Polyalanine expansion and frameshift mutations of the paired-like homeobox gene *PHOX2B* in congenital central hypoventilation syndrome. Nat Genet 33:459–461

Brunet JF, Pattyn A (2002) *Phox2* genes: from patterning to connectivity. Curr Opin Genet Dev 12:435–440

Gabriel SB, Salomon R, Pelet A, Angrist M, Amiel J, Fornage M, Attie-Bitach T, Olson JM, Hofstra R, Buys C, Steffann J, Munnich A, Lyonnet S, Chakravarti A (2002) Segregation at three loci explains familial and population risk in Hirschsprung disease. Nat Genet 31:89–93

Gaisie G, Oh KS, Young LW (1979) Coexistent neuroblastoma and Hirschsprung's disease: another manifestation of the neurocristopathy? Pediatr Radiol 8:161–163

Maris JM, Matthay KK (1999) Molecular biology of neuroblastoma. J Clin Oncol 17:2264–2279

Maris JM, Weiss MJ, Mosse Y, Hii G, Guo C, White PS, Hogarty MD, Mirensky T, Brodeur GM, Rebbeck TR, Urbanek M, Shusterman S (2002) Evidence for a hereditary neuroblastoma predisposition locus at chromosome 16p12-13. Cancer Res 62:6651–6658

Matera I, Bachetti T, Puppo F, Di Duca M, Morandi F, Casiraghi GM, Cilio MR, Hennekam R, Hofstra R, Schober JG, Ravazzolo R, Ottonello G, Ceccherini I (2004) *PHOX2B* mutations and polyalanine expansions correlate with the severity of the respiratory phenotype and associated symptoms in both congenital and late onset central hypoventilation syndrome. J Med Genet 41:373–380

Nemecek ER, Sawin RW, Park J (2003) Treatment of neuroblastoma in patients with neurocristopathy syndromes. J Pediatr Hematol Oncol 25:159–162

Pattyn A, Morin X, Cremer H, Goridis C, Brunet JF (1999) The homeobox gene *Phox2b* is essential for the development of autonomic neural crest derivatives. Nature 399:366–370

Perri P, Longo L, McConville C, Cusano R, Rees SA, Seri M, Conte M, Romeo G, Devoto M, Tonini GP (2002) Linkage analysis in families with recurrent neuroblastoma. Ann NY Acad Sci 963:74–84

Trochet D, Bourdeaut F, Janoueix-Lerosey I, Deville A, De Pontual L, Schleiermacher G, Coze C, Philip N, Frebourg T, Munnich A, Lyonnet S, Delattre O, Amiel J (2004) Germline mutations of the paired-like homeobox 2B (*PHOX2B*) gene in neuroblastoma. Am J Hum Genet 74:761–764

Weese-Mayer DE, Berry-Kravis EM, Zhou L, Maher BS, Silvestri JM, Curran ME, Marazita ML (2003) Idiopathic congenital central hypoventilation syndrome: analysis of genes pertinent to early autonomic nervous system embryologic development and identification of mutations in *PHOX2B*. Am J Med Genet 123A:267–278

Address for correspondence and reprints: Dr. John M. Maris, Division of Oncology, The Children's Hospital of Philadelphia, Abramson Pediatric Research Center 902A, 3516 Civic Center Boulevard, Philadelphia, PA 19104-4318. E-mail: maris@email.chop.edu

0002-9297/2004/7504-0025$15.00

Am. J. Hum. Genet. 75:730–731, 2004

Comparative Frequency of Fragile-X (*FMR1*) and Creatine Transporter (*SLC6A8*) Mutations in X-Linked Mental Retardation

To the Editor:

The study by Rosenberg et al. (2004), in the July 2004 issue of *The American Journal of Human Genetics,* and the previous work by Dr. Salomons's laboratory on the implications of the creatine transporter gene, *SLC6A8,* for X-linked mental retardation (XLMR) are very important contributions to the field (Salomons et al. 2001; Rosenberg et al. 2004). I wish, however, to qualify the concluding sentences in the abstract and the discussion section of the study by Rosenberg et al. (2004), which may lead readers to overestimate the incidence of mutations in the creatine transporter gene in mental retardation (MR). The authors write in the abstract that the "frequency of *SLC6A8* mutations in XLMR is close to that of CGG expansions in *FMR1*" (Rosenberg et al. 2004, p. 97). This is certainly incorrect. Rosenberg et al. (2004) found a 2.2% prevalence of *SLC6A8* mutations in families with proven or possible XLMR (the latter are families with at least two males affected by MR and compatible with X-linked inheritance). On the other hand, the *FMR1* expansion mutation associated with fragile-X syndrome is found in ~2%–3% of males with MR who were not selected for family history (these figures are based on cohorts with little clinical preselection apart from the exclusion of clearly chromosomal or syndromic forms of MR) (see de Vries et al. 1997; Hecimovic et al. 2002; Pandey et al. 2002; Grønskov et al. 2004; Biancalana et al., in press). In fact, when selection is based on possible X-linked inheritance, the proportion of individuals with fragile-X syndrome is much higher. For instance, in the study by Fishburn et al. (1983), fragile-X syndrome accounted for MR in 12 of 45 male sib pairs with "nonspecific" MR, a proportion (27%) that is thus >10 times higher than the reported incidence of *SLC6A8* mutations in a cohort containing sib pairs such as these as well as families with even more obvious XLMR. In fact, we have proposed recently that, unless there are clear hotspots of mutations and/or a very large mutation target size (such as for Duchenne muscular dystrophy, Rett syndrome, and hemophilia A), the population incidence of X-linked diseases implicating genes of average size that lead to highly decreased reproductive fitness is 10–20 times lower than the incidence of fragile-X syndrome (1/50,000–1/100,000 for most X-linked diseases, compared with 1/~5,000 males for the fragile-X syndrome) (Chelly and Mandel 2001). Thus, one expects that the contribution to XLMR of an average gene that does not present mutation hotspots would be 10–20 times lower than that of *FMR1*.

SLC6A8 is such a gene (with 13 exons, a 635-aa coding sequence, and no indication of highly recurring mutations), and thus we predict that its incidence in "nonsyndromic" MR will be in the range of 0.1%–0.3%. Indeed, even for the *ARX* (*X-linked Aristaless*) gene, which has a clear mutation hotspot that accounts for ~6.6% of families with X-linked "nonsyndromic" MR, the incidence of this *ARX* recurring mutation in cohorts of patients with MR is much lower (~0.15%) than that of *FMR1* mutations (Grønskov et al. 2004; Mandel and Chelly 2004).

I also suggest that, in reporting prevalence estimates that are based on relatively small numbers of positive cases, it would be useful to give confidence intervals (CIs). Thus, the observed prevalence, in the study by Rosenberg et al. (2004), of 2.2% may indeed be an underestimate, since some mutations may have been missed and some variants of uncertain significance at present may prove pathogenic, or it may be an overestimate of the true prevalence, since, for the reported data, the CI for the prevalence of proven mutations is 1.0%–4.4%.

J. L. MANDEL
Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC)
CNRS/INSERM/Université Louis Pasteur/Collège de France
Illkirch, France

References

Biancalana V, Beldjord C, Taillandier A, Szpiro-Tapia S, Cusin V, Gerson F, Philippe C, Mandel JL, French National Working Group on Fragile X Syndrome. Five years of molecular diagnosis of fragile X syndrome (1997–2001): a collaborative study reporting 95% of the activity in France. Am J Med Genet (in press)

Chelly J, Mandel JL (2001) Monogenic causes of X-linked mental retardation. Nat Rev Genet 2:669–680

de Vries BB, van den Ouweland AM, Mohkamsing S, Duivenvoorden HJ, Mol E, Gelsema K, van Rijn M, Halley DJ, Sandkuijl LA, Oostra BA, Tibben A, Niermeijer MF (1997) Screening and diagnosis for the fragile X syndrome among the mentally retarded: an epidemiological and psychological survey. Collaborative Fragile X Study Group. Am J Hum Genet 61:660–667

Fishburn J, Turner G, Daniel A, Brookwell R (1983) The diagnosis and frequency of X-linked conditions in a cohort of moderately retarded males with affected brothers. Am J Med Genet 14:713–724

Grønskov K, Hjalgrim H, Nielsen IM, Brøndum-Nielsen K (2004) Screening of the *ARX* gene in 682 retarded males. Eur J Hum Genet. http://www.nature.com/cgi-taf/dynapage.taf?file=/ejhg/journal/vaop/ncurrent/full/5201222a.html (electronically published June 16, 2004; accessed August 16, 2004)

Hecimovic S, Tarnik IP, Baric I, Cakarun Z, Pavelic K (2002) Screening for fragile X syndrome: results from a school for mentally retarded children. Acta Paediatr 91:535–539

Mandel JL, Chelly J (2004) Monogenic X-linked mental retardation: is it as frequent as currently estimated? the paradox of the ARX (Aristaless X) mutations. Eur J Hum Genet (in press)

Pandey UB, Phadke S, Mittal B (2002) Molecular screening of FRAXA and FRAXE in Indian patients with unexplained mental retardation. Genet Test 6:335–339

Rosenberg EH, Almeida LS, Kleefstra T, deGrauw RS, Yntema HG, Bahi N, Moraine C, Ropers HH, Fryns JP, deGrauw TJ, Jakobs C, Salomons GS (2004) High prevalence of SLC6A8 deficiency in X-linked mental retardation. Am J Hum Genet 75:97–105

Salomons GS, van Dooren SJ, Verhoeven NM, Cecil KM, Ball WS, Degrauw TJ, Jakobs C (2001) X-linked creatine-transporter gene (*SLC6A8*) defect: a new creatine-deficiency syndrome. Am J Hum Genet 68:1497–1500

Address for correspondence and reprints: Dr. Jean-Louis Mandel, IGBMC, BP10142, 67404 Illkirch, CU Strasbourg, France. E-mail: mandeljl@igbmc.u-strasbg.fr

0002-9297/2004/7504-0026$15.00

Am. J. Hum. Genet. 75:731–732, 2004

Reply to Mandel

To the Editor:

On behalf of all the authors of our recent study (Rosenberg et al. 2004), we thank Dr. Mandel for his comments (Mandel 2004 [in this issue]), to which we fully subscribe, and we apologize for the misleading statement in our article. Indeed, *SLC6A8* mutations, although probably more common than mutations in other known nonsyndromic X-linked mental retardation (MRX) genes except *ARX,* must be much less frequent than pathogenic CGG expansions in the *FMR1* gene. As pointed out by Mandel (2004 [in this issue]), this is convincingly illustrated by the relative paucity of *ARX* mutations in nonselected cohorts of males with mental retardation (MR) (Grønskov et al. 2004). Mandel's second argument, which implies that mutation rates in X-linked genes can be inferred from their lengths and must be intrinsically much lower than the rate of CGG expansions in *FMR1,* is less compelling in view of the evidence for mutational hotspots in many disease genes, including *ARX* and *PQBP1,* a recent addition to the growing list of genes involved in MRX (Kalscheuer et al. 2003). Therefore, the existence of another common but hitherto-unknown cause of nonsyndromic MR cannot be ruled out yet, even though ongoing large-scale mutation screening in regions known to carry many