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Denaturing HPLC-Based Assay for Detection of ATRX Gene Mutations

To the Editor:
In 2003, we described (1) a broad-range denaturing gradient gel electrophoresis method for mutation scanning of the entire open reading frame and canonical splice sites of the ATRX gene (OMIM 300032), a zinc finger transcriptional regulator undergoing X inactivation and probably involved in chromatin remodeling, DNA methylation, and gene expression in mammalian development (2, 3). Mutations affecting the ATRX gene lead to the α-thalassemia/mental retardation syndrome (ATRX syndrome; OMIM 301040).

We now propose a simpler, rapid mutational approach based on denaturing HPLC (DHPLC) (4), with which we were able to confirm all of the nucleotide variations described in our first report (1) and to detect 5 other mutations in 7 of 15 unrelated Italian patients with a clinical suspicion of ATR-X syndrome. Segregation of the syndrome was sporadic in all but 2 individuals. X-inactivation status at the human androgen receptor locus was tested in all patients’ mothers as described previously (5).

PCR primers (Table 1 of the Data Supplement that accompanies the online version of this letter at http://www.clinchem.org/content/vol51/issue7/) were designed to amplify all 35 exons and the consensus splicing sites of the ATRX gene (Entrez Gene ID 546). A total of 44 reactions were performed at a single annealing temperature (57 °C) with Optimase DNA polymerase in 1× buffer (both from Transgenomic); exon 9 was amplified as 10 overlapping fragments. Heteroduplex formation was obtained by mixing together, denaturing, and gradually reannealing equimolar quantities of PCR products for patients and controls. DHPLC analysis was performed with the WAVE™ 3500HT System (Transgenomic). Each crude PCR product (50 µL) was eluted by a 2.5-min run at 3 different analysis temperatures, and the buffer gradients were chosen as suggested by the Navigator software (Transgenomic; Table 1 of the online Data Supplement). Wild-type controls were included in each run. For the heterozygous elution profiles, genomic DNA was reamplified with the DHPLC primers and sequenced using Big-Dye sequencing chemistry (Applied Biosystems). No false-positive results were reported. In the 8 patients negative by DHPLC analysis, direct sequencing failed to detect any deleterious variations. The DHPLC results are summarized in Table 1 of the online Data Supplement.

The new p.L195P (c.584T>C) mutation identified in this study was not found in 200 apparently healthy females; moreover, this amino acid belongs to the zinc finger domain, was conserved during evolution (data not shown), and is segregated in a 3-generation pedigree with a classic ATR-X phenotype and a skewed X-inactivation status in carrier females.

The chromatograms for all mutations found in our patients, including those described in our first report (1), are shown in Fig. 1. Taking into account the time needed to set up and run the PCR...
Whole-Blood Hypercholinemia and Coronary Instability and Thrombosis

To the Editor:

Whole-blood choline (WBCHO) and plasma choline (PLCHO) have been reported to be predictive for cardiac events in patients with suspected acute coronary syndromes (1, 2). The differential information of whole-blood vs plasma choline offers insights into the biochemistry and pathophysiology of acute coronary syndromes. A previous study has shown that mean (SD) WBCHO concentrations are significantly increased in patients with non-ST-elevation myocardial infarction [31.1 (18.8) μmol/L] and high-risk unstable angina [47.4 (22.8) μmol/L] compared with patients with noncardiac chest pain [19.4 (6.8) μmol/L] (1), or healthy volunteers [15.8 (9.5) μmol/L] (1). For interpretation of WBCHO, a cutoff of 28.2 μmol/L has been proposed (1), which also represents the 90th percentile of a reference population. For PLCHO, the optimum cutoff has not been determined, and 25 μmol/L (99th percentile of a reference population) and lower cutoffs (18.5 μmol/L) have been used for risk stratification. We have selected 3 cases with the constellation of increased WBCHO in combination with low PLCHO to discuss potential pathophysiologic implications. All choline analyses were performed with HPLC–mass spectrometry (1), and choline concentrations were not available for clinical decision-making.

Case 1. A 68-year-old man presented to the emergency department with...