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Multiple Imprinted Genes Associated with Prader-Willi Syndrome and Location of an Imprinting Control Element

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INTRODUCTION

Our studies aim to identify the mechanisms and genes involved in genomic imprinting in mammalian development and human disease. Imprinting refers to an epigenetic modification of DNA that results in parent-of-origin specific expression during embryogenesis and in the adult. This imprint is reset at each generation, depending on the sex of the parental gametogenesis. Prader-Willi (PWS) and Angelman (AS) syndromes are excellent models for the study of genomic imprinting in humans, since these distinct neurobehavioural disorders are both associated with genetic abnormalities (large deletions, uniparental disomy, and imprinting mutations) of inheritance in chromosome 15q11-q13, dependent on the parental origin (reviewed in ref. 1). Some AS patients have biparental inheritance, consistent with a single imprinted gene (active on the maternal chromosome), whereas similar PWS patients are not found suggesting that at least two imprinted genes (active on the paternal allele) may be necessary for classical PWS. We have previously shown that the small ribonucleoprotein associated protein SmN gene (*SNRPN*), located in the PWS critical region [2], is only expressed from the paternal allele and is differentially methylated on parental alleles [3]. Therefore, *SNRPN* may have a role in PWS. Methylation imprints have also been found at two other loci in 15q11-q13, PW71 [4] and *D15S9* [5], which map 120 kb and 1.5 Mb proximal to *SNRPN*, respectively. We have now characterized in detail the gene structure and expression from two imprinted loci within 15q11-q13, *SNRPN* and *D15S9*, which suggests that both loci are surprisingly complex, with important implications for the pathogenesis of PWS.

SNRPN gene structure and allele-specific DNA methylation

By isolation of genomic phage and cDNA clones, RT-PCR, and 5' RACE, we have isolated two previously unknown 5' exons of the *SNRPN* gene, exons 0 and –1, and the promoter. The putative promoter lies within a strong CpG-island and is currently being tested for transcriptional activity. Southern analysis of DNA from peripheral blood of PWS and AS patients, digested with the methyl-sensitive enzymes NotI, HpaII, and HhaI, has shown that the promoter is completely methylated on the inactive maternal allele, but unmethylated on the active paternal allele.

The *SNRPN* gene may encode a bicistronic mRNA

Surprisingly, we have found that exons –1, 0 and 1 encode a novel open reading frame (ORF) upstream of the SmN protein ORF, which we term *SNURF* (*SNRPN* upstream open reading frame). Notably, the majority of base substitutions in this ORF from the human, rat and mouse, occur in the codon wobble positions, suggesting strong selection for protein coding function. In addition, Southern analysis detects a putative *SNURF* gene homolog in DNA from *Drosophila*, and we have isolated a *SNURF*-related gene and pseudogene from human, both latter genes mapping to chromosome Xq. Protein expression studies will test whether two proteins are translated from the *SNURF-SNRPN* transcript.

An imprinting control element (ICE) is located at the 5' end of the *SNRPN* gene

We and others have identified rare AS and PWS patients with biparental inheritance but abnormal methylation imprints at multiple 15q11-q13 loci (6-8; this report). We suggest that these imprinting mutation patients have mutations in a regulatory element, the ICE, that co-ordinately regulates in *cis* the expression of imprinted genes. There may an ICE for both the paternal and maternal chromosomes [1], or alternatively, one ICE with parental origin determining the state. Consistent with the ICE hypothesis, we have found small deletions of about 15-25 kb in several PWS patients with imprinting mutations, and these small deletions also remove the *SNURF-SNRPN* CpG-rich promoter and exon –1.

Overlapping transcripts at the *D15S9* locus and imprinting of *ZNF127* in human and mouse

A 3.1-kb cDNA and genomic clones were isolated from *D15S9*, and found to contain 2 partial 3' exons split by a 2-kb intron. This gene, termed *DN34*, was expressed in human fetal tissues only, as 7– and 11-kb mRNAs, but was not detectable in adult tissues preventing analysis of whether it's expression is imprinted. Surprisingly, an ORF encoding a putative 505 amino acid polypeptide containing a C_3HC_4 zinc-finger motif as well as

other nuclear motifs was found on the antisense strand of the *DN34* cDNA. This intronless gene was named *ZNF127* and homologs from several species have been isolated. A CpG-island precedes the *ZNF127* gene, which is expressed in all tissues in the mouse and human. Using F_1 crosses, the mouse *ZNF127* gene was shown to be expressed from the paternal allele only in brain and other tissues. Using tissue culture cells from PWS and AS patients [3], *ZNF127* is also only expressed from the paternal chromosome in the human.

CONCLUSION

Our studies show that *SNRPN* [3] and *ZNF127* are functionally imprinted and that both represent candidate genes for the imprinted disorder PWS. This is consistent with the hypothesis that multiple genes cause PWS. Combined with DNA methylation and DNA replication asynchrony data [3-8, 9, 10], these results suggest that 15q11-q13 comprises an imprinted domain. Our data have mapped a potential imprinting control element (ICE) within 15q11-q13, and suggest further complexity in gene and chromatin structure and imprinted expression in 15q11-q13.

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REFERENCES

1. Nicholls RD (1994): Am J Hum Genet 54, 733-740.
2. Özçelik T et al. (1992): Nature Genet 2, 265-269.
3. Glenn, CC et al. (1993): Hum Mol Genet 2, 2001-2005.
4. Dittrich B et al. (1993): Hum Mol Genet 2, 1995-1999.
5. Driscoll DJ et al. (1992): Genomics 13, 917-924.
6. Glenn CC et al. (1993): Hum Mol Genet 2, 1377-1382.
7. Reis A et al. (1994): Am J Hum Genet 54, 741-747.
8. Buiting K et al. (1994): Hum Mol Genet 3, 893-895.
9. Kitsberg D et al. (1993): Nature 364, 459-463.
10. Knoll JHM et al. (1993): Nature Genet 6, 41-46.

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