Erasure of *MLH1* methylation in spermatozoa—implications for epigenetic inheritance

To the Editor:

Recent discourse concerning the mode of inheritance of germline epimutations has centered on whether the altered epigenetic state may be passed directly through the gametes to offspring (bona fide transgenerational epigenetic inheritance), or whether, alternatively, the epigenetic mark is cleared in the germ cells and reimposed on the affected allele after fertilization[1–4]. Pivotal to this debate is the report by Suter *et al.* that a male individual (TT) with a soma-wide *MLH1* epimutation retained the methylation mark in a small proportion (≤1%) of his spermatozoa[5]. Spermatozoa are notoriously difficult to separate from somatic cells, and neither FACS nor microscopy provides absolute reassurance that the methylated *MLH1* alleles were not derived from the proband's somatic tissues, in which the epimutation was widespread[2]. Because a stringent molecular control was not included in the original analysis, this remained a possibility. The *SNRPN* gene is monoallelically expressed from the paternally inherited allele and methylated specifically on the maternal allele in somatic tissues, but it is unmethylated in mammalian spermatozoa[6], allowing any source of methylation from somatic cells to be traced. We have now reassessed the original spermatozoa sample from individual TT, this time including the *SNRPN* control, using two quantitative techniques: the same colony-hybridization method originally employed[5] and the more recently developed fluorescence-based real-time methylation-specific PCR f-MSP method[7]. Irrespective of technique, we detected low levels of *MLH1* methylation consistent with the original report, but we found the level of *SNRPN* methylation marginally exceeded that of *MLH1*, indicating the methylation most likely derived from residual somatic DNA (data presented in the Addendum to the original report by Suter *et al.*). Futhermore, we acquired a new spermatozoa sample from individual TTthat was devoid of *MLH1* methylation using f-MSP. In light of this new evidence, we suggest that the data originally reported should not be proffered as evidence that *MLH1* methylation persists in male germ cells[5]. The data presented here, and in our more recent study[7], show that the spermatozoa from individuals with soma-wide *MLH1* epimutations are, in fact, devoid of *MLH1* methylation. The epigenetic manifestations of this defect are thus likely to be cleared from the affected allele during spermatogenesis. This demethylation is most likely to occur contemporaneously with the removal of epigenetic marks from imprinted genes in primordial germ cells. Although this argues against the notion that *MLH1* epimutations are directly transmissible through the male germ line through 'transgenerational epigenetic inheritance,' it does not necessarily nullify the risk of paternal transmission of this defect to offspring. Epimutations may be re-established postzygotically, albeit associated with a single parental allele and present in all somatic cell types. A precedent for this caveat is the paternal transmission of microdeletions of the *SNURF-SNRPN* imprinting center on the paternal 15q12 allele, which cause the normally unmethylated allele to assume the fully methylated imprint of the maternal allele across the imprinted 15q12 region in the somatic tissues of offspring, resulting in Prader-Willi syndrome[8]. In such cases, the spermatozoa of fathers harboring the imprinting center microdeletions are unmethylated, and the allele only becomes fully methylated postzygotically[6]; hence the methylation status of the spermatozoa themselves provides no indication of transmission risk. Although we have shown that *MLH1* epimutations are unlikely to be caused by a fully penetrant genetic alteration *in cis*[7], the possibility that genetic interplay between a *cis*- or *trans*-acting modifier predisposes the allele to epimutation cannot be ruled out at present. Our recent report of stochastic maternal transmission of an *MLH1* epimutation indicates that the 'signal' underlying this defect is passed directly through the female germ line to a proportion of offspring[7]. Whether this takes the form of a co-inherited genetic factor or a fundamental epigenetic aberration in the oocyte remains to be elucidated. Should the latter be the case, the risk of inheritance of *MLH1* epimutations will depend on the sex of the transmitting parent, and we would expect to see a significant bias in the rate of maternal transmission. However, until the mechanism underlying *MLH1* epimutations is identified, we caution against using the epigenetic state in spermatozoa as a predictor of paternal inheritance risk. The children of epimutation carriers should be offered testing irrespective of the sex of the affected parent.

Megan P Hitchins & Robyn L Ward

School of Medicine, University of New South Wales, Sydney NSW 2052, Australia. Correspondence should be addressed to R.L.W. (robyn@unsw.edu.au).

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