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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

Genetics Quality Network Steering Group in July 2008, can be found at the following website: http://cmgsweb.shared.hosting.zen.co.uk/BPGs/Best_Practice_Guidelines.htm.

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