Letters to the Editor

Am. J. Hum. Genet. 62:484, 1998

The −75A→C Substitution in the 5′ UTR of the Wilson Disease Gene Is a Sequence Polymorphism in the Mediterranean Population

To the Editor:

In their haplotype and mutation analysis of Wilson disease (WD) in Japanese patients, Nanji et al. (1997) report an A→C substitution at position −75 in the 5′ UTR of the WD gene, found in 1/42 WD chromosomes investigated. The authors considered this substitution to be a disease-causing mutation, and they postulated that the mutation adversely affects WD-gene expression, either by abolishing ribosome binding or by interfering negatively with transcriptional factor(s)–DNA binding. However, Nanji et al. (1997) did not report screening for the presence of the A→C mutation in normal chromosomes, to exclude the possibility that this mutation is a simple polymorphism.

Of 228 WD chromosomes analyzed in our study of WD in Mediterranean populations, we found the −75A→C substitution in 23 WD chromosomes that carry an unquestionable disease-causing mutation, as well as in 16 WD chromosomes in which the mutation has not yet been defined. The A→C substitution at position −75 was also detected in 15 (28%) of 54 normal chromosomes from the same Mediterranean population.

These data clearly indicate that −75A→C is a sequence polymorphism that most likely does not affect the function of the WD gene. We previously reported the same A→C substitution in the 5′ UTR (Figus et al. 1995); however, because of erroneous numbering of the nucleotide sequence in the sequence ladder, we incorrectly indicated its position as −74 instead of −75.

Acknowledgments

We want to thank Associazione Baschirotto and Società Italiana di Gastroenterologia Pediatrica for providing WD families. This research was supported by Telethon Italy grant E129 and by Assessorato Igiene e Sanità, Regione Sardegna, Legge Regionale grant 30.04.1990.

GEORGIOS LOUDIANOS,[1] VALERIA DESSI,[2] MARIO LOVICU,[3] ANDREA ANGIUS,[4] ANTONIO CAO,[1,2,3] AND MARIO PIRASTU[4]

[1]*Ospedale Regionale per le Microcitemie, ASL 8,* [2]*Istituto di Clinica e Biologia dell' Età Evolutiva, Università degli Studi di Cagliari, and* [3]*Istituto di Ricerca sulle Talassemie ed Anemie Mediterranee, CNR-Cagliari, Cagliari, Italy; and* [4]*Istituto di Genetica Molecolare, CNR-Alghero, Italy*

References

Figus AL, Angius A, Loudianos G, Bertini C, Dessi V, Loi A, Deiana M, et al (1995) Molecular pathology and haplotype analysis of Wilson disease in Mediterranean populations. Am J Hum Genet 57:1318–1324

Nanji SM, Nguyen VTT, Kawasoe JH, Inui K, Endo F, Nakajima T, Anezaki T, et al (1997) Haplotype and mutation analysis in Japanese patients with Wilson disease. Am J Hum Genet 60:1423–1429

Address for correspondence and reprints: Dr. Georgios Loudianos, Ospedale Regionale per le Microcitemie, ASL 8, Cagliari Via Jenner s/n, 09121 Cagliari, Italy. E-mail: gloudian@mcweb.unica.it

0002-9297/98/6202-0033$02.00

Am. J. Hum. Genet. 62:484–485, 1998

Reply to Loudianos et al.

To the Editor:

We appreciate receiving additional information on the A→C substitution at position −75 in the 5′ UTR of the Wilson disease gene. When we reported this substitution (Nanji et al. 1997), we were careful to indicate that it was in the "putative promoter" region and that it *might* be associated with the disease. We pointed out that direct testing of the effect of the mutation on expression would be required to confirm the nature of the mutation. We described the results of the analysis of 21 normal chromosomes from a Japanese group, which is the same ethnic group as that of the patient. These normal-chromosome results were obtained from the analysis of the normal chromosomes in the heterozygous parents of the patients. None of the putative promoter mutations were identified in the normal sample. We did report, in table 3 of our previous study (Nanji et al. 1997), some alterations that we felt were definitely polymorphisms. Ap-

parently, the A→C substitution is more common in the normal Mediterranean population. We had missed this because of the error in the study by Figus et al. (1995), as is noted in the letter by Loudianos et al. (1998 [in this issue]). Promoter studies are currently in progress to determine the nature of the mutations reported by us.

MANOJ S. NANJI AND DIANE W. COX
Department of Medical Genetics
University of Alberta
Edmonton

References

Figus AL, Angius A, Loudianos G, Bertini C, Dessi V, Loi A, Deiana M, et al (1995) Molecular pathology and haplotype analysis of Wilson disease in Mediterranean populations. Am J Hum Genet 57:1318–1324

Loudianos G, Dessi V, Lovicu M, Angius A, Cao A, Pirastu M (1998) The 75A→C substitution in the 5′ UTR of the Wilson disease gene is a sequence polymorphism in the Mediterranean population. Am J Hum Genet 62:000–000 (in this issue)

Nanji SM, Nguyen VTT, Kawasoe JH, Inui K, Endo F, Nakajima T, Anezaki T, et al (1997) Haplotype and mutation analysis in Japanese patients with Wilson disease. Am J Hum Genet 60:1423–1429

Address for correspondence and reprints: Dr. Diane Cox, Department of Medical Genetics, University of Alberta, 660 Heritage Medical Research Centre, Edmonton, Alberta T6G 2S2, Canada. E-mail: diane.cox@ualberta.ca

0002-9297/98/6202-0034$00.00

Am. J. Hum. Genet. 62:485–486, 1998

Reply to Burghes

To the Editor:

In his recent editorial entitled "When Is a Deletion Not a Deletion? When It Is Converted" Burghes (1997) correctly ascribes the cause of spinal muscular atrophy (SMA) to the loss or mutation of the telomeric copy of the *SMN* (survival motor neuron) gene. The reduction in SMN protein, as Burghes recognizes, most likely leads to motor-neuron death, by unknown mechanisms (Coovert et al. 1997; Lefebvre et al. 1997). He also outlines the probable role of centromeric copies of SMN in the modulation of disease severity (Campbell et al. 1997; Velasco et al. 1996; McAndrew et al. 1997). However, concerning a second SMA candidate gene, known as *NAIP* (neuronal apoptosis–inhibitory protein), Burghes states that "it appears likely that the deletion of *NAIP* marks the extent of the [genomic] deletion and that different forms of SMN_{cen} modify the SMA phenotype," thereby rejecting a role for *NAIP* in SMA pathogenesis.

On this final point we strongly disagree. During the past 2 years, our group, our collaborators, and other laboratories have shown that NAIP could be involved in SMA pathogenesis in several ways. First, in most populations the *NAIP* gene is deleted in the majority of type I SMA individuals. In some type I SMA populations, the deletion of SMN_{tel} extends to *NAIP* in >80% of affected chromosomes (Morrison 1996; Samilchuk et al. 1996; Velasco et al. 1996). Second, in the CNS, NAIP is expressed in at least eight distinct neuronal populations, including the motor neurons, all of which are affected in type I SMA (Towfighi et al. 1985; Murayama et al. 1991; Peress et al. 1986; Xu et al. 1997*b*). A number of NAIP-positive neuronal types (e.g., cholinergic neurons of the striatum), when subjected to ischemia, demonstrate both a significant increase in NAIP levels (Xu et al. 1997*a*) as well as a marked resistance to apoptotic death. Third, NAIP exerts an antiapoptotic effect in cultured cells (Liston et al. 1996) and affords hippocampal neuroprotection in vivo when overexpressed from a transgene (Xu et al. 1997*a*).

In view of these data, we find the assertion surprising that the *NAIP* gene serves merely as a marker of genomic-DNA deletion size. Clearly, formal proof of NAIP involvement in SMA pathogenesis must await further analysis (e.g., exacerbation of an SMA phenotype in SMN-deficient mice when expression of NAIP is compromised). However, we feel that it is 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.

ALEX E. MACKENZIE
Division of Genetics
Children's Hospital of Eastern Ontario
University of Ottawa
Ottawa

References

Burghes AHM (1997) When is a deletion not a deletion? when it's converted. Am J Hum Genet 61:9–15

Campbell L, Potter A, Ignatius J Dubowitz V, Davies K (1997) Genomic variation and gene conversion in spinal muscular atrophy: implications for disease process and clinical phenotype. Am J Hum Genet 61:40–50

Coovert DD, Le TT, McAndrew PE, Strasswimmer J, Crawford TO, Mendell JR, Coulson SE, et al (1997) The survival motor neuron protein in spinal muscular atrophy. Hum Mol Genet 6:1205–1214

Lefebvre S, Burlet P, Liu Q, Bertrandy S, Clermont O, Munnich A, Dreyfuss G (1997) Correlation between severity and