RESEARCH LETTER

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Molecular Classes in 209 Patients With Prader–Willi or Angelman Syndromes: Lessons for Genetic Counseling

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TO THE EDITOR:

Prader–Willi (PWS; OMIM 176270) and Angelman (AS; OMIM 105830) syndromes are clinically and genetically distinct congenital neurobehavioral disorders, each occurring at a frequency of 1/10,000–30,000 births. PWS and AS were the first recognized human genomic imprinting disorders and the first recognized as resulting from uniparental disomy (UPD). Both syndromes are caused by several different genetic alterations in chromosome region 15q11.2–q13, which can be classified in three or five molecular classes depending on diagnosis and genetic mechanism. The molecular classes of PWS are I, II, or III, each of which can be subdivided into "a" or "b." The molecular class Ia accounts for 65–75% of PWS due to a 5–6 Mb deletion; class Ib reflects a chromosomal rearrangement that involves chromosome 15 and is observed in <1% of PWS patients. On the other hand, classes IIa and IIb represent 20–30% and <1% of patients with PWS, respectively, and in both the genetic mechanism is maternal UPD, although in class IIb this disomy may be due to a Robertsonian translocation. Finally, the classes IIIa and IIIb account for <0.5% and 2% of PWS, respectively; in the first one, the mechanism is a deletion in the imprinting center (IC) and for the second one, the mechanism is an epimutation of the IC. Regarding AS, the molecular classes are I, II, III, IV, or V, and only the first three can be subclassified into "a" or "b". The percentages and mechanisms for these three classes are similar to those for PWS, except for class II, which explains fewer cases. Class IV denotes those patients with a mutation in *UBE3A* and class V groups all patients with an AS phenotype, but whose molecular diagnosis could not be determined. The molecular classification in both syndromes is important because it implies a recurrence risk for PWS/AS patients' sibs. For example, the recurrence risk for Ia class is less than 1% but it may be as high as 100% in families whose child was diagnosed as class IIb. Thus, a correct determination of the molecular class in PWS and AS patients is essential for accurate genetic counseling for parents seeking a future pregnancy [Jiang et al., 1999; Cassidy et al., 2012].

The Laboratory of Genetics and Metabolic Diseases at the Institute of Nutrition and Food Technology (INTA), University of Chile, is one of the largest laboratories for clinical and molecular diagnosis in Chile, including PWS and AS. It has the greatest experience diagnosing these syndromes, having performed *SNRPN* methylation studies via methylation-sensitive PCR analysis (MS-PCR), since 1998, for samples referred from the entire country. After a positive test result, our laboratory offers fluorescence in situ hybridization (FISH) using the LSI Prader–Willi/Angelman probe *D15S10* (Vysis). If there is no deletion, microsatellite marker analysis with small tandem repeats (STRs) is performed to define the existence of UPD or IC defects in PWS and AS patients. Our laboratory also evaluates mutations in exons 9 and 16 of *UBE3A* of AS patients not diagnosed through MS-PCR. We reviewed the results of samples referred from 1998 to 2013, from patients with suspected cases of PWS or AS. We also surveyed clinical geneticists and other laboratories in Chile that perform similar kinds of PWS and AS analyzes, asking for their results in order to clarify the PWS/AS patients' molecular class diagnosed in our laboratory. Several of these laboratories evaluate only the Prader–Willi Syndrome Critical Region using FISH with probe *D15S10* and one performs Methylation-Specific Multiplex Ligation-dependent

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Probe Amplification (MS-MLPA) for PWS/AS and microsatellite markers analysis as well.

We found 209 patients who were positive for MS-PCR; 152 patients were diagnosed with PWS (72.7%), of which 79 were females (52%). Concerning AS, 57 individuals were identified (27 were males) and none were diagnosed through *UBE3A* analysis. The median age of diagnosis in PWS patients was 0.83 year (Interquartile range [IR] 0.1–5.0 years), which was significantly earlier than in AS patients (median age 3 years, IR 1.6–5.7 years; $P < 0.001$). However, there were no statistically significant differences between males and females by diagnosis or age of presentation. Overall there was a high prevalence of patients with either an AS or PWS diagnosis that appear to have had no genetic studies specific for AS/PWS (Fig. 1). Considering only studied patients, the largest molecular class was Ia for both syndromes (50.8%, n = 33 for PWS; 88%, n = 14 for AS). We added the additional classification, UPD/IC, as several patients were only studied by FISH or MS-MLPA after MS-PCR. This group represented 43.1% (n = 28) of PWS patients and 12.5% (n = 2) of AS patients and this difference was statistically significant ($P = 0.007$). Other comparisons between both syndromes were not relevant.

These results deserve several comments. First, patients with AS manifest a generally normal prenatal and birth history, with normal head circumference and absence of major birth defects. Feeding difficulties may be present in the neonate and infant, but are not usually more severe than in PWS patients, which delays the diagnosis [Williams et al., 2006; Cassidy et al., 2012]. On the other hand, the molecular class of most of the patients was not studied, which reflects that many of them could not receive accurate genetic counseling. Although the reasons for this lack of determination were not investigated, the Chilean public health system does not cover the costs of successive analyzes other than karyotype and MS-PCR. This, in part, explains why our laboratory's approach does not complete the full axis of AS genetic evaluation sequencing all *UBE3A* coding exons, and therefore, none AS patient with mutations in exons 9 and 16 of *UBE3A* was diagnosed. Nevertheless, this study should be carried out in case of high suspicion of AS and where MS-PCR was normal. Despite this issue, our laboratory was the first Chilean laboratory in incorporating chromosomal microarray analysis since last year. Thus, in the future we may diagnose more individuals with AS/PWS referred for genetic study of developmental delay. Apart from the lack of health coverage, other clinicians stopped the evaluation after MS-MLPA analysis arguing that the test revealed an IC defect. Although the practice guidelines for the molecular testing of PWS and AS [Ramsden et al., 2010] validate this technique to detect IC deletions, MRC Holland explicitly stated in the description of SALSA MS-MLPA probemix ME028-B2 for PWS and AS that it cannot discriminate between UPD and IC defects [MRC Holland, 2014]. Therefore, it is necessary to perform microsatellite analysis in the patient and parents, and the guidelines proposed by Ramsden et al. [2010] should be updated. Finally, the much higher likelihood for women to have a non-disjunction event compared to men should explain the difference in UPD/IC classes between PWS and AS patients, and the dissimilar proportions of patients with UPD specifically. Indeed, the high prevalence of PWS patients due to UPD/IC defects might be explained by higher maternal age in Chilean women, compared to other Latin American countries [Nazer and Cifuentes, 2011]. It is well demonstrated that higher maternal age at childbirth is a predisposing factor for the development of UPD (15) mat because of increased meiosis 1 errors [Matsubara et al., 2011]. Thus, we emphasize the importance of a correct determination of the molecular class in PWS and AS patients and the effect of maternal age for accurate genetic counseling to the parents of PWS and AS patients who may be seeking a future pregnancy.

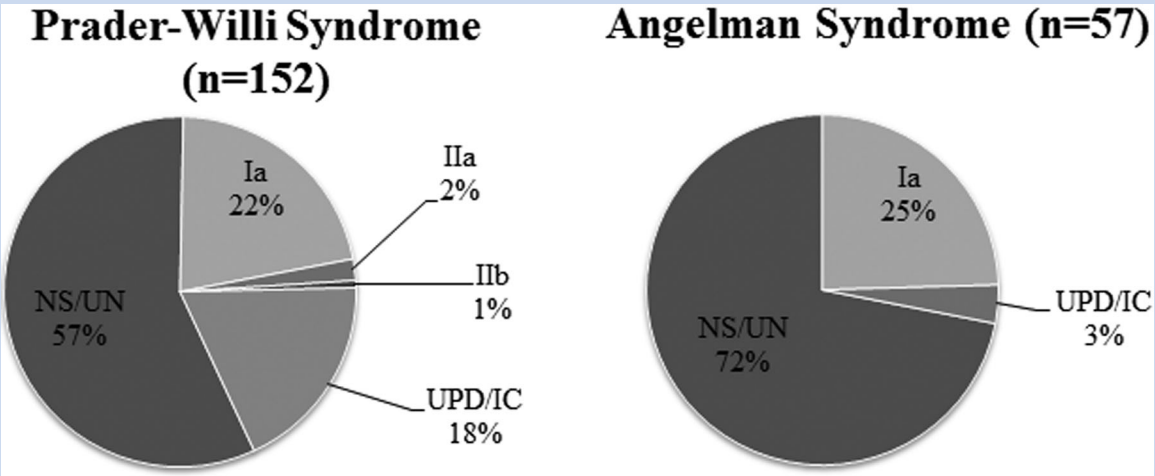

FIG. 1. Molecular classes in 209 Chilean patients diagnosed with PWS or AS. There were no PWS patients with molecular classes Ib and III, whereas there were no AS patients with classes Ib, II, III, IV, and V. NS/UN: Not studied/Unknown results of other tests, UPD/IC: Uniparental Disomy/Imprinting Center Defect.

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