2005-07-07983B Supplementary Discussion A

DISCUSSION OF THE DOWN SYNDROME CRITICAL REGION

Defining the boundaries of the Down Syndrome Critical Region (DSCR) is important to determine which genes have the potential to elicit DS phenotypes when triplicated. Unfortunately, rigorously determining the borders of the DSCR is complicated by many difficulties that necessarily arise in a complex human genetic disease such as DS. This supplementary discussion will examine published human and mouse data concerning the DSCR as it pertains to the possible roles of *DSCR1* and *DYRK1A* in DS pathogenesis.

The concept of a DSCR is compelling and supported by several thorough reports. However, a number of factors impede a comprehensive definition of the boundaries of the DSCR. First, the number of individuals with partial trisomy 21 that have had their regions of chromosomal triplication rigorously mapped is small. Second, the major studies mapping the DSCR were performed more than 10 years ago, prior to complete sequencing of chromosome 21 and with markers that are relatively far apart. Third, some partial trisomy 21 cases result from chromosomal translocations, thus while they carry three copies of part of chromosome 21, they may also be aneuploid for other chromosomal regions, with either duplications or deletions of parts of other chromosomes. Fourth, as DS comprises a constellation of phenotypes, most with widely varying levels of penetrance even in the presence of complete trisomy 21, it is difficult to conclusively rule in or out triplication of a particular gene as a causative factor for a given phenotype. For example, although congenital heart disease (CHD) is found in

50% of DS cases, it is also present in 0.5% of the general population[1]. Here we analyze partial trisomy patients and mouse models reported in the literature with respect to their phenotypes and number of copies of *DSCR1* and *DYRK1A,* based on chromosomal markers cited in those studies.

While several patients in a study of CHD excluded *DSCR1* from their region of trisomy, all but one had three copies of *DYRK1A*. The exceptional case also had partial trisomy 13 and did not meet other criteria for a diagnosis of DS[2]. The results of that study suggest that the development of CHD in partial trisomy 21 may not require three copies of *DSCR1,* and on the basis of the single patient with partial trisomy 13, the authors conclude that *DYRK1A* may also be excluded. On the other hand, 80% of full trisomy 13 cases have CHD, with septal defects being the most common. Four of 9 patients had CHD in a study of partial trisomy 13 involving the same pter-q14 region as the patient in the DS CHD study[3]. Overall, *DYRK1A* is triplicated in all three partial trisomy 21 patients with CHD in one study[4] and 7 out of 8 patients in another[2], the one outlier having significant other chromosomal abnormalities. Therefore, it appears likely that increased dosage of *DYRK1A* plays a role in CHD in DS patients.

Partial trisomy 21 has been linked to neurological deficits and CHD, but no significant studies to date have reported a relationship between partial trisomy 21 and placental insufficiency. Of the other phenotypes reported in partial trisomy 21 patients, in an initial study of 10 patients, the 3 individuals with triplication of *DYRK1A* but not *DSCR1* had fewer than half of the 23 features comprising the Jackson phenotypic score[5], implying an intermediate likelihood of DS, while 2 of 6 with triplication of both genes had a 100% probability of DS. Of the two patients in that study whose Jackson score was in the normal (i.e. non-DS) range, one was disomic for both *DSCR1* and

DYRK1A while the other had three copies of each gene, but had an X-translocated chromosome 21 fragment which may have been subject to X-inactivation[4]. In another study, 2 of 16 individuals had duodenal stenosis; both of those had three copies of both *DSCR1* and *DYRK1A*. Four of 6 patients with brachycephaly had three copies of both genes[6]. Of the four new patients characterized in the CHD study, one patient with brachycephaly and Hirschsprung Disease (HSCR) had triplication of *DYRK1A* but not *DSCR1*, while of the three patients without brachycephaly or HSCR, two had triplication of both genes while one had triplication of *DYRK1A* but not *DSCR1*[2]. Given that the prevalence of duodenal stenosis and HSCR are 4.6% and 1.4%, respectively, in DS patients and 0.02% and 0.01%, respectively, in the euploid population[1], a larger sample size of partial trisomy patients would need to be analyzed to confidently predict the boundaries of the DSCR in determining those pathologies. Collectively, these data suggest that *DYRK1A* triplication is essential for many major DS phenotypes while *DSCR1* may be dispensable for CHD but may be required for other phenotypes.

Mouse models of DS in which a portion of the mouse genome homologous to parts of human chromosome 21 including the DSCR is triplicated (segmental trisomy 16) successfully recapitulate certain DS phenotypes such as defects in learning and memory and altered craniofacial morphology[7-11]. However, they do not have significant cardiac malformations, immunodeficiencies, gastrointestinal malformations, genitourinary anomalies, or placental defects[11], each of which is common in human DS (Fig. 1h). Thus while segmental trisomy 16 mice may be useful models for the traits they express, they are difficult to reconcile with a complete portrait of DS, as developmental pathways affected by gene dosage may be regulated differently in mice and humans. That said, one of the most penetrant DS phenotypes and certainly the most

obvious phenotype to a casual observer is the characteristic craniofacial dysmorphology. An analogous phenotype has been documented by thorough morphometric analysis in two segmental trisomy 16 mouse strains[8, 9] (Ts65Dn and Ts1Cje). The triplicated region in these mice extends centromeric to what is commonly accepted to be the DSCR on human chromosome 21 (HSA21) and includes *DSCR1* (Fig. 1i). An attempt to more closely define the centromeric border of the DSCR on mouse chromosome 16 (MMU16) omitted *DSCR1* from the region of triplication (Ts1Rhr). Another transchromosomic mouse model with a nearly complete HSA21 in addition to the full mouse genome has a small gap omitting a third copy of *DSCR1*[12]. These mice lack the characteristic craniofacial dysmorphology of Ts65Dn and Ts1Cje mice[13] and *NFATc2/c4* DKO mice (Fig. 1a-c, h, and i). It is therefore tempting to speculate that the centromeric border of the DSCR extends at least to *DSCR1* with the significant caveat that this is only with regard to the craniofacial phenotype in mice.

Absent the rigorous analysis of a substantially larger population of partial trisomy 21 patients, it is difficult to definitively rule in or out *DSCR1* for human analogues of the phenotypes we have characterized in NFATc mutant mice. Our mathematical modelling predicts that a 1.5-fold increase in gene dosage of either *DSCR1* or *DYRK1A* may be sufficient to significantly decrease NFAT transcriptional activity, depending on the relative expression levels of each component in the system and the threshold of activity required for target gene activation (Fig. 4 and Supplementary Discussion B). Therefore, considering variations in expression patterns, it is possible that at specific developmental windows in various tissue types, NFAT activity may be sufficiently inhibited by increased levels of *DSCR1, DYRK1A,* or both simultaneously, to produce DS pathologies. Accordingly, it appears that three copies of

DYRK1A alone are sufficient to create conditions that predispose humans to CHD, while three copies of both *DSCR1* and *DYRK1A* may be necessary for other NFAT-related phenotypes.

What is clear from this analysis is that the variability of penetrance of DS phenotypes in the human population, the differences in phenotypic penetrance between humans and mice, and the small sample size of partial trisomy 21 humans combine to make defining precise borders of the DSCR difficult for each individual DS phenotype. Furthermore, it suggests that *DSCR1* and *DYRK1A* may contribute variably to dysregulation of the NFAT genetic circuit in different DS phenotypes.

REFERENCES

1. Torfs, C. P. & Christianson, R. E. Anomalies in Down syndrome individuals in a large population-based registry. Am J Med Genet 77, 431-8 (1998).
2. Barlow, G. M. et al. Down syndrome congenital heart disease: a narrowed region and a candidate gene. Genet Med 3, 91-101 (2001).
3. Schinzel, A. Catalogue of Unbalanced Chromosome Aberrations in Man (Walter de Gruyter, Berlin, 2001).
4. Delabar, J. M. et al. Molecular mapping of twenty-four features of Down syndrome on chromosome 21. Eur J Hum Genet 1, 114-24 (1993).
5. Jackson, J. F., North, E. R., 3rd & Thomas, J. G. Clinical diagnosis of Down's syndrome. Clin Genet 9, 483-7 (1976).
6. Korenberg, J. R. et al. Down syndrome phenotypes: the consequences of chromosomal imbalance. Proc Natl Acad Sci U S A 91, 4997-5001 (1994).
7. Reeves, R. H. et al. A mouse model for Down syndrome exhibits learning and behaviour deficits. Nat Genet 11, 177-84 (1995).
8. Richtsmeier, J. T., Baxter, L. L. & Reeves, R. H. Parallels of craniofacial maldevelopment in Down syndrome and Ts65Dn mice. Dev Dyn 217, 137-45 (2000).
9. Richtsmeier, J. T., Zumwalt, A., Carlson, E. J., Epstein, C. J. & Reeves, R. H. Craniofacial phenotypes in segmentally trisomic mouse models for Down syndrome. Am J Med Genet 107, 317-24 (2002).
10. Sago, H. et al. Ts1Cje, a partial trisomy 16 mouse model for Down syndrome, exhibits learning and behavioral abnormalities. Proc Natl Acad Sci U S A 95, 6256-61 (1998).
11. Sago, H. et al. Genetic dissection of region associated with behavioral abnormalities in mouse models for Down syndrome. Pediatr Res 48, 606-13 (2000).
12. O'Doherty, A. et al. An aneuploid mouse strain carrying human chromosome 21 with down syndrome phenotypes. Science 309, 2033-7 (2005).

13. Olson, L. E., Richtsmeier, J. T., Leszl, J. & Reeves, R. H. A chromosome 21 critical region does not cause specific Down syndrome phenotypes. Science 306, 687-90 (2004).