and allele-specific distribution[3]. At any rate, the epimutation is also present in germ cells. Horsthemke's intimation that contaminating somatic cells were responsible for its apparent presence in spermatozoa of individual TT[3] is at odds with the evidence. As stated[3], we sorted semen by FACS and then checked the sorted spermatozoa for purity by FACS and microscopy; contamination with even a very small fraction of the 1% found to carry the epimutation would have been obvious. The more recent demonstration of inheritance of the *MLH1* epimutation[4] provides further compelling evidence that it is present, and can be maintained, in germline cells.

The *MLH1* germline epimutations are significant because they provide evidence that intact loci can undergo stable epigenetic silencing in the animal germline (as they can in plants). This has potentially broad biological significance. Unlike the other examples of transgenerational epigenetic inheritance that we and others have studied, the *MLH1* epimutation is not associated with a transgene[9] or a retroelement[10]. We sought these epimutations as a test of a specific hypothesis: that constitutive activity of mechanisms that initiate and maintain epigenetic silencing in higher eukaryotes will result in sporadic germline epimutations. Thus far, the results are at least consistent with that hypothesis, despite all attempts to fit them into the procrustean bed of mendelian genetics.

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The authors declare no competing financial interests.

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Leung *et al.* reply:
Regarding the heritable germline (soma-wide) methylation of *MSH2* (ref. 1) and *MLH1* (ref. 2) genes observed in the families reported by us and others, we agree that although they may represent an example of transgenerational epigenetic inheritance, the possibility of an underlying genetic change that causes the heritable methylation cannot be excluded. Whitelaw *et al.* specified the strict requirements necessary to document transgenerational epigenetic inheritance in non-human organisms; these include experimental conditions that can never be recapitulated in human studies. Interestingly, the mode of inheritance in two new families with germline *MLH1* methylation recently reported by Ward and colleagues[2] is in sharp contrast to our reported family with *MSH2* methylation. As Martin *et al.* pointed out, the new results[2] provide stronger evidence in support of transgenerational epigenetic inheritance for *MLH1*. However, the results may still be explained by low penetrance or by the presence of an unlinked modifier. Thus, it is almost impossible to unequivocally document a case for transgenerational epigenetic inheritance in humans even if such a phenomenon exists. Despite the difficulties in distinguishing between primary and secondary epimutations, our study[1] as well as those by Ward and colleagues[2,3] have demonstrated the diverse spectrum of heritable germline methylation, showing, for instance, a range of propensities for transmission to offspring and variation in the degree of mosaicism in tissue distribution of the methylated alleles. The existence of these forms of heritable methylation (either due to incomplete erasure in germ cells or subsequent re-establishment in somatic development), as distinguished from heritable germline mutation, and their role in the causation of the most common hereditary cancer syndrome in humans, deserves recognition. Specifically, the problems they create in genetic diagnosis and their possible role in the causation of other forms of disease that may mimic polygenic or complex traits warrant further study. Also, a common unique feature for these heritable epimutations is the presence of an intact underlying gene that may make modification of the epigenetic states possible as a therapeutic strategy. For example, it has been shown in animal studies that maternal diet during pregnancy may modulate methylation status at epigenetically labile promoter regions in offspring[4]. Last, development of a unified language that can be easily understood by both geneticists and epigeneticists may be necessary to classify these phenomena.

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