

Sintesi di: **Towards a molecular understanding of Prader-Willi and Angelman syndromes**

Melissa R. W. Mann, Marisa S. Bartolomei
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In mammals, both parents contribute equal genetic information to their offspring. This normal diploid complement means that most autosomal genes will be expressed from both the maternal and paternal alleles. A small group of genes defies this normal Mendelian mode of inheritance. Instead, imprinted genes are expressed from only one of the two alleles in a parent-of-origin-dependent manner. These genes are designated as imprinted since they retain the parental identity they acquired during gametogenesis. The regulation of imprinted genes is orchestrated by an epigenetic modification to DNA. As such, imprinted genes are not only susceptible to changes in the genetic sequence but also to disruptions in the epigenetic program that controls these genes.

The mechanisms controlling genomic imprinting are likely to be complex and at present are poorly understood (1-4). What is clear is that deviation from appropriate parent-of-origin-dependent expression may have dire consequences for the organism. Aberrant imprinted gene expression has now been determined to be the cause of a number of human diseases, including Prader-Willi syndrome (PWS) and Angelman syndrome (AS), emphasizing the importance of correct parental-specific expression of imprinted genes. PWS and AS are two classic examples of imprinting in humans (5, 6).

GENETIC ETIOLOGY OF PWS AND AS

PWS and AS are clinically distinct neurological disorders. Several molecular mechanisms have been identified that lead to PWS and AS, in all cases, loss of expression of at least one paternally expressed or one maternally expressed gene, respectively, at 15q11-q13 is the causative event in the development of these syndromes. The most common molecular defect giving rise to these syndromes is a large chromosomal deletion (~4 Mb) that includes a large cluster of imprinted genes (2-3 Mb) and a non-imprinted domain (1-2 Mb) (9, 10). Paternal inheritance of the deletion results in PWS while maternal inheritance produces AS. In addition to the same rate of occurrence (~70%), deletions in PWS and AS occupy the same cytogenetic position, 15q11-q13. Molecular analysis of the breakpoint ends indicates that the vast majority of deletions cluster at distinct sites (9, 11). Two breakpoint clusters have been mapped centromeric to ZNF127, with the more proximal breakpoint accounting for ~65% of deletions (12). The distal breakpoint cluster has been mapped telomeric to the P locus. The inherent instability of 15q11-q13 may be attributed to an expressed low copy repeat sequence which is located in the vicinity of the breakpoint clusters (12, 13). This sequence appears to be housed within the HERC2 gene, which encodes a gigantic HECT (homologous to E6-AP C-terminus) and RCC1 (regulator of chromatin condensation) domain protein and is located telomeric to P (13). At least seven expressed pseudogenes arising from genomic duplication of HERC2 are present in the human genome, including two copies that are located adjacent to the HERC2 locus at 15q13, three pseudogenes that were translocated to 15q11 and two pseudogenes that reside at 16p11. 2.

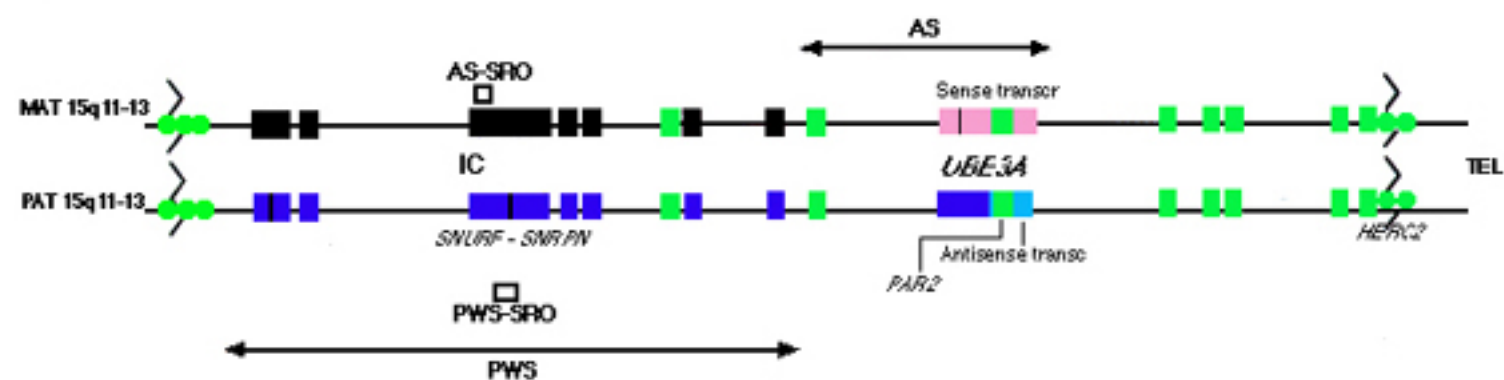


Figura1. Genetic map of human chromosome 15q11-q13. The translocation breakpoint clusters (zigzag line) associated with chromosome 15q11-q13 deletions, the critical PWS and AS regions (arrows) and the essential imprinting elements (AS-SRO and PWS-SRO) are shown. Maternal and paternal chromosomes are indicated. Genes expressed from the maternal allele are shown as pink boxes. Paternally expressed alleles are indicated by blue boxes. The silent, non-expressed allele is shown as a black box. Non-imprinted genes (i. e. expressed from both alleles) are in green. Imprinting of UBE3A, the sense transcript and the antisense transcript are tissue-specific. In the brain, the UBE3A and the sense transcript are expressed from the maternal allele while the antisense transcript is transcribed in the opposite orientation from the paternal allele. Other tissues do not express the antisense transcript but express UBE3A biallelically. The size of the antisense

transcript has yet to be determined (light blue). PAR2 is contained within the UBE3A gene (10, 41). The expressed HERC2 pseudogenes are indicated by circles.

Unequal crossover events between repeat sequences at 15q11 and 15q13 likely generate the large deletions observed in PWS and AS (14, 15). The HERC2/Herc2 gene is unlikely to be imprinted given that

1- it is expressed in somatic cell hybrids with a single maternal or a single paternal chromosome 15, [n. b. E' possibile ottenere degli ibridi che contengono come contributo umano oltre ad altri cromosomi un solo dei due crom. 15. Se il gene e' espresso dalle cellule in coltura si puo' dimostrare la funzionalita' di entrambi gli alleli, Naturalmente tramite polimorfismi si deve poter distinguere fra gli omologhi].

2- it is located in a non-imprinted region in humans and mice and

3- mutations in murine Herc2 are inherited as a recessive trait (13, 16).

In addition to large chromosomal deletions, smaller microdeletions situated upstream of the SNRPN gene have been identified (17-21). These localized deletions appear to disrupt the epigenetic program that regulates imprinted gene expression across 15q11-q13, defining a putative cis-acting imprinting control center (IC) (20). This means that while chromosome 15 exhibits a normal biparental mode of inheritance, AS patients have two chromosomes with a paternal identity (hypomethylation and biallelic expression of paternally expressed genes) and PWS patients have two chromosomes with a maternal identity (hypermethylated and silent paternal genes). Molecular characterization of the IC has established that this region covers ~100 kb of genomic sequence and consists of a bipartite structure (17, 19, 21). Deletion of the proximal portion of the IC (25-30 kb upstream of the SNRPN promoter) results in AS (Fig. 1). Recently, the AS imprinting control element has been narrowed to a region of 1. 15 kb [AS shortest region of overlap (AS-SRO) cfr figura 4 dell'articolo Genetics of Angelman syndrome]. (22). This element is hypothesized to be involved in the imprinting process that establishes the maternal epigenotype of 15q11-q13 (23, 24). In PWS, it is the distal portion of the IC that is deleted. The PWS imprinting control element spans the SNRPN promoter and exon 1 and is estimated to be <4. 3 kb in size, as determined by the shortest region of overlap (PWS-SRO) of microdeletions in PWS individuals [cfr figura 4 dell'articolo: Genetics of Angelman syndrome] (25). This element is hypothesized to function in the germline to establish the paternal identity of 15q11-q13 by switching the grandmaternal imprint to a paternal imprint (20, 24, 26). These imprinting elements act to regulate imprinted expression across a domain of 2-3 Mb. Although still poorly understood, several models have been proposed for the role that the IC may play in the imprinting process (4, 20, 22-28).

CANDIDATE GENES FOR PWS

The PWS critical region extends over nearly half of 15q11-q13 and contains multiple paternally expressed genes. (Fig. 1). In addition, several paternally expressed SNRPN upstream exons have been localized to this region and are spliced in various combinations to produce the IC transcripts (5, 10, 20, 29). Murine homologs of these genes/transcripts map to the syntenic region of mouse central chromosome 7 (5, 31-33).

Since the PWS critical region is so large it is likely that more than one paternally expressed gene is involved in the pathogenesis of PWS. However, it is uncertain which of these genes is involved as no intragenic mutation affecting expression of only one PWS gene has been described and loss of expression of a single specific candidate gene has not been correlated with PWS. Some PWS patients with rare balanced translocations show loss of expression of a subset of paternally expressed genes while others exhibit normal imprinted expression of these same genes (34-37). Recent identification of a novel protein contained within the 5'-portion of the SNRPN gene may help to explain these data (31). In two of the four translocation patients the novel protein-encoding exons, termed SNURF (SNRPN upstream reading frame), are severed while the SNRPN exons remain intact (35, 37).

[n. b.: gli autori che hanno descritto uno di questi pazienti portatore di una t(15; 19)de novo, hanno trovato che il punto di rottura sul cromosoma 15 che era quello paterno, cadeva fra l'esone 0 e l'esone 1 al di fuori della regione codificante di SNRPN. Il prodotto del gene e' stato ritrovato intatto utilizzando RT-PCR con primer degli esoni 2 e 8, probabilmente perche' espresso grazie all'azione di un regolatore del cromosoma 19 attivo su cellule in coltura su cui si era traslocato. Inoltre hanno trovato utilizzando primer degli esoni -1 e 0 il trascritto corrispondente agli esoni -1, 0 che mappano sul derivativo 15. Quello che manca in questo paziente e' proprio SNURF che e' codificato a partire dall'esone -1, fino ad 8. Per chiarire questo guardare la figura 3 dell'articolo intitolato Conversione dell'imprinting, in cui sono descritti i CDNA che costituiscono i trascritti alternativi del gene SNRPN. La figura 2 spiega i dati sopra descritti ricavati da 35].

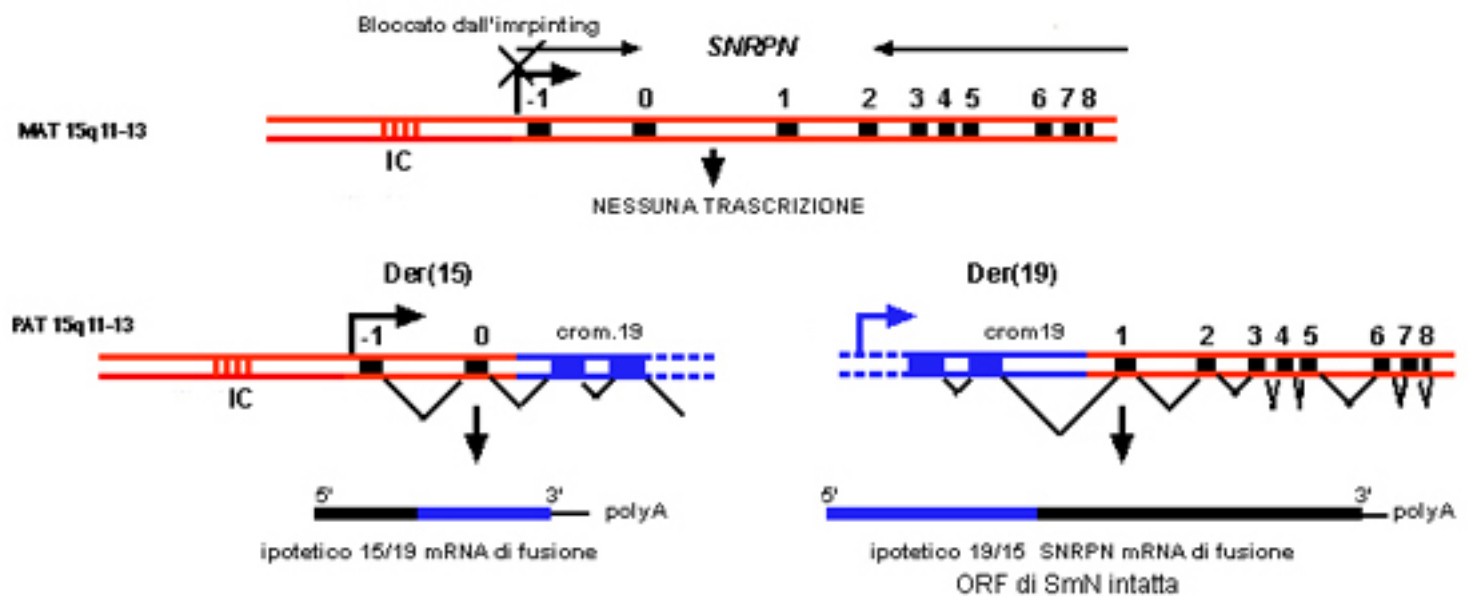


Figura1. This locus has been renamed SNURF-SNRPN to depict the atypical, bicistronic nature of this gene (i. e. a single SNURF-SNRPN mRNA transcript from which the proteins SNURF and SmN [SmN e' uno splicing factor ed e' il prodotto della ORF dall'esone 1 all'esone 8] are translated). SNURF-SNRPN is expressed from the paternal allele and, like SmN, SNURF protein is not detected in PWS patients. The SNURF exons are completely contained within the 4.3 kb PWS-SRO, perhaps suggesting an important but not exclusive role for SNURF in the genesis of PWS: in the other translocation patients the breakpoint occurs downstream of the SNURF-SNRPN locus (34, 36). Currently, at best PWS can be characterized as a contiguous gene syndrome involving multiple paternally expressed genes. Complex mutant mice carrying targeted deletion of the various mouse homologs (see below) may identify which genes play a role in PWS.

CANDIDATE GENES FOR AS

In contrast to PWS, ~20% of AS cases are predicted to have intragenic mutations in the putative AS gene. The E6-AP ubiquitin-protein ligase (UBE3A) gene has been strongly implicated as the AS gene since genomic mutations and an inversion breakpoint have been identified in UBE3A in AS patients (38-40). In addition, the entire 120 kb genomic UBE3A sequence is contained within the 250 kb AS critical region (Fig. 1) (41). UBE3A/Ube3a exhibits tissue-specific imprinting with preferential maternal expression in sub-regions of the brain in humans and mice (42-45). A fair number of AS patients have now been examined for the presence of mutations in UBE3A. Only 30% of AS patients in this class had loss-of-function mutations in UBE3A (46, 47). The remaining 70% of patients had no identifiable defect in UBE3A. While this may be explained by misdiagnosis of AS, it is also possible that additional genes or silencing elements in the AS critical region are involved in the pathogenesis of AS. Recently, additional transcripts have been detected in this region, including a 3.5 kb sense transcript whose promoter is embedded in the 3'-UTR of the UBE3A gene (48). This transcript is preferentially expressed from the maternal allele in brain. Mutations in this candidate transcript/gene could account for the remaining patients not possessing mutations in UBE3A.

In addition to the sense transcript, an antisense transcript has also been identified (48). This transcript begins ~6.5 kb from the UBE3A stop codon, includes sequences corresponding to the sense transcript and is coincident with the 3'-half of UBE3A. The size of this transcript has yet to be determined. In brain, the antisense transcript is expressed predominantly from the paternal allele. A competition model has been proposed where transcription of the antisense gene would exclude paternal allele-specific UBE3A expression (4, 48).

EPIGENETIC MODIFICATION OF 15q11-q13

It is widely believed that the mechanism governing the imprinting of the PWS/AS domain is likely to involve parent-of-origin-specific epigenetic modification of the DNA. Studies have focused on epigenetic modifications such as allele-specific DNA methylation, replication timing and chromatin structure. SNRPN methylation patterns, which have been studied in the most detail, are likely to be an important part of the mechanism that controls imprinting at this locus. Both the human and mouse genes are hypermethylated on the inactive maternal allele at the promoter and first exon (corresponding to the PWS-SRO) and in the 3'-portion of the gene on the active paternal allele (18, 32, 52, 56-59). There is also evidence to suggest that these methylation patterns are established in the gametes, thereby representing candidate sequences for conferring the allelic imprinting mark (57, 58).

The well-established association between regulatory elements and nuclease hypersensitivity has led investigators to use

chromatin analyses to search for regulatory elements. [n. b. La sensibilita' alle nucleasi e' legata probabilmente al fatto che nei geni trascritti la cromatina e' meno condensata per permettere l'azione dei fattori necessari alla trascrizione, rendendo il DNA piu' facilmente accessibile all'azione degli enzimi]. Consistent with the methylation patterns observed throughout the SNRPN locus, the promoter and exon 1 (PWS-SRO) are hypersensitive to nucleases on the paternal allele. While the paternal allele-specific hypersensitivity could merely reflect the transcriptionally active state of the SNRPN gene, it is also possible that this part of the IC is controlling the paternal-specific epigenotype. Interestingly, the AS-SRO is hypersensitive to nucleases on the maternal allele (60). Thus, the nuclease hypersensitivity of the PWS-SRO and AS-SRO in the IC supports the proposal that these regions serve to mediate the switching between paternal and maternal epigenotypes.

SNRPN and the PWS/AS region display other properties that are characteristic of imprinted genes.

- 1- Many genes in the region harbor repetitive elements. For example, the first intron of the mouse and human SNRPN genes contains structurally conserved G-rich repeats (32, 59). As proposed for other imprinted genes, the repeats may be involved in establishing the imprinting or DNA methylation patterns of this gene (61).
- 2- The human chromosome 15 homologs replicate asynchronously and exhibit preferential association during late S phase of the cell cycle (62, 63).

MOUSE MODELS OF PWS AND AS

The first candidate mouse models for PWS and AS were described by Cattanaach et al. (65, 66). They used intercrosses between mice harboring translocations to derive progeny with uniparental disomy of the PWS/AS homologous region in mice. While these mice display phenotypic characteristics indicative of the two syndromes, the large region of uniparental disomy makes it difficult to assign the phenotype to individual genes.

Yang et al. have generated a mouse model for PWS and IC mutations by using homologous recombination in embryonic stem (ES) cells to engineer a deletion of the Snrpn gene and the region that corresponds to the distal portion of the IC (including the PWS-SRO) (67). Chimeric males with a mutation in their maternally derived allele were not capable of reversing the maternal epigenotype of the mutant allele in their germline and, as such, the progeny inheriting this allele from the chimeric male failed to express the genes normally transcribed exclusively from the paternal allele (i. e. Snrpn, Zfp127, Ndn and Ipw). These mice displayed some of the phenotypes characteristic of PWS. Thus, in addition to generating a mouse model of PWS, this mutation mimics human IC mutations, indicating that the position and hypothesized role of the IC are conserved between mice and humans.

The mouse models and data from PWS patients provide compelling evidence that perturbations in multiple paternally expressed genes are involved in the pathogenesis of PWS. In agreement with this proposal, mice that harbor an intragenic deletion of the Snrpn gene are phenotypically normal (67). Thus, perturbations in Snrpn gene expression alone are not sufficient to cause PWS symptoms in the mouse. To prove that more than one gene is involved, mice with mutations in multiple paternally expressed genes will have to be derived.

ES cell technology has also been used to generate mutations in two candidate genes for AS, Ube3a and the b3 subunit of the GABAA receptor (Gabbr3). Mice with maternal deficiency of the imprinted Ube3a gene display a phenotype that mimics AS, including motor dysfunction, inducible seizures and a context-dependent learning deficit (45). Although absence of an imprinted gene that is expressed exclusively from the maternal allele (i. e. UBE3A) is the most likely AS candidate gene, the non-imprinted b3 subunit of the GABAA receptor (GABRB3) gene is located in the large deletion region of the majority of AS patients and may also contribute to the phenotype. Mice lacking the Gabrb3 gene exhibit seizures, learning and memory deficits, poor motor skills and hyperactivity, features that are common to AS (68, 69). Additionally, heterozygous Gabrb3 mutant mice exhibit a partial phenotype, suggesting that haploinsufficiency of the GABRB3 gene could be a contributing factor in AS deletion patients.

During the last year, many research contributions have advanced our understanding of the pathogenesis of PWS and AS. Identification of the HERC2 gene and pseudogenes provided a molecular explanation for 15q11-q13 being a hotspot for recombination and thereby generating one of the most common interstitial deletions in humans. The lack of pseudogenes also explains why the same chromosomal instability is not observed in the mouse.

Many questions arose from the data. A cursory glance at the genetic maps reveals a paucity of genes/transcripts in mouse when compared with humans. 1- Will the novel sense and antisense Ube3a transcripts be found in mouse? More to the point, if the IC contains information that confers the primary imprinting mark, will sequence identity between humans and mice be revealed at the genomic level?

2- While sequence conservation is certainly an attractive idea, no conserved cis-acting elements have yet been identified in other imprinted genes. The generation of murine mutations that mimic imprinting mutations in humans suggests functional conservation of the IC. Will the upstream Snrpn exons that compose the IC transcripts be conserved in the mouse?

3- Alternatively, does anonymous transcription play a role in determining parental identity? Nuclease hypersensitivity studies may help to determine whether a similar underlying chromatin structure exists in the mouse. With the advent of murine PWS and AS models, these questions may be addressed, providing greater understanding of the complex

molecular mechanism that governs PWS and AS imprinted gene expression.

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