). Here, we report on cytogenetic and molecular studies of an apparently balanced t(8;10)(p11.2;p13) initially thought to be de novo, identified in a Caucasian male (designated DGAP016) at age 26 in 1975, who presented at a regional hospital with “hypogonadism and testicular atrophy”. He had “orchitis” at ages 15 and 17 years. Testosterone levels in serum were extremely low (1.4 nmol/L normal range 14–18). Other endocrinological tests were normal except for very mild hypocalcemia. The patient was started on a testosterone derivative, Sustanon depot, 250 mg by injection every other week. The patient continued to visit the clinic of the hospital until March of 1996 at which point he was referred to a local health care center closer to his home. During a telephone follow up in 2006, the patient indicated that he was married, childless, and well.

The mother and maternal grandmother of DGAP016 were found to have the same translocation. His mother was the only child of her parents. DGAP016 had maternal great uncles; however, they were not evaluated. This case was obtained from the NIGMS Human Genetic Cell Repository (NIGMS GM01512).

The 8p11 breakpoint was FISH-mapped to clone RP11-473J6 (AC100818) and then further narrowed

to an approximately 20 kb interval (not shown). Abundance of highly repetitive sequence in the breakpoint area hindered further refinement using Southern blot analysis. No known genes are disrupted by the rearrangement in this region (UCSC genome browser, <http://genome.ucsc.edu>, March 2006 assembly). The breakpoint is 30 kb 3' to *UNC5D*, unc-5 homolog D (*C. elegans*) that participates in neuronal guidance, apoptosis and tumorigenesis [Mehlen and Mazelin, 2003]. The 10p13 breakpoint has been localized to BAC RP11-455B2 (AL590365), and further FISH mapping with overlapping BAC clones demonstrated disruption of N-myristoyltransferase 2 (*NMT2*) between exons 1 and 3 (Fig. 1a).

NMT2, belonging to the family of N-myristoyltransferases (NMTs), consists of 12 exons (Fig. 1b). N-myristoyltransferase (NMT_HUMAN) catalyzes the reaction of N-terminal myristoylation of many signaling proteins, oncogenes, and viral maturation proteins. It transfers myristic acid from myristoyl coenzyme A to the amino group of a protein's N-terminal glycine residue. Biochemical evidence indicates the presence of several distinct NMTs, varying in apparent molecular weight and subcellular distribution [Boutin, 1997]. Humans and mice possess two distinct but structurally similar enzymes,

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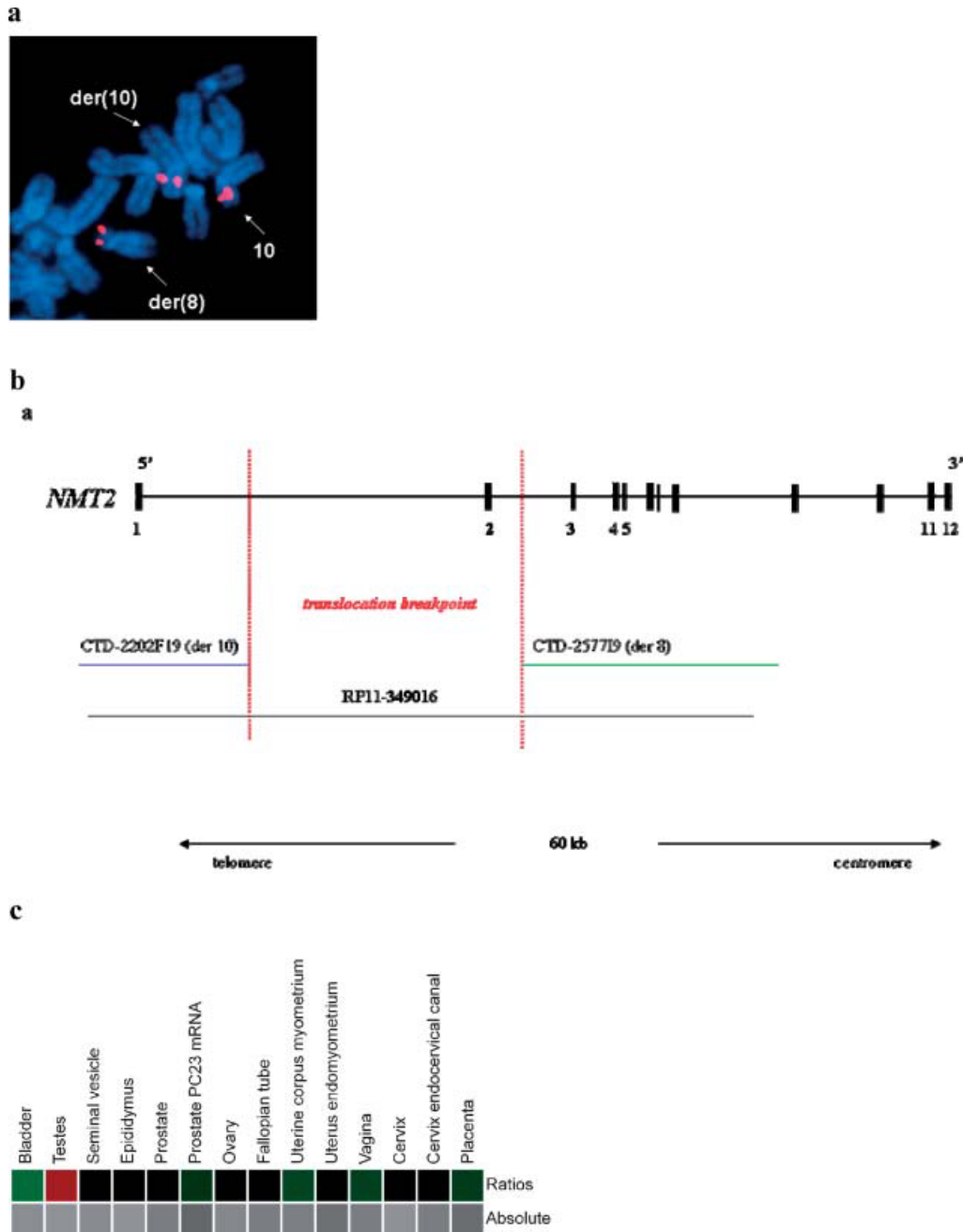


FIG. 1. Mapping the 10p13 breakpoint within *NMT2* and expression in different tissues. Cytogenetic analysis of DGAP016: (a) Representative metaphase spread following FISH with BAC clone RP11-455B2 labeled by SpectrumRed (Abbott Molecular/Vysis, Inc., Downers Grove, IL). RP11-455B2 spans the breakpoint region on chromosome 10, producing signals on the normal 10, der(10) and der(8). (b) *NMT2* exon-intron structure is shown with select exons numbered and the relevant contig below. Location of the 10p13 translocation breakpoint in DGAP016 is indicated by the red dotted vertical lines between exons 1 and 3 of *NMT2*. (c) Expression of *NMT2* in testes, ovary and other tissues. Human cDNA microarrays reveal that *NMT2* is highly expressed in testes (red), with less expression in seminal vesicle, epididymus, and prostate. Green represents lower expression. Adapted from http://genome.ucsc.edu/cgi-bin/hgGene?hgg_gene=NM_004808&hgg_prot=NMT2_HUMAN&hgg_chrom=chr10&hgg_start=15189908&hgg_end=15250663&hgg_type=knownGene&db=hg18&hgsid=81689704%20TITLE=

NMT1 and *NMT2*. The predicted 498-amino acid of human *NMT2* protein shares 77% and 96% sequence identity with human *NMT1* and mouse *Nmt2*, respectively [Giang and Cravatt, 1998].

Nmt1 and *Nmt2* are expressed in a wide variety of adult mouse tissues. *Nmt2* is mainly present in skin, liver and thymus; it is also expressed in testis but

the expression is low [Yang et al., 2005]. *Nmt2* is expressed later during mouse embryonic development (17dpc), whereas *Nmt1* expression is detected at an early time during embryogenesis (7dpc).

Recently, the two rat *Nmt* cDNAs, *Nmt1* and *Nmt2*, were cloned [Rioux et al., 2006], and contain 1491 and 1590 nucleotides, respectively, with high amino

acid identity with their mouse homologs. Studies of native protein expression revealed that the level and sizes of NMT proteins vary greatly amongst rat tissues although both *Nmt1* and *2* do not display tissue-specific expression at the mRNA level. However, human cDNA microarrays (U133A, GNF1H and normal human tissues chips, UCSC genome browser) [Kent et al., 2002] have shown that *NMT2* is highly expressed in testis, with substantially lower expression in ovary, fallopian tube, uterine corpus myometrium, vagina, cervix, and cervix endocervical canal tissues (Fig. 1c). Moreover, *NMT1* is not expressed in testis. Expression of *NMT1* is higher in uterine corpus myometrium and vagina compared to seminal vesicle and prostate tissues where the expression is low [Su et al., 2004]. In addition, data from a Northern blot (not shown) of different human tissues hybridized with a probe spanning exons 1 and 7 of *NMT2* show that the full length 2.8 kb transcript (Refseq: NM_004808.1) is highly expressed in prostate and testis compared to uterus, spleen, thymus, small intestine, colon, and peripheral blood.

Gonadal development is a complex process that involves the tightly regulated differentiation of a bipotential embryonic gonad into either testis or ovary. Once differentiated, the gonadal sex of an individual has been determined [Dewing et al., 2002]. In the human male fetus, testes develop by the 7th week of gestation and begin to secrete two hormones: anti-mullerian hormone (AMH), which induces the regression of mullerian ducts (the anlagen of the uterus, fallopian tubes, and upper vagina) upon binding to a specific membrane receptor; and testosterone, which induces the differentiation of the wolffian ducts into the epididymus, vas deferens, and seminal vesicles. This process, however, may not always proceed correctly, and may result in abnormal development. In addition, an intact hypothalamic-gonadal axis must be present for development and maintenance of steroid biosynthesis and gametogenesis, with Kallmann syndrome as the major model disorder [Bhagavath et al., 2006]. The proband's hypogonadism may be a consequence of viral insults, or as both his mother and grandmother have the same translocation, it is

possible that this is a gender-specific disorder resulting either from abnormal function of a critical signaling protein, or an interaction of cellular proteins with viral infections.

To summarize, we have found disruption of the gene for NMT2 by a t(8;10) translocation in a male with hypogonadism. This gene is highly expressed in the testis, making dysfunctional N-myristoylation a possible mechanism for testicular failure, either directly or in concert with one or more viral insults.

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