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LETTERS

Prader–Willi and Angelman syndromes: genetic counseling

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We read with great interest the recent Prader–Willi syndrome (PWS) and Angelman syndrome (AS) review articles by Cassidy and Driscoll (2009)[1] and by Van Buggenhout and Fryns (2009),[2] respectively. We completely agree with most of the contents. However, we consider it important to point out certain comments appearing in the genetic counseling section of both articles.

Knowledge of the specific genetic cause is essential to offer genetic counseling. In the genetic counseling section of the PWS article, the authors mention that 'deletions 15q11q13 are sporadic except in rare cases where a chromosome rearrangement is present in the father'. Next, the authors assert that 'fathers of children with deletion should be offered chromosomal and FISH analysis of the 15q11-q13 region as the recurrence risk is significantly increased in these cases'. With this assertion it is not clear whether the authors recommend studies on fathers in those cases in which a deletion resulted from a chromosome rearrangement or in any PWS case caused by a deletion. Although chromosome rearrangements are the most infrequent genetic cause ($<1\%$), it is important to always analyze the karyotype of patients suspected of PWS to identify chromosome 15 rearrangements, plus other chromosomal anomalies.[3,4] Small supernumerary marker chromosomes (sSMCs) have been reported in $\sim 0.3\%$ of mentally retarded patients,[5] and in most cases, the sSMCs could be derived from chromosome 15, resulting in a UPD.[6] We consider it important to point out that the karyotype and FISH analyses carried out in the affected child give enough information to suspect whether the deletion comes from a chromosome rearrangement. Only in these cases are studies on fathers recommended to offer a thorough genetic counseling.

We also have certain disagreements with regard to the PWS genetic counseling in the case of a matUPD. The authors assert that 'maternal UPD 15 is typically *de novo* except if a Robertsonian translocation is present in either parent, so a chromosomal analysis is indicated. If this is normal, then the father of the child should be offered a chromosomal analysis to ensure that he does not have a Robertsonian translocation'. We do not agree with this affirmation because, if the patient chromosomal analysis is normal, it must be expected that the matUPD be sporadic. It is suitable to point out two important considerations. First, as we commented above, the patient chromosomal analysis is also important to identify the presence of sSMCs that could explain some matUPD cases. Second, if a Robertsonian translocation is identified in the patient karyotype and suspected as the origin of a matUPD, then it is the mother karyotype that must be analyzed instead of the father one.

We also do not agree with the comments made by Van Buggenhout and Fryns (2009)[2] regarding genetic counseling in those AS cases caused by a 15q11q13 deletion or by paternal UPD 15 (patUPD 15). Maternal 15q11q13 deletion or patUPD 15 could be considered *de novo* if a chromosome 15 rearrangement is not identified analyzing the patient's karyotype.

In those confirmed imprinting defect (ID) cases, an imprinting center (IC) quantitative analysis must be carried out to identify a possible IC deletion as the cause of ID.[7,8] These deletions could be sporadic or inherited from the father, who will carry the deletion on his maternal chromosome in the case of PWS, and from the mother who will carry the deletion on her paternal chromosome in the case of AS. As the IC region deleted in PWS corresponds to the *SNRPN* promoter region, the father analysis in these cases is as easy as performing the methylation test. If the father carries the deletion, he will show an abnormal methylation pattern (AS like) with a 50% recurrence risk. The methylation test is not useful to identify the IC deletion in AS patients' mothers, as the deleted IC region in these cases does not correspond to the *SNRPN* promoter region. In such PWS and AS IC deletion cases, familial studies are needed to confirm whether the IC deletion is a *de novo* event in the parent or if it is inherited from grandparents. It is important to note also the possibility of a grandparent being a mosaic germline carrier of the IC deletion. Thus, the PWS patient's uncles and the AS patient's aunts must be always warned about the possibility of having affected descendants if they are also carriers of the IC deletion. In the same context, the PWS patient's aunts and AS patient's uncles could also be carriers of the IC deletion, although they will not have affected descendants. However, the IC deletion could be transmitted to their next generations, who must be offered genetic counseling. The PWS patient's male cousins and the AS patient's female cousins will have a 50% recurrence risk if they have inherited the IC deletion from his mother or her father, respectively.

Finally, we want to emphasize that prenatal diagnosis is recommended to rule out a possible germ line mosaic carrier of a 15q11q13 deletion[9,10] or IC deletion in the case of PWS and AS, so as to rule out a *UBE3A* mutation in the case of AS.

As cytogenetic and genetic specialists, we go into these comments and considerations in depth, as the review articles have an important impact in the clinical and genetic community and the contents must be of as much help as possible.

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Reply to Camprubí *et al*

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Camprubí *et al* have raised important issues regarding the genetic counseling for families with children who have Prader–Willi syndrome (PWS) that bear further discussion. PWS is a complex genetic condition with multiple possible etiologies, but with all the mechanisms resulting in a loss of expression of key imprinted genes in the paternally inherited 15q11.2–q13 region. We agree with Camprubí *et al* that knowing the specific genetic etiology in individuals with PWS is essential for the appropriate genetic counseling of affected families, as we state in our review. However, we stand by our original recommendations for the specific testing of parents. Unfortunately, due to space limitations in our review article,[1] the rationale for some of our recommendations may not have been clear to all readers.

As we state in our review, for genetic counseling purposes, a chromosome analysis should be performed in individuals with a deletion, as occasionally the deletion is the result of a chromosomal rearrangement. This could have occurred *de novo* in the proband's father's gamete or the father may carry a balanced rearrangement. The statement by Camprubí *et al* 'that the karyotype and FISH analysis done in the affected child gives enough information to suspect if the deletion comes from a chromosomal rearrangement' needs further clarification. This is true in many cases, but in some cases, a parental chromosomal rearrangement may not be obvious from the proband's chromosomal and FISH analyses. For example, a paternal paracentric inversion within or including the 15q11.2–q13 region with an unequal crossing over in paternal meiosis could result in a deletion in the offspring.[2] Furthermore, a parent could be the carrier of a cryptic translocation that could result in either a child with Angelman syndrome (AS) or PWS, depending on the parent of origin of the cryptic translocation (father for PWS and mother for AS). One illustrative example would be the report of a family with a child with AS who had a deletion that was the result of an unexpected familial cryptic translocation between chromosomes 14 and 15 (break points 14q11.2 and 15q11.2).[3] The true etiology of the deletion in the patient was not identified until the mother's chromosomes were examined, thus changing the recurrence risk dramatically. Many cytogenetics laboratories would not have discerned the true etiology of this deletion from examining only the proband's chromosomes, as the typical FISH analysis for AS and PWS in many laboratories only includes *SNRPN* (or *D15S10*) and *PML* probes. For this reason, we would recommend FISH analysis in individuals with AS and PWS (and subsequently the father in PWS and the mother in AS deletion cases) to include the simultaneous use of a centromeric probe (for example, *D15Z1*), two critical region probes (for example, *SNRPN* and *D15S10*) and a distal control probe (for example, *PML* at 15q22). Two critical region probes are important for evaluating the possibility of an inversion in the parent and an atypical deletion in the proband. The use of a chromosome 15 centromeric probe is crucial in diagnosing a cryptic translocation, particularly between two acrocentric chromosomes.

We agree with Camprubí *et al* that in rare instances of maternal uniparental disomy (UPD) 15, a small marker chromosome is also present, and then it is important to examine the mother's karyotype, as it appears that these small marker chromosomes may increase the risk of nondisjunction if present in the mother. However, we state in our review that if the chromosomal analysis is normal in a proband with a maternal UPD 15 'then the father should be offered a chromosomal analysis to ensure that he does not have a Robertsonian translocation.' This is because we presume that the mother does not have a Robertsonian translocation as the two maternal chromosome 15s are normal in the proband. However, we cannot rule out whether the father has a Robertsonian translocation involving chromosome 15, which led to aberrant segregation at meiosis I, resulting in a sperm that was nullisomic for 15. This, combined with the known maternal nondisjunction, would result in an embryo with maternal UPD 15.

We also need to clarify the assertion made by Camprubí *et al* with respect to imprinting center (IC) deletions in PWS that 'if the father carries the deletion he will show an abnormal methylation pattern.' Although the DNA methylation analysis that targets the 5′ end of the *SNRPN* locus has proven to be extremely reliable since its first introduction over a decade ago,[4,5] there are rare polymorphisms inside restriction sites used for Southern blot analysis and others that affect the primer-binding sites in methylation-specific PCR techniques that can lead to a false-positive result. For this reason, Karin Buiting and Bernhard Horsthemke (personal communication), who have extensive experience with IC deletion families, recommend that an abnormal DNA methylation result in the father be confirmed to be an IC deletion by an independent method (for example, dosing analysis or sequencing), which assesses the PWS-IC region.[6,7] Alternatively, the newest version (ME028-B1) of the recently developed methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) assay by MRC-Holland has been tested and will pick up all cases of PWS-IC deletions (Karin Buiting and Bernhard Horsthemke, personal communication). The MS-MLPA assay combines both DNA methylation analysis and dosing analysis across the PWS region. The latest kit has a particularly dense probe coverage for dosing and DNA methylation analysis in the PWS critical region.

Testing for an IC deletion should be carried out in an experienced laboratory. If an IC deletion is found in the proband, then the father can be tested using the appropriate strategy to determine whether he is a carrier for an IC deletion. As we state in our review, an IC deletion 'can be familial and has a 50% recurrence risk when it is.'

Finally, we completely agree that all affected families should be aware that prenatal diagnosis for PWS is available and that germ cell mosaicism in the father is always a rare but distinct possibility. As we state in our review, various genetic tests for PWS have been validated in prenatal diagnosis, but only DNA methylation analysis at the 5′ *SNRPN* locus 'will identify the imprinting defects'.[8,9]

A thorough discussion of Best Practice Guidelines for genetic testing in PWS (and AS), which was approved by the European Molecular