ANCIENT DNA ANALYSIS IN AN ITALIAN MEN1 KINDRED

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Multiple Endocrine Neoplasia type 1 (MEN1) is an autosomal dominant inherited disorder with a high penetrance and an equal sex distribution, characterised by tumours of the parathyroids, neuroendocrine cells of the gastro-entero-pancreatic tract and anterior pituitary. The MEN1 locus was originally mapped to chromosome 11q13, flanked centromerically by PYGM and telomerically by D11S449. The MEN1 gene was cloned in 1997 (80-90% of MEN1 patients harbour mutations in its coding region). Approximately 10-20% of MEN1 affected individuals escape from the identification of mutation in the coding region of the gene. When any MEN1 mutation cannot be detected in the germline of a MEN1 pedigree the genetic ascertainment can be performed by haplotype analysis in at least two generations of affected members. Haplotype analysis can be performed using specific DNA markers linked to and flanking the MEN1 region, reaching a higher degree of confidence if a substantial number of affected members can be analysed. We performed haplotype analysis on an Italian MEN1 family with two clinically MEN1 affected members (III-1 and III-2) whose germline mutation was not previously detected either in the coding region or the exon-intron boundaries of MEN1 gene. Consequently, 9 individuals spanning four generations were analysed: 8 living family members and the early deceased affected father (II-1) of the two probands. Modern DNA was extracted from peripheral blood samples. Ancient DNA of II-1 was extracted from powdered small pieces of exhumed bone by means of silica-base protocol (Krings M et al 1997) in an ancient DNA-dedicated laboratory. To exclude possible modern DNA contamination we quantified the hypervariable region I (HVRI) of the mitochondrial genome from deceased patient, using Real-time PCR amplification. All collected family members were haplotyped by PCR-microsatellite analysis, using 4 specific markers for 11q13 locus (D11S480, PYGM, D11S449, D11S913). Microsatellite amplifications for the deceased subject were performed separately, in an ancient DNA-dedicated laboratory, using appropriate no-template controls. Three (two living brothers III-1 and III-2 and their deceased father II-1) out of the 9 analysed family members showed clinical features consistent with those of MEN1 syndrome. Their father early died before the introduction of MEN1 gene genetic test. Mutational analysis of exons 2-10 and exon-intron junctions of the MEN1 gene, in III-1 and III-2 subjects, failed to detect a germline mutation responsible for the disease in this family. Haplotype analysis found that all three clinically affected cases shared a common 11q13 haplotype co-segregating with the disease. This haplotype was not found in any other analysed member of this family. Results coming out from haplotyping analysis allowed us to identify the siblings of III-1 and III-2 as non-carriers. Thus, it has been possible to reconstruct haplotype family history of MEN1 even if one family members was deceased, allowing the early identification of disease haplotype carriers in this family without germline mutation in the MEN1 coding region, avoiding further biochemical and clinical screenings to non-carriers.