Hum Genet (1997) 99 : 421–423

RAPID COMMUNICATION

Burton L. Shapiro

Whither Down syndrome critical regions?

Received: 15 November 1995 / Revised: 11 September 1996

For about 25 years attempts have been made to learn which segments of chromosome 21, when triplicated, are responsible for the clinical condition Down syndrome (DS). That viable human autosomal trisomic conditions involve chromosomes with relatively small amounts of G-negative bands was an early clue that G-negative bands are rich in structural genes. This was the first indication that in DS the distal half of the long arm of chromosome 21 (21q22, the major G-negative segment of 21) would be the band most associated with the syndrome. In the mid-1970s a quest began, which continues today, based on an assumption that seemed to me to be counterintuitive: that a very small segment or even locus of 21q22 would be *the* critical region responsible for the multiple findings in DS. Why would there be an expectation that only one or a few of the hundreds or thousands of loci on 21 contribute to the DS phenotype? Besides, except for the inconstant increased SOD1 activity in DS cells, no single locus has ever been demonstrated to account for any phenotypic finding in DS. Nevertheless, regions of 21 said to be "critical to" or "responsible for" findings in DS have been reduced in the literature to 21q22; 21q22.1 and 22.2; 21q22.1; 21q22.3; the SOD1 locus (proximal 21q22.1); and finally to the single subregion identified by the DNA marker, D21S55, located on subband 21q22.2 or very proximal 21q22.3. Several of these sites have been referred to as the "Down syndrome critical region," the chromosomal region that, if triplicated, results in phenotypic characteristics that permit the diagnosis of DS. Unfortunately, a minimal or critical region *cannot* be different regions identified by different investigators. Inevitably, this approach has been challenged.

Korenberg et al. (1994), whose laboratories, in a series of technically elegant and influential papers, had been among those contributing to the critical region notion, reversed that trend when they proposed that different segments over most of 21q were responsible for different phenotypic expressions of DS. They used a model (whose basis is not apparent from their text) that invoked phenomena such as penetrance and expressivity of clinical features in individuals with full and partial trisomy of 21 and employed chromosomal overlap procedures of partial trisomies. They created what they referred to as "a phenotypic map"of chromosome 21, plotting sites of "possible contribution of one, two, or three and greater numbers of loci to the phenotype." For nearly all traits thus "mapped", the potential location of chromosomal region(s) responsible for a given phenotype spanned from proximal 21q11.2 to 21qter, nearly the entirety of 21q. The work of Korenberg et al. (1994) reopened the potential contribution of most of the long arm of 21 to a role in the pathogenesis of the DS phenotype. Rather than attributing the DS phenotype in general to a restricted subsegment of 21q22 as had previous groups including their own, Korenberg and her colleagues (1994) proposed to "associate particular regions (of 21q) with specific phenotypes." In fact, particular regions of 21q were not noted in their phenotypic map of 25 features. Rather, in their analyses they demonstrated that each of the physical findings can be found with triplication of loci occurring someplace along nearly the entire long arm of chromosome 21. They suggested also that DS should be considered a "contiguous gene syndrome."

Contiguous gene syndrome describes rare conditions caused by microdeletion or (theoretically) microduplication of two or more consecutive loci that result in diverse phenotypic effects. The use of this phrase for triplication of nearly an entire long arm of a chromosome is without precedent and has no basis as applied here. The subject with DS and tetrasomy of all *but* 21q22 described subsequently by Daumer-Haas, Korenberg and their coworkers (1994) possessed no segment overlap between her partial tetrasomy and the cases of partial trisomy on which claims of DS critical regions were based and had little overlap with the phenotypic map of Korenberg et al.

B. L. Shapiro
Departments of Oral Science and Laboratory Medicine and Pathology and Institute of Human Genetics, University of Minnesota, 17-220 Moos Tower, 515 Delaware Street SE, Minneapolis, MN 55455, USA
Fax: +1-612-626-2651; e-mail: burt@mailbox.mail.umn.edu

(1994). A different set of loci involved in this case would of course speak against a contiguous gene explanation for the clinical findings in DS and must call into question the presumption that specific loci lead to most of the abnormalities in DS.

Korenberg et al. (1994) analyzed congenital heart defects (CHD) in their subjects and concluded that "the penetrance (percentage of DS with CHD) and expressivity (percentage of DS CHD that is an atrioventricular septal defect) of CHD are similar in duplications of distal 21q22 and full trisomy. This suggests a single locus responsible for most of the variability of the trait". This conclusion is a leap of faith, not of logic. The use of a concept such as penetrance may be questionable even in the context of describing phenotypes of single gene traits; it is inappropriate for traits for which a single gene basis has not yet been demonstrated and in any case provides no evidence for a single locus being responsible for most of the variability of this or any trait. Of course the possibility exists that triplication of a locus or region can be responsible for a particular abnormal trait (or its variability) in some individuals with DS. It may be true that a product of a locus or loci (on distal 21q22) adversely affects cardiac development. But this has yet to be demonstrated for any clinical sign in DS including CHD.

The search for a minimal region on chromosome 21 (the so-called DS critical region) responsible for producing DS has come full circle back to almost the entire chromosome. No evidence exists that individual loci on 21 are singularly responsible for individual phenotypic abnormalities in DS. The association of particular segments of chromosome 21 and different components of the clinical phenotype of DS as well as the "phenotypic map" (Korenberg et al. 1994) were based on assumptions that many clinical findings in DS are specific to DS (which is not true) and are a direct expression of triplicated loci on chromosome 21. That is, the linear single mutant gene – direct phenotypic expression paradigm of classical genetics was invoked. To carry this thinking to its logical extreme would require finding on chromosome 21 specific loci for nearly all human congenital malformations, inflammatory responses and degenerative processes since these are all more common in DS.

What has not been kept in mind in attempting to relate specific chromosomal segments or loci to particular phenotypic expressions in DS is that 1) no single finding in DS (except, probably, mental retardation) occurs in all affected subjects and 2) without exception every abnormality other than the extra chromosome or chromosomal segment (except in phenocopies) occurs in the general population, albeit much less commonly. None of the physical findings of DS associated with full trisomy 21 is found in all cases; most are not even found in a majority of cases.

The finding that tetrasomy for 21p–21q proximal permits the diagnosis of DS (Daumer-Haas et al. 1994), when trisomy for this segment usually does not, supports the idea that it may be the amount of superfluous transcribing genetic material that contributes significantly to the DS phenotype. Continued mapping of chromosome 21 is certainly worthwhile as part of the acquisition of complete knowledge of the human genome. Nevertheless, the expectation of a linear (gene locus to phenotype) explanation for the highly complex anomalies that comprise DS is unlikely just as complete knowledge of the genome will be insufficient to fully understand development of an organism. The former is a disruption and the latter a realization of the effects of a balanced genome on development, neither of which is a simple gene-phenotype phenomenon.

The assumption persists that because a chromosomal accident, i.e., nondisjunction or unbalanced translocation, is involved, that clinical findings in DS are a direct and singular result of products of loci on chromosome 21. This is no more true of DS (where mutant genes are not at issue) than that an abnormal gene product in hypomelanotic conditions causes sun-induced skin carcinoma. In the presence of different segments of chromosome 21, the ability to make a clinical diagnosis of DS (most of the time) has led investigators to attribute responsibility for signs of the syndrome to particular loci on the segment studied. The fact is that more commonalities exist among the autosomal trisomy syndromes than distinctions. Besides, the distinctions (and their clinical recognition) among the autosomal trisomies and between them and the general population cannot form the basis for understanding the development of aneuploid-associated anomalies since the ability to diagnose a condition is far removed from understanding its pathogenesis. The effects of disrupted evolved gene product balance (as occurs in autosomal trisomic states) are too complex to justify a simple single gene dose – phenotypic expression model for most if not all traits observed in DS. Developmental and physiological systems most liable to deviations from "normal" are those most vulnerable to abnormality. What is special in DS is that multiple unstable systems are affected with relatively (compared with the general population) high frequencies.

I concur with Korenberg et al. (1994) that the vogue of a narrow critical DS region is untenable. On the other hand, the complexity and variability of findings in DS and their parallel with the general population and other autosomal trisomies, makes the notion of an association of particular regions (or more narrowly, loci) of 21 with purported specific phenotypes equally unlikely for most if not all traits associated with DS.

One may ask what is gained by debunking attempts to narrow the effects of trisomy to specific loci? The heuristic value I would hope is the recognition that in trisomy 21 excessive gene products exist that interact, in doses not tested by evolution, with products of numerous loci involved in developmental and physiological pathways. This loss of genetic balance predisposes affected individuals to susceptibility to genetic and (pre- and postnatal) environmental insults and stochastic errors that organisms with balanced genomes usually buffer (Shapiro 1994). This is substantially different from assuming that a particular locus and its product causes or is responsible for most of the variability of a particular trait. To the extent that the

clinical diagnosis of DS can often be made there must be some differential effects of chromosome-21 loci on the phenotype. These loci may have more or less of an effect on development of different traits and may have pleiotropic effects. Yet no rationale exists for assuming that the pathogenesis of the abnormalities that characterize DS is any different from that of comparable traits in the general population. Without exception, traits that characterize DS are complex; they should be viewed and analyzed accordingly. I would urge those interested in DS to recognize the complexity of development of the signs that permit its diagnosis. To do otherwise would be a return to "beanbag genetics", the misleading tendency of early Mendelians to equate genes and characters, as if there were a one-to-one relation with no interaction among them.

References

Daumer-Haas C, Schuffenhauer S, Walther JU, Schipper RD, Porstmann T, Korenberg JR (1994) Tetrasomy 21pter→q22.1 and Down syndrome: molecular definition of the region. Am J Med Genet 53:359–365

Korenberg JR, Chen XN, Schipper R, Sun Z, Gonsky R, Gehwehr S, Carpenter N, Daumer C, Dignan P, Disteche C, et al (1994) Down syndrome phenotypes: the consequences of chromosomal imbalance. Proc Natl Acad Sci USA 91:4997–5001

Shapiro BL (1994) The environmental basis of the Down syndrome phenotype. Dev Med Child Neurol 36:84–90