staining of agarose gels. In cases where clinical suspicion is high, for example in patients with a strong maternal history of diabetes, isotopic-PCR or silver staining of either standard gels or PhastSystem gels may be appropriate, but only if non-isotopic methods are negative.

Yours sincerely,

A. W. Thomas, R. Morgan, A. Majid, A. Rees, J. C. Alcolado

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Selection of primers for detection of A to G mutation at nucleotide 3243 of the mitochondrial gene

Dear Sir,

Blanché and colleagues [1] report a sensitive method for the diagnosis of maternally inherited diabetes with an A-to-G mutation at nucleotide 3243 of the mitochondrial gene. We also used almost the same method to detect this mutation (unpublished data). However, we think another selection of the primers for polymerase chain reaction (PCR) amplification may lead to an increased possibility of detecting the mutation. We compared the detection rate of this mutation of two sets of primers for amplification: (1H) AG-GACAAGAGAAATAAGGCC (3130–3149), (1L) CAC-GTTGGGGCCTTTGCGTA (3423–3404), and (2H) CGTTTGTTCACGATTAAAG (3035–3054), (2L) AG-CGAAGGGTTGTAGTAGCC (3456–3437). Using the second set of primers the two cleaved fragments (212bp and 214 bp) moved to almost the same position in the gel, resulting in a more distinct single band. Because the population of the mu-

tant DNA of the mitochondrial genome in the peripheral blood is relatively low, thin bands may be overlooked in the screening for this mutation. Although the use of labelled primers is also useful for the detection of this mutation, the procedures are more complicated. From our experience we recommend using the second set of the primers. At the first screening of 300 Japanese patients with non-insulin-dependent diabetes using the (1H) and (1L) primers, we only found two patients positive for the mutation. However, the second set of primers detected one more patient with this mutation.

Retrospectively, the mutation was found to be overlooked because of the thinness of the two bands. We also recommend using polyacrylamide gel or PhastSystem (Pharmacia, Uppsala, Sweden), for the analysis rather than agarose gel, because although expensive it can obtain more distinct bands.

Yours sincerely,

M. Odawara, K. Sasaki, Y. Tachi, K. Yamashita

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Response from the authors

Dear Sir,

Dr. Odawara and colleagues report the selection of a new set of primers to detect the 3243 bp A to G mutation in the mitochondrial genome associated with maternally inherited diabetes and deafness. They suggest that the use of primers giving rise to two cleaved fragments of almost the same size will increase the sensitivity of the test. We agree that these primers could increase the intensity of the band corresponding to the mutation, but we think that the important point to improve this technique is the coloration step, the silver nitrate detection method being more sensitive than an ethidium bromide staining.

However, to decrease the cost of this test and the problems associated with heteroduplexes, we have established an allele specific method using 5 primers. Allele-specific polymerase chain reaction (PCR) is obtained using primers 5' CCG GAG TAA TCC AGG TCG G3' (3077–3095) and 5' TTT ATG

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Genetic analysis of HLA DRB1, DQA1 and DQB1 alleles and susceptibility to IDDM in Chilean subjects

Dear Sir,

The incidence of insulin-dependent diabetes mellitus (IDDM) in Santiago de Chile has been reported to be 2.49 per 100,000 per year [1], which is much lower than that reported for Madrid (11.3 per 100,000 per year) [2] or Barcelona (11.5 per 100,000 per year) [3]. These differences may be due to genetic variation between the populations (i.e. frequencies of IDDM susceptibility alleles, DQA1*Arg-52 and DQB1*non-Asp-57) in Spain and Chile [4–7], partly sharing a Spanish ancestry. Due to the lack of previous HLA studies in IDDM and in non-diabetic subjects in the general population of Chile, we have recently completed a molecular analysis [6] of DRB1, DQA1 and DQB1 alleles for 63 unrelated IDDM patients (26 male, 37 female; mean age, 12.6 years, diabetes duration 7.99 ± 3.95 years), randomly chosen from the Santiago de Chile IDDM Registry for 1991–1992 [1] and 74 healthy non-diabetic control subjects (29 males, 45 females; mean age, 13.6 years) from the same geographical area according to a standardized protocol [7]. IDDM and control children were ethnically similar, belonging to the strata III of Valenzuela [8], which represents a 40% aborigine admixture with European genetic pools, primarily of Spanish origin.

IDDM patients showed a significant decrease in the frequency of HLA-DR2 and DR14 and an increase in DR3 and DR4 compared to non-diabetic control subjects (Table 1). DR14, one of the strong protective alleles, was common among non-diabetic patients (6.8%). DQA1*0101–102–103 and DQA1*0201 were significantly decreased, and

CGA TTA CCG GGC T/C (3261–3243) giving rise to a 185 bp product specific of wild type or mutant allele. In each reaction, an internal positive control primer pair is included: 5' AAT TGA CCT GCC CGT GAA GA3' (2677–2696) and 5' CCC AAC CGA AAT TTT TAA TGC A3' (2816–2795) giving rise to a 140 bp product. The PCR reaction mixture consists of 100 ng genomic DNA, 1 × PCR buffer, 200 mmol/l of each dNTP, 50 ng of each primer and 1 unit Taq polymerase (Perkin Elmer, Branchburg, USA). Cycling conditions were: a first denaturation at 94°C for 5 min, then 30 cycles of 10 s at 94°C, 60°C and 72°C and a final extension at 72°C for 6 min. PCR reaction was checked on a 2% agarose gel.

We checked the patients previously tested by the PCR-RFLP method and the same positive and negative results were obtained by both methods. Allele-specific PCR is less expensive and less time consuming than PCR-RFLP, both advantages being very important for the diagnosis of patients affected with maternally-inherited diabetes associated with deafness.

Yours sincerely,

H. Blanché, N. Cohen, P. Froguel

DQA1*0301 allele was significantly increased among cases compared to control subjects. On the other hand, only allele DQB1*0302 was significantly increased among the cases compared to control subjects. The commonest high-risk genotype found in diabetic patients was DQB1*0302/*0201–DQA1*0301/*0501 (43%), associated with DR4/DR3 alleles in 22% of the cases.

Also, DQA1*Arg-52 homozygotes had 18 times more risk developing IDDM than DQA1*non-Arg-52 homozygotes. Heterozygosity for DQA1*Arg52 conferred only a threefold increased risk. DQB1*non-Asp-57 homozygotes had more than a fivefold increase in IDDM risk as compared to DQB1*Asp57 homozygotes.

The highest relative and absolute IDDM risks were associated with homozygosity for both DQA1*Arg-52 and DQB1*non-Asp-57 alleles, reflecting the potential formation of diabetogenic DQα/DQβ heterodimers, as predicted by the model of Khalil et al. [9]. Only 19% of the general population in Chile were capable of generating four diabetogenic heterodimers, with an absolute risk for developing IDDM of 7.6 per 100,000 per year. A fivefold increase in risk was observed for those who could form two diabetogenic heterodimers while individuals (about 50% of non-diabetic patients) able to generate one or zero diabetogenic heterodimers showed a very low risk of developing IDDM.

Despite the dramatic variation in IDDM incidence in Chile as compared to Spain, the overall frequencies of DQB1 and DQA1 alleles were similar among diabetic patients and control subjects in both countries [6]. DQB1*0602 and DQB1*0301, considered to be protective for IDDM, occurred less frequently in the non-diabetic Chilean children than among those from Madrid (9.5 vs 1.9% and 20.9% vs 1.6%, respectively), but these differences were not statistically significant. DQA1*0301, the most common DQA1 allele in the IDDM Chileans also had, in the Chilean general population, a frequency higher than that reported in non-diabetic Spaniards (27.7 vs 1.7%, respectively) [6], but not compared to other Caucasian populations.

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