BCG vaccine was routinely given at birth in East Germany (6), and the incidence of type 1 diabetes resembled that of countries listed above that give BCG at birth. We believe the findings of Neu et al. can be explained by BCG immunization status and believe this factor should be considered in any study on the incidence of type 1 diabetes.

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Response to Classen and Classen

Incidence of IDDM in Germany higher than expected

As a result of an epidemiological study, incidence rates of childhood diabetes in Germany according to internationally accepted standards were presented for the first time (1).

Previously reported incidence estimations referred to a registry kept in the former German Democratic Republic (2). Although these incidence rates were not validated by a secondary data source, the data were commonly quoted assuming the incidence to be 7.5 per 100,000 for Germany as a whole. With 10.6 and 11.6 per 100,000, the Baden-Wuerttemberg incidence rates were markedly higher than those reported from former East Germany.

The hypothesis of Classen and Classen according to which bacille Calmette-Guérin (BCG) immunization status could explain these differences is interesting, although other studies could not confirm this observation and reported incidence rates unaffected by BCG vaccination (3).

In fact, BCG immunization was done more frequently in East Germany than in West Germany. However, there are considerable differences between various regions. In Baden-Wuerttemberg, the federal state in which our incidence study was done, 60.7% of all children are vaccinated with BCG (4). Therefore, it is definitely not correct to put Baden-Wuerttemberg in a line with countries not using BCG.

Many environmental factors such as coffee intake (5) and rainfall (6) as well as immunization status (7) have been associated with the occurrence of type 1 diabetes. Yet the complex pathogenesis of the disease seems to be ignored by Classen and Classen, reducing this process to one single cause. As a matter of fact, between federal states in East and West Germany, many environmental conditions are obviously different and could be correlated with the incidence of diabetes. Whether there is a causal relationship is, however, questionable.

The first objective of an epidemiological study is the collection of data on the frequency of a disease. A further step is to consider underlying causes for the data found. At present, we are concentrating our work on the documentation of incidence rates and not the analysis of the cause of the disease. The preliminary results of our ongoing study indicate a rising incidence in Baden-Wuerttemberg in the last decade without any change in BCG immunization activity in this area.

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Mitochondrial DNA 3243 Mutation Is Infrequent in Japanese Diabetic Patients With Auditory Disturbance

In a recent article, Di Leo et al. (1) concluded that cochlear dysfunction is common in type 1 diabetes. Sensorioneural auditory disturbance has been indicated to be associated with diabetes with mitochondrial mutation at an A-to-G transition at position 3243 of $tRNA^{leu(UUR)}$ (2–4). The frequency of the diabetic patients with this mutation has been reported to be ~0.9–2.0% in Japanese patients with type 2 diabetes (5,6). A previous report (6) has shown that as many as 3 out of 5 (60%) diabetic patients with auditory disturbance

were found to have the mutation of $tRNA^{leu(UUR)}$, suggesting that the mitochondrial mutation is frequent among the patients with diabetes and auditory disturbance. However, there have been no reports, to our knowledge, about the real frequency of this mutation in diabetic patients with auditory disturbance.

To investigate whether sensorioneural auditory disturbance could be a good index to detect the mitochondrial mutation, we examined the mutation in Japanese type 2 diabetic patients with auditory disturbance. Subjects included 35 Japanese patients (21 men and 14 women; mean [±SD] age, 61.6 ± 12.8 years) with type 2 diabetes (n = 33) or impaired glucose tolerance (n = 2), according to World Health Organization criteria, presenting with hearing loss, which was confirmed to be sensorioneural auditory disturbance with audiometry by one of the investigators (H.S.). Genomic DNA was extracted from peripheral leukocytes of the patients and submitted to the detection of an A-to-G transition at the 3243 nucleotide of $tRNA^{leu(UUR)}$, using polymerase chain reaction and restriction fragment length polymorphisms methods with restriction endonuclease *Apa* I. The mutation of the 3243 nucleotide of $tRNA^{leu(UUR)}$ was found only in one female patent with type 2 diabetes (2.9%). There was no statistically significant difference (χ^2 test) between this frequency and the reported ones of the mutation among Japanese patients with type 2 diabetes: 0.9% (5) or 2.0% (6).

A previous report (6) showed that 3 out of 5 cases (60%) of Japanese diabetic patients with hearing loss were found to have the mitochondrial DNA mutation. However, all of those patients also had a family history of the association of diabetes and hearing loss. Our result suggests that auditory disturbance is often associated with NIDDM and that mitochondrial DNA mutation is infrequent in Japanese diabetic patients with auditory disturbance.

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Response to Fukunaga et al.

Fukunaga et al. found that the mutation at np 3243 in the mitochondrial $tRNA^{LEU(UUR)}$ gene was rare in Japanese nonselected type 2 diabetic patients with hearing loss. Although this point mutation is commonly present in patients with the syndrome of mitochondrial myopathy, encephalopathy, lacticacidosis, and stroke-like episodes (1,2), the presence of the 3243 mutation in diabetic patients with maternal inheritance and deafness was noted in a group of Dutch and Japanese populations (3,4). It is clear that no obvious relationship between the 3243 mutation and the onset or the type of diabetes is evident, as postulated by Maassen and Kadowaki (5). The low prevalence of the $tRNA^{LEU(UUR)}$ mutation is in keeping with some studies (6–8) where the mutation is likely to account for <1% of type 2 diabetes. As concerns the effect of the $tRNA^{LEU(UUR)}$ gene mutation on the nature of diabetes (i.e., type 2 diabetes), the situation is still less clear.

Although in our study our type 1 diabetic patients were not tested for the $tRNA^{LEU(UUR)}$ gene mutation, they underwent an audiological examination to exclude the presence of hearing loss before performing the evoked otoacoustic emissions. Thus, the impairment of cochlear receptor function found in some of our patients may indicate an early damage of peripheral nervous system that does not necessarily lead to deafness. Moreover, the finding of mitochondrial DNA mutation by using polymerase chain reaction techniques in peripheral leukocytes may not accurately reflect the number of mutated mitochondria in β-cells, which play an important role in pathogenesis of type 1 diabetes.

An interesting observation is that we showed that no mother of our type 1 diabetic patients with cochlear dysfunction was affected by diabetes. Two of our patients had diabetic fathers (type 1 diabetes in one case and type 2 in the other). We conclude that the $tRNA^{LEU(UUR)}$ mutation does not have a prominent place in the association with type 1 diabetes.

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