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INVITED EDITORIAL
Parental Origin Effects, Genome Imprinting, and Sex-Ratio Distortion: Double or Nothing?

Carmen Sapienza

The Fels Institute for Cancer Research, Temple University School of Medicine, Philadelphia

In this issue of *The American Journal of Human Genetics*, Carlson et al. (1994*a*) report two interesting and potentially important findings on the development of multiple endocrine neoplasia type 2B (MEN 2B). This disease, together with multiple endocrine neoplasia type 2A (MEN 2A) and familial medullary thyroid carcinoma (FMTC), is one of three disorders for which medullary thyroid tumors are a common clinical feature and mutations in the RET proto-oncogene a common genetic feature. While a number of different mutations in RET are associated with MEN 2A and FMTC (Donis-Keller et al. 1993; Mulligan et al.1993*a*, 1993*b*), all MEN 2B mutations characterized to date have yielded the same amino acid substitution (threonine for methionine) at RET codon 918 (Carlson et al. 1994*b*; Eng et al. 1994; Hofstra et al. 1994).

The homogeneous nature of RET mutations in MEN 2B and the existence of polymorphic markers that are very tightly linked to MEN 2B have enabled Carlson et al. to determine unequivocally whether the de novo mutation occurred on the maternal or the paternal chromosome. The result obtained by these investigators is striking: all 25 of the mutations analyzed occurred in the paternal allele. Therefore, MEN 2B may be added to the list of neoplastic diseases that already includes Wilms tumor, bilateral retinoblastoma, osteosarcoma, embryonal rhabdomyosarcoma, and neurofibromatosis type I, for which the relevant genetic alteration occurs either predominately or exclusively on the paternally derived chromosome (reviewed in Sapienza and Hall, in press).

Carlson et al. entertain two possible explanations for their finding. The first is based on the difference in the number of cell divisions required to form the male versus the female germ line. The authors calculate that, in their study population, a mean of 496 cell divisions should have occurred in the collective male germ line, while only 23 cell divisions would have taken place in the female germ line. Assuming equivalent rates of mutation per cell division in each sex, the authors calculate that only one case of MEN 2B associated with mutation of the maternal allele should have been observed in their study. Their findings may be interpreted, then, as a simple result of the arithmetic difference in the physiology of male and female germline formation. The authors present data in support of this hypothesis by demonstrating that the mean age of fathers in their study population is significantly greater than the mean age of fathers in the general population, during the period when the affected individuals reported in their study were born.

In the face of these data, the alternative hypothesis—that the strong parental origin effect observed in this disease may be the result of genome imprinting—becomes much less compelling. The authors point out that imprinting, "in the classic sense" (the description of a poorly understood process, whose existence has been appreciated for only a decade, as "classic" demonstrates that scientific writing need not be humorless), involves the inactivation of an allele as a consequence of having passed through gametogenesis in one parent (for discussion, see Barlow 1994; Sapienza and Hall, in press). In contrast to the simple expectations of this model, the authors demonstrate that all of the individuals examined carry a RET allele that has been altered in sequence and fail to find any evidence that RET is monoallelically expressed in tumors. Although the authors are careful to point out that failure to find monoallelic expression does not prove that RET is not imprinted, these data will provide a large measure of comfort to those who prefer a standard (classical?) explanation for the parental origin effects observed in MEN 2B, as well as Wilms tumor, retinoblastoma, osteosarcoma, embryonal rhabdomyosarcoma, and neurofibromatosis type I.

If Carlson et al. had been content to provide us with only this installment of the story, most members of the human genetics community, myself included, would be tempted to pronounce the paternal mutation/genome-imprinting debate dead. However, interesting stories often have a way of becoming more interesting, and, at this point in their narrative, the authors detail their second unusual finding: of the 43 patients presenting with de novo disease, 28 are female and 15 are male. This departure from the sex

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Address for correspondence and reprints: Dr. Carmen Sapienza, The Fels Institute for Cancer Research, Department of Pathology and Laboratory Medicine, Temple University School of Medicine, 3307 North Broad Street, Philadelphia, PA 19140.

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ratio expected for a trait that is caused by a mutation in an autosomal gene does not appear to be the result of chance, because a similar level of sex-ratio distortion has been reported by another group (Takami et al. 1994). Together, these reports include 45 females and 24 males affected by de novo MEN 2B.

Sex-ratio distortion, per se, is interesting, but it need not have an "interesting" cause. Carlson et al. propose two explanations, although, to be fair, they do not argue very strongly in favor of either: (1) sperm bearing both a Y chromosome and a mutant RET allele are less fit than their X/RET-bearing counterparts; or (2) mutant RET alleles have a greater negative effect on male embryos than on female embryos, when paternally derived.

Both of these hypotheses are, a priori, plausible, but neither one derives great support from the pedigrees presented. If Y/RET-bearing sperm are less fit than X/RET-bearing sperm, one might also expect to observe sex-ratio distortion in favor of females, among the offspring of males who carry a germ-line RET mutation. The available numbers are small (affected males have 10 affected female and 8 affected male offspring; see both fig. 1 in Carlson et al. [1994*a*] and additional pedigrees supplied by P. J. Goodfellow), but there is little indication that this expectation will be met. Of course, the proviso that another gene, not linked to RET, may allow these particular males to transmit the Y/RET combination with the expected frequency must be attached to this criticism. These data also provide limited evidence against the authors' contention that RET mutations have a greater negative effect on XY embryos than on XX embryos, whenever the mutation is paternally derived. Examination of a larger number of pedigrees may help to clarify these possibilities.

If all MEN 2B mutations have a greater negative effect on the survival of XY embryos than on that of XX embryos, irrespective of which parent transmits them (this possibility is not the contention of Carlson et al., but I consider it here because males with inherited disease appear to have as many affected male offspring as affected female offspring), this effect must be hypothesized to operate only in embryos that harbor the de novo mutation. As mentioned above, there is no apparent distortion of the sex ratio when the mutant RET allele is inherited from an affected male. When the trait is inherited from an affected female, twice as many affected males as affected females are observed. Again, the numbers are small (9 affected females vs. 18 affected males; see both fig. 1 in Carlson et al. [1994*a*] and additional pedigrees supplied by P. J. Goodfellow), but the direction of the distortion is the opposite of that predicted by this hypothesis. Although these latter numbers do not differ statistically from a sex ratio of 1:1, they are not significantly different from a sex ratio of 1:2, either; and if there is a 2:1 excess of females among the ~50% of cases that are categorized as de novo, it is an algebraic requirement that an excess of affected males must appear among individuals with inherited disease, if the overall sex ratio of MEN 2B cases is truly 1:1.

In some respects, the observations reported by Carlson et al. resemble those reported for bilateral retinoblastoma (Naumova and Sapienza 1994). If there is a common thread that connects the unexpected observations on the genetics of these two diseases (besides the bias in parental origin of disease alleles), it is the apparent distortion of sex ratio among founders and next-generation offspring. In bilateral retinoblastoma, the direction of the distortion in the founder generation is opposite (in favor of males in retinoblastoma, but in favor of females in MEN 2B) and the magnitude of the distortion is much smaller, but in the subsequent generation, in both diseases, sex-ratio distortion is observed among the affected offspring of the founder sex who appear in greater numbers and not among the affected offspring of the founder sex who appear in smaller numbers.

Two aspects of this comparison are of great potential interest. The first is the fact that sex-ratio distortion among the affected offspring of the founders occurs regardless of whether the founder sex present in larger number is male (in retinoblastoma) or female (in MEN 2B). The second point concerns the significance of the 1:2 (female: male) ratio observed among the affected offspring of the founder sex who appear in greater numbers in both diseases (in retinoblastoma, males with de novo bilateral disease give rise to 36 affected females and 72 affected males; Naumova and Sapienza 1994; A. Naumova and C. Sapienza, unpublished data) and the 2:1 ratio among the founders themselves in MEN 2B. In the absence of an additional disease-associated gene and any proved sex-specific, embryonic-lethal effect of MEN 2B or RB-1 mutations in the heterozygous state (the available pedigrees provide some evidence against this hypothesis), there are no standard genetic models that predict such a ratio for an autosomally determined trait.

It is fair to say that these observations pose more questions than they answer, although one can hardly fault the investigators for generating provocative data. For example, it is difficult to say (because of ascertainment bias, pedigrees that may be incomplete, etc.) whether the sex-ratio distortion observed in the disease pedigrees applies to all offspring or only to those who are affected—i.e., does the disease trait merely serve as a marker for some other defect in these families, or is the sex-ratio distortion accompanied by concurrent transmission-ratio distortion for the chromosome that carries the mutant allele? Sex-of-offspring–specific transmission-ratio distortion has been observed for autosomal markers in the mouse (through interspecific F_1 hybrid females), but, in this case, the transmission-ratio distortion was not accompanied by significant distortion of the sex ratio (Siracusa et al. 1991). An excess of male carriers of cystic fibrosis mutations has also been reported (Kitzis et al. 1988), but whether families in which cystic

fibrosis mutations are segregating also show sex-ratio distortion is a subject of some debate (Gloria-Bottini et al. 1980; Gibson 1988; Pritchard 1991).

These concerns are all subcategories of the more general questions of whether the processes that give rise to imprinted genes, preferential mutation of paternal alleles, parental origin effects (writ large), and sex-ratio and/or transmission-ratio distortion are unrelated or are different aspects of some process that is not recognized as genome imprinting "in the classic sense." In this regard, it may be instructive to reexamine existing genetic and epidemiological data on traits that are thought to be influenced by parental origin, with a careful eye toward the genetic classification of affected individuals, by position in the pedigree, sex of all individuals in the pedigree, and sex of transmitting parent. It may be that the overall collection of unusual observations that have been reported for many of these traits are due to completely unrelated processes, but jumping to this conclusion may be as "dangerous" (P. M. Goodfellow, quoted in Barlow 1994) as jumping to the opposite conclusion, without a more thorough investigation. The report by Carlson et al. may prove to be a very important piece of the puzzle.

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