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Invited Editorial: dNORs and Meiotic Nondisjunction

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Three papers in this issue of the *Journal* address different aspects of the continuing investigation of perhaps the oldest recognized etiologic mechanism in human genetics, i.e., nondisjunction and its resulting chromosomal imbalance. This three-pronged attack utilizes cytological (Spinner et al. 1989), epidemiological (Olshan et al. 1989), and statistical (Chakravarti 1989) methodologies to clearly demonstrate the complexity of the issue and how far we must go before understanding the problem. This editorial will concentrate on the paper by Spinner et al. (1989), which presents additional data on the possible role of nucleolar-organizing region (NOR) variants in trisomy 21.

Meiotic nondisjunction leading to both trisomy and monosomy is the most common etiologic mechanism underlying human cytogenetic abnormalities. Among spontaneous abortuses, it is responsible for 74% of the recognizable chromosomal errors (Chandley 1982), while comprising some 70% of the cytogenetic pathology observed in newborns. That a chromosomal error was the basis for Down syndrome was initially suggested some 50 years ago by Penrose (1933), and its cytologic corroboration occurred in 1959 (Lejeune et al. 1959).

Despite intense investigation during the past 3 decades, with ever-increasing levels of sophistication (i.e., chromosomal banding and special staining, computer programs, and RFLP analysis), there remains an astonishing vacuum concerning the etiology of nondisjunction. This lack of understanding may have been best expressed by Bond and Chandley (1983), who stated: "Among all the aspects of human aneuploidy which have been considered, none has received more attention than the aetiological factors which might play a role in their production. Yet on this subject alone there is probably a greater amount of equivocal data than on any other. The fact is that we are really not very much nearer today to pinning down the responsible mechanisms than we were twenty years ago when the human aneuploid conditions were first identified."

Down syndrome has always been of special interest to human geneticists, perhaps because of its historical perspective but, more important, owing to the strong correlation among advanced maternal age, increased meiotic nondisjunction, and the birth of an affected child, which provide an interesting paradigm for the investigation of chromosomal anomalies (Hassold 1985). Estimates utilizing chromosomal heteromorphisms suggest that 75%–80% of the nondisjunctional events leading to trisomy 21 are maternal in origin, while the remaining 20%–25% can be traced to an error in paternal meiosis (Juberg and Mowrey 1983). Paternal errors are distributed approximately equally between meiosis I and meiosis II, while the majority of maternal nondisjunctions result from meiosis I errors.

The cytogenetics literature is replete with possible etiologies for nondisjunction. These suggested causalities include maternal and paternal age effects, genetic predisposition, seasonal variation (preovulatory overripeness ovopathy), viral infection, parental irradiation, environmental chemical insults (including alcohol ingestion and smoking), DNA haplotypes, and intrachromosomal effects (for review, see Hassold and Jacobs 1984; Hassold 1985). In the past year alone, numerous publications have appeared concerning chemical or X-ray induction, genetic predisposition, and DNA and/or HLA haplotype–associated causes of nondisjunction. In addition, several articles have discussed the utility of DNA methodology in determining the origin of nondisjunction, leading to the hypothesis of a reduction in crossing-over (recombination) as a contributing mechanism. Because substantial information gaps still exist, it is imperative to carefully examine any and all potential factors suggested as etiologic causes of nondisjunction. Such is the case with the double NOR (dNOR), proposed by Jackson-Cook in the *Journal* in

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1985 and further analyzed by Spinner et al. (1989) in this issue.

The NOR present on the short-arm stalks of the 10 acrocentric chromosomes contains numerous copies of the genes coding for 18S and 28S ribosomal RNA. Silver staining of cytological preparations has been used for more than 100 years but was introduced for the differential staining of the human NORs in 1975 (Howell et al. 1975). The quantity of deposited silver is directly associated with the NOR activity, an apparent heritable trait as demonstrated by twin and family studies (Mikelsaar et al. 1977; Markovic et al. 1978; Weltens et al. 1985).

It was initially thought that silver-stained NORs bore no clinically associated implications. At the 1984 American Society of Human Genetics meeting, Jackson-Cook first described a possible association between the dNOR variant and nondisjunction. In subsequent studies, she reported that in the majority (three of five) of families with two Down syndrome children, the parent in whom nondisjunction occurred possessed a dNOR (Jackson-Cook and Brown 1985). More recently, several other investigators have indicated that the NOR might be a diagnostic discriminator of malignancy (Underwood and Giri 1988).

The relationship of the dNOR variant to nondisjunction has been questioned by Spinner et al. (1989). A review of the available data on this subject clearly indicates discrepant results and significant variability in the observations. The initial report described a 30% (15/50 couples) dNOR-positive rate among parents of Down syndrome children, compared with none (0/50) in control couples (Jackson-Cook et al. 1985). Furthermore, of the 41 couples in whom the parental origin of nondisjunction was determined, 13 (32%) had a dNOR, compared with only one (2%) whose chromosomes 21 had undergone normal disjunction. Cases of "nonconcordance" (i.e., nondisjunction in one parent and the presence of the dNOR in the other) will be discussed below. Hassold et al. (1987) studied parents of spontaneously aborted trisomic fetuses or chromosomally normal fetuses. Among the 150 individuals in the study (both "cases" and controls), not a single dNOR was observed! The data of Spinner et al. (1989) revealed frequencies of dNOR "positivity" of 12% among cases (parents of trisomy 21 children) and 14% in the controls, a nonsignificant difference. Our own data (Roulston et al., in press) are in agreement with those of Spinner et al. (1989) in that dNORs were demonstrated in both groups, the rates being 20% (8/40) of case and 14% (2/14) of control couples (again, a nonsignificant difference). In addition, Jones et al. (1988) have implicated the dNOR in nondisjunction of the sex chromosomes as well. In a study of 33 cases of Turner syndrome (45,X or variant karyotypes, 14 (43%) affected individuals possessed a dNOR, compared with 9% (4/41) among the controls.

An overview of these data raises several basic questions: What possible common mechanism(s) could account for nondisjunction of both chromosome 21 and the sex chromosome? Why do such gross differences exist in the frequencies of the dNOR observations among these studies? And—perhaps most puzzling—what could account for the differences between the "controls" in the various investigations?

Regarding the first question, Jackson-Cook et al. (1985) proposed nucleolar persistence and the facilitation of nonhomologous pairing and/or crossing-over among the acrocentrics (via satellite association) as the mechanisms whereby dNORs may be associated with nondisjunction. The observations of dNORs in individuals expressing only meiosis I errors and mostly in females (in whom meiosis I and acrocentric acssociations would last longer by virtue of normal oogenesis) fits well with the maternal age effect. Jones et al. (1988) stated that "in both interphase mitotic nuclei and meiotic prophase I, the sex chromosomes are often physically associated with nucleoli." This association as a factor influencing nondisjunction may provide a possible hypothesis for the nondisjunction of both chromosome 21 and the sex chromosomes. However, there is no proof that the dNOR increases the association of the sex chromosomes with nucleoli.

Hassold et al. (1987) advanced four possibilities to explain the discrepancies among the reported studies: (1) it is due to the actual definition and scoring of the dNOR—i.e., technical variability; (2) the effect of the dNOR is limited to live-born trisomic individuals (as they did not find the effect among aborted fetuses); (3) the dNOR effect is limited to only certain acrocentric chromosomes; and (4) the initial finding (Jackson-Cook et al. 1985) may have been fortuitous and coincidental. It seems unlikely that this effect is limited to live-born individuals, since no explanatory mechanism or proof to support such a selection hypothesis is currently available (as indicated by Hassold et al. 1987). While it is possible that dNOR-influenced nondisjunction might be limited to certain acrocentric chromosomes, the likelihood of such an effect is limited, given the possible analogous effect on the sex chromosomes (Jones et al. 1988). Last, that the finding of Jackson-Cook et al. (1985) may indeed have been fortuitous and coincidental gains cre-

dence, since, to date, no confirmatory report for Down syndrome has been forthcoming, despite active investigation. However, the study of Jones et al. (1988), suggesting a dNOR effect on sex chromosome nondisjunction, cannot be overlooked.

Perhaps the most perplexing component of all these studies is the difference in the frequency of dNORs among the control populations. These differences suggest variability on at least two levels—scoring difficulty (i.e., technical) and inherent interindividual variation in dNOR expression (i.e., biological). Probably the foremost reason for the intra- and interlaboratory variation lies in the definition and subjective identification of the dNOR itself. The frequency of dNOR-positive cells appears increased in prometaphase as opposed to metaphase cells, with the majority of dNORS having been noted in chromosomes with large single NOR regions. The microscopic appearance of the NOR often poses difficulties in trying to determine whether it is just large or indeed represents a double NOR; might a single large NOR with a slight indentation be interpreted as a dNOR, or is this an artifact? Finally, the dNOR frequency in a given individual is quite variable, as demonstrated by complete absence in many metaphases. Are we missing dNORs when only one positive cell is found in an individual, or is that one observed dNOR an artifact?

Obviously, extreme care must be applied to the design and analysis of any study in which the variable in question is so subjective. All slides must be coded, and the use of appropriate and concurrent controls is imperative. Who constitutes the appropriate control groups for such investigations—individuals, couples, couples with normal fertility, etc.? As so little is known concerning the frequency of dNORs in the general population, any analysis must include contingency testing. Last, one of the major difficulties of these studies is the variability of the silver-staining technique itself. Considerable inter- and intraindividual variability has been documented; the variation in the number of silver-stained NORs is 2–10/cell examined, and the range of cells demonstrating the mean or modal number of active NORs is 24%–61% (Bloom and Goodpasture 1976; Goodpasture et al. 1976; Weltens et al. 1985). Although it can be argued that technical factors most likely account for much of the observed variation, this is difficult to prove. Sozansky et al. (1984) showed that cell-to-cell differences in NOR staining are regularly seen, even with minimal experimental variation. Inactive NORs remain inactive over generations, whereas active NORs vary (i.e., they may stay active or may become inactive) (Jotterand-Bellomo and van Melle 1981). There also seems to be a natural intercellular variability of the NOR activity, which compensates for differential activity between nucleolar organizers.

If such wide variability exists for NOR expression, it is also most likely the case for dNORs. In a recent study, Perez-Castillo et al. (1986) described four related individuals with the identical 15p+ chromosome derived from a common source. Not only were there obvious differences within and among the individuals as to the amounts of silver deposited, but two individuals demonstrated a dNOR on this chromosome, a third showed but a single NOR region, and in the fourth individual the 15p+ NOR was completely inactive. These findings support the existence of significant inter- and intraindividual variation of NOR activity and specifically in the behavior of the dNOR.

In the investigation of the inherent or biological variability of the dNOR phenomenon in relationship to nondisjunction, one salient observation cannot be overlooked. Spinner et al. (1989) described three of five families in which the parental origin of nondisjunction and the presence of the dNOR are nonconcordant, i.e., the carrier of the dNOR did not experience the meiotic error. In our studies, two of six cases were similarly discordant (Roulston et al., in press). That five (45%) of 11 informational families demonstrate an almost random distribution of the two observations severely compromises the etiologic association of the dNOR with increased meiotic nondisjunction and diminishes its utility as a predictive risk factor.

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