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Reply to Pragliola et al.

To the Editor:

In their letter to the editor, Pragliola et al. (1997 [in this issue]) have noted that in our manuscript (Gottlieb et al. 1997) we assume that the *GSCL* gene is deleted in patient G (Levy et al. 1995), a patient with DiGeorge syndrome who has an atypical deletion boundary in chromosome 22. Their FISH analysis indicates that *GSCL* in fact is not deleted in this patient. Since patient G was unavailable to us for analysis, we were unable to verify the deletion status of *GSCL*. We based our estimate of the deletion boundary in patient G on the information provided in a previous paper published by the same senior investigator (Rizzu et al. 1996): in an article by Rizzu et al. (1996), the deletion endpoint of patient G is described as 100–150 kb telomeric to the ADU/VDU breakpoint, "close to the 5′ end" of the *DGSI/ES2* gene. Since we had the complete sequence of the ~120-kb region extending from the ADU breakpoint to *GSCL,* and since we knew that the 5′ end of *GSCL* is ~6 kb from *DGSI/ES2,* we felt that it was reasonable to assume that *GSCL* was disrupted or deleted in this patient. Pragliola et al. indicate in their letter that, although the deletion endpoint in patient G is within a fosmid containing *GSCL,* the breakpoint is distal to the 5′ end of the gene. However, as they note—and as we pointed out in our paper—a deletion or translocation breakpoint can easily affect the expression of genes in the vicinity. Therefore, the expression of *GSCL* could still be affected in patient G. Moreover, as we stated in our paper, the definition of a minimal critical region does not exclude a role for genes outside the region. Thus, although we consider *GSCL* to be a strong candidate gene for some of the abnormalities associated with DiGeorge syndrome/velocardiofacial syndrome, we have not excluded the possibility that genes outside the regions that we have called the "DiGeorge minimal critical region," or the smallest region of deletion, play a role in the disease phenotype. We appreciate the additional data from Pragliola et al., clarifying the location of the proximal breakpoint in patient G.

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The Frequency of the Methylenetetrahydrofolate Reductase–Gene Mutation Varies with Age in the Normal Population

To the Editor:

Cardiovascular disease and cerebrovascular disease are second only to cancer as the leading causes of death in Japan, and their mortality rates rise with the age of the population (Health and Welfare Statistics Association 1995). In addition to environmental factors such as smoking, genetic factors are involved in determining an individual's susceptibility to vascular disease. Because low frequency of genetic risk factors for vascular disease could contribute to a population's longevity, the frequency of potentially protective genotypes may be higher in the oldest segment of the population.

For the past decade, mild hyperhomocysteinemia has been recognized as a risk factor for occlusive arterial disease and thrombosis. Methylenetetrahydrofolate reductase (MTHFR) is the key enzyme in the methylation of homocysteine. A homozygous mutation of the MTHFR gene (677C→T; A→V) alters a highly conserved amino acid, resulting in decreased specific MTHFR activity and elevated levels of homocysteine (Frosst et al. 1995). According to a recent report on patients with premature vascular disease, this mutation is associated with a threefold increase in risk for premature cardiovascular disease (Kluijtmans et al. 1996).

Individuals with the homozygous 677C→T mutation have been reported to show increased thermolability of MTHFR (Frosst et al. 1995). One study attributed the abnormal homocysteine metabolism in 28% of the homocysteinemic patients with premature vascular disease to thermolabile MTHFR (Engbersen et al. 1995). Another study indicated that an association between the thermolabile MTHFR defect and coronary artery disease is independent of other known risk factors for coronary artery disease (Kang et al. 1993).

These observations led us to speculate that the MTHFR mutation may be partly responsible for cardiovascular disease, thus decreasing the probability of an individual's reaching old age. To test this hypothesis, we investigated the frequency of the MTHFR mutation in 945 unrelated, healthy subjects 14–99 years old, all Japanese living in a single local area. Information about health condition (e.g., history of hypertension, hyperlipidemia, diabetes mellitus, stroke, and heart disease) was obtained by semistructured interview of 506 subjects who were >60 years old. We assessed blood pressure at rest twice during the interview.

Taking account of the rising rate of mortality due to cardiovascular and cerebrovascular diseases in people >80 years old, we stratified the subjects into three age groups: younger ($\leqslant$54 years; $n = 311$), older (55–79 years; $n = 486$), and oldest ($\geqslant$80 years; $n = 148$, of whom 22 were $\geqslant$90 years of age). DNA was extracted from peripheral leukocytes obtained from each subject, and MTHFR genotyping was performed as described by Frosst et al. (1995).

As shown in table 1, the MTHFR mutation decreased as the ages of the subjects increased. The homozygous MTHFR mutation occurred in 19% of the younger group and in 14% of the older group, whereas we found the mutation in only 7% of the oldest group. The homozygous mutation occurred significantly less often in both the older and oldest groups than in the younger group, especially among males (total—df = 2, $\chi^2 = 10.39$, $P = .006$; males—df = 2, $\chi^2 = 7.25$, $P = .027$; females—df = 2, $\chi^2 = 3.59$, $P = .166$). The MTHFR-genotype distributions for the younger and older age groups were in Hardy-Weinberg equilibrium, but the distribution for octogenarians and nonagenarians was not

Table 1

MTHFR Genotype in Healthy Japanese Stratified by Age Groups and Gender

	Younger (<55 years)			Older (55–79 years)			Oldest ($\geqslant$80 years)		
		MTHFR Allele[a]			MTHFR Allele[a]			MTHFR Allele[a]	
	n	+/+	+/− or −/−	*n*	+/+	+/− or −/−	*n*	+/+	+/− or −/−
Male[b]	147	.21	.79	227	.15	.85	79	.08	.92
Female	164	.17	.83	259	.13	.87	69	.07	.93
Total[c]	311	.19	.81	486	.14	.86	148	.07	.93

[a] A plus sign (+) denotes the mutant allele; and a minus sign (−) denotes the wild-type allele.
[b] $\chi^2 = 7.25$, $P = .027$, for differences among the three age groups.
[c] $\chi^2 = 10.39$, $P = .006$, for differences among the three age groups.