A new aldolase B variant, N334K, is a common cause of hereditary fructose intolerance in Yugoslavia

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Hereditary fructose intolerance (HFI) is an autosomal recessive disease that results from a deficiency of aldolase B, the enzyme responsible for the assimilation of dietary fructose (1). Recently we have identified three common point mutations that together account for >85% of HFI alleles in Western Europe (2). In this paper we describe the analysis of 6 unrelated patients from the vicinity of Belgrade.

Aldolase B exons 5 and 8 were amplified by the PCR and probed for the presence of three prevalent HFI lesions (2). Of the 12 Yugoslav alleles, three were found to be A149P and two were A174D (not shown); seven alleles remained unidentified. Patient Y3, who had unknown aldolase B lesions in both genes, was selected for further analysis. Exons 2–9, including splicing signals and lariat branch points, were amplified and sequenced as described (2). A single point mutation was identified: a G→C change in exon 9 which results in the normal asparagine (AAC) at position 334 being replaced by a lysine (AAG). We have therefore termed this mutation N334K.

To confirm the presence of this lesion in patient Y3, allele-specific oligonucleotides (wild type: 5'GCTAACTGCCAGG-CGG3'; mutant: 5'GCTAAGTGCCAGGCGG3') were used to probe amplified exon 9 sequences. Hybridisation conditions were as described (2), with the discriminatory wash at 59°C for 4 mins. Y3 hybridised only to the mutant probe, indicating homozygosity for this lesion. The N334K mutation was detected in 4 Yugoslav patients and accounted for all 7 unidentified alleles. These probes were used to investigate the genotypes of other individuals with HFI: the N334K lesion was also found in an Austrian and in a British HFI patient, both of whom were compound heterozygotes. The use of probes specific for N334K should appreciably enhance the detection of HFI by direct analysis of genomic DNA.

REFERENCES

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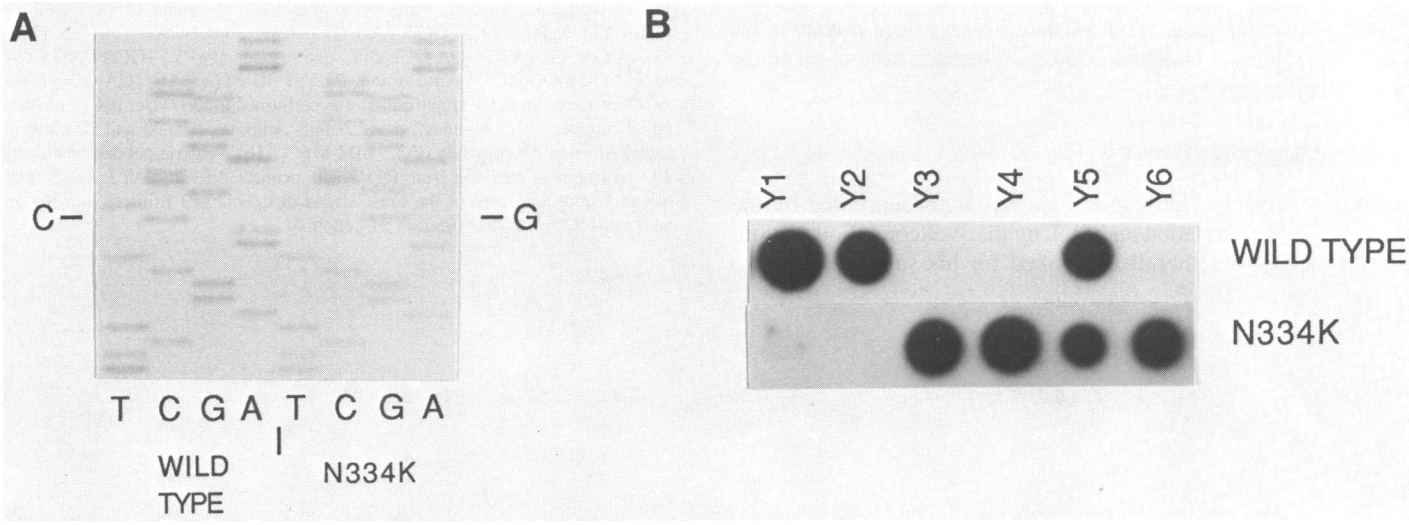

Figure 1. A. Sequencing gel showing N334K mutation. B. Allele specific oligonucleotide hybridisation to 6 Yugoslav HFI patients. Y3, Y4 and Y6 are homozygous for N334K; Y5 is heterozygous; Y1 and Y2 do not carry this lesion.