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Reply to Mackenzie

To the Editor:

MacKenzie suggests that, in my editorial "When Is a Deletion Not a Deletion? When It Is Converted" (Burghes 1997), I have not ascribed sufficient significance to the role of the neuronal apoptosis inhibitory protein (NAIP) gene in spinal muscular atrophy (SMA). In particular, MacKenzie takes issue with the following statement: "Further work is required to clearly define the mechanism by which the converted alleles modify phenotype, and it is possible that deletion of adjacent genes, such as NAIP, could influence the exact severity of the phenotype. However, it appears most likely that the deletion of NAIP marks the extent of the deletion and that different forms of SMN^C modify the SMA phenotype" (Burghes 1997, p. 13).

It is my opinion that this is a fair reflection of our current knowledge of the situation and that, at present, there is not adequate evidence to implicate NAIP as a major SMA-modifying gene. The first and foremost argument against involvement of NAIP comes from genetic studies. MacKenzie indicates that, in some type I SMA populations, the rate of NAIP deletion approaches 80%. However, in most cases in which a noninbred population has been studied, the rate of NAIP deletion in type I SMAs is 45%–50% (Cobben et al. 1995; Hahnen et al. 1995; Roy et al. 1995; Thompson et al. 1995; Velasaco et al. 1996; DiDonato et al 1997*b*). MacKenzie states that "we feel it likely that motor neurons from SMA individuals with deletions of both NAIP and SMN_{tel} are prone to apoptosis. As a result, the cells are less able to withstand the stress of SMN depletion and die earlier than they would otherwise, resulting in a more severe form of SMA." This would predict that the disease in those patients without a deletion of the NAIP gene would be mild, whereas the disease in those patients with a NAIP gene deletion would be severe. Patients with SMN^T intragenic mutations that still retain the NAIP gene would also be predicted to have a mild form of the disease. So a critical question is, Do the type I SMA cases without NAIP deletions show a clinical progression different from that seen in patients with an NAIP deletion? There is no clear difference between these two populations. In addition, type II/III SMA cases can have deletions of NAIP, as can carriers with no clinical phenotype (Cobben et al. 1995; Hahnen et al. 1995; Roy et al. 1995; Thompson et al. 1995; Rodrigues et al. 1996; Campbell et al. 1997; DiDonato et al. 1997*b*). Therefore, it does not always seem to be the case that motor neurons lacking NAIP are more sensitive to the loss of SMN^T. It could be argued that the type I SMA cases with an intact NAIP gene have another mutation, which is not detectable by current assays—and that they therefore are phenotypically equivalent—whereas type II/III SMA patients with a deletion of NAIP somehow make the NAIP protein in the motor neurons. This indicates two critical studies that are needed to substantiate NAIP as a major modifier of SMA. First, there must be detection of intragenic NAIP mutations in the type I SMA patients who have an intact NAIP gene. Second, there must be studies at the protein level that show reduction of the NAIP protein in motor neurons of type I SMA patients who do not have a deletion of the NAIP gene.

Apart from deletions of SMN^T, type I SMA patients with intragenic mutations of SMN^T and no detectable alteration in the NAIP gene have been identified (Burghes 1997, table 1). These patients have a clinical phenotype indistinguishable from those type I SMA cases who have NAIP deletions. If NAIP were a major modifier of SMA, it would be difficult to explain type I SMA cases who had an intact NAIP gene, since NAIP would be expected to exert its protective effect and to modulate the phenotype.

The identification of the NAIP gene in the SMA region gave an appealing candidate gene, since it indicated that apoptosis could have a key role in SMA. However, at the current time, in my view, it is not clear what role apoptosis plays in SMA. Does the motor-neuron cell activate the apoptotic pathway because the cell lacks a critical element or is SMA due to an actual defect in an apoptotic pathway? Mice do not have two SMN genes (SMN^C and SMN^T) on a chromosome; rather, they have one SMN gene on a chromosome (DiDonato et al. 1997*a;* Violet et al. 1997). Mice that lack this SMN gene die early in embryogenesis (Schrank et al. 1997), and the cells show definite apoptotic changes. This clearly highlights the importance of the SMN^C gene in human SMA patients, but it does not explain why the loss of SMN^T causes SMA. Indeed, the SMN protein has recently be shown to be important for snRNP biogenesis, presumably a critical function in a cell (Liu et al. 1997). Interestingly, snRNPs appear to be particularly enriched in motor neurons. So is SMA caused by disruption of snRNP formation in neurons or by disruption of apoptotic pathways? What is the role of SMN^C in motor neurons? These questions remain to be resolved.

I should also note that phenotypic modification in SMA is not entirely resolved by the conversion model that I have presented elsewhere (Burghes 1997). In particular, there are rare type II/III SMA families in which two sibs inherit the identical 5q13 haplotypes (Burghes et al. 1994; Cobben et al. 1995; Hahnen et al. 1995; Wang et al. 1996; DiDonato et al. 1997*b*); both sibs have no detectable SMN^T gene, but they show remarkably discordant phenotypes. This is not fully explainable by the conversion model and could indicate that genes outside the 5q13 region act as phenotypic modifiers. If alteration of apoptotic death is implicated in SMA, then genes such as bcl2 become candidates as phenotypic modifiers. However, it is equally likely that phenotypic modifiers of SMA effect the level of SMN expression in motor neurons by up-regulating the SMN^C gene to increase the level of SMN protein. If this is the case, an intriguing possibility is that up-regulation of the SMN^C gene serves as a therapeutic intervention in SMA. In conclusion, one component that modifies phenotype in SMA is the type of mutation in SMN^T; but, as yet, it is not clear what other factors can modify phenotype.

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Evidence for Paleolithic and Neolithic Gene Flow in Europe

To the Editor:

In recent Letters to the Editor, Cavalli-Sforza and Minch (1997) and Richards et al. (1997) discuss the relative contributions of the first Paleolithic colonizers of Europe, and of later Neolithic immigrants, to the gene pool of current Europeans. Using the method of median networks (Bandelt et al. 1995), Richards et al. (1996) demonstrated that most mitochondrial lineages coalesce at ancestors who presumably lived in the Paleolithic period, which, in Europe, means >10,000 years ago. Through an analysis of the geographic distribution of these lineages, they reached the conclusion that most mitochondrial alleles spread in Europe prior to the Neolithic period. Two implications of this finding were that (1) farming was essentially a local development, the spread of which was not accompanied by extensive gene flow, and (2) the gradients of allele frequencies described in many studies (starting with Menozzi et al. [1978] and reviewed in Cavalli-Sforza et al. [1994]) were not due to a Neolithic demic diffusion from the Near East (Ammerman and Cavalli-Sforza 1984), as is generally believed. Richards et al. (1996) interpreted the results of a simulation study of various population-expansion mechanisms (Barbujani et al. 1995) as supporting a Paleolithic origin of these clines.

Cavalli-Sforza and Minch (1997) argued that sequences of the mtDNA hypervariable region are not suitable for reconstructing evolutionary processes at this scale, because the high mutation rates at some sites cause an excess of random noise. In addition, a high female mobility might have blurred some previously existing geographic patterns. They suggested that a figure of ~25% might realistically represent the contribution of Neolithic immigrants to the gene pool of Europeans, because, in principal-component analyses of allele frequencies, a clinal component accounts for one quarter of the genetic variance (Menozzi et al. 1978; Piazza et al. 1995). If that were the case, there would be little overall disagreement; given the approximate nature of any such estimates, the figure (15%) proposed by Richards et al. (1997) may not differ significantly. We would like to suggest a third possibility—namely, that the available mitochondrial data do not contradict a much larger Neolithic contribution and that envisaging the current European gene pool as essentially a product of an Upper Paleolithic colonization may create more problems than it solves.

There are four traditional reasons to believe that there was a major Neolithic contribution to the European gene pool: (1) the continentwide gradients of allele frequencies; (2) their correlation with the archaeological record; (3) their overlapping with areas defined by linguistic criteria; and (4) their similarity to the gradients theoretically predicted under, or generated in simulation studies of, a model of demic diffusion. None of these pieces of evidence is proof, but in this field there is little that one can really prove. The point, at this stage, is to find the simplest explanation that accounts for most (or, possibly, for all) observed population characteristics. Of course, speaking of Paleolithic versus Neolithic processes is an oversimplification of phenomena that were certainly more complicated. However, such a highly schematic opposition is useful for the sake of clarity.

As for the gradients detected for roughly one third of the alleles studied in Europe (Sokal et al. 1989) (point 1), few doubt that they result from some form of population movement. Indeed, random genetic drift alone cannot generate nonrandom patterns on such a broad scale, and major selective effects on many independent loci appear unlikely (Ammerman and Cavalli-Sforza 1984; but see also Fix 1996). The problem is *when* those movements took place. As Richards et al. (1996, 1997) pointed out, the correlation with archaeological gradients (point 2) and, specifically, with the first evidence of farming activities (Sokal et al. 1991) now seems less cogent. Indeed, evidence is emerging that, not only in the Neolithic but also in the Paleolithic period, the main population movements occurred along a southeast-northwest axis (Richards et al. [1997] and references therein). If so, whatever the relative importance of the two temporal phases, both should have determined similar clines of gene frequencies. On the contrary, however, if it were shown that Paleolithic populations moved