EVIDENCE FOR A MUTATION AT 3'HVR COL2A1 LOCUS - SEGREGATION ANALYSIS AND SEQUENCE DATA

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Type II collagen is the major structural protein of cartilage and its encoding locus (COL2A1, 12q1.23-3.21) is the candidate gene for the heritable chondrodysplasia. Single base mutations and partial gene deletions in this sequence are possibly responsible for the clinical syndrome.
Downstream of this exone, a hypervariable region exists composed of tandemly arranged, imperfect repeats, each spanning 31-34 base pairs (1). This marker is highly polymorphic (2) and is used for forensic applications.
We report a mutational event at this interesting locus, met during a paternity analysis.

CASE OUTLINE

A family trio showed a parenthood exclusion at this locus. A higher molecular weight allele (COL2A1 14), present in the child, was absent in both parents, who shared in turn the only segregating child's allele. Analysed for a variety of conventional systems (ABO, Rh haplotype, MNSs, Gc, PI, Hp) Single Locus Probes (SLPs; D2S44, Ms43a, Ms1, Ms31 and g3), Amplified Fragment Length Polymorphisms (AmpFLPs; APOB, COL2A1, D1S80) and Short Tandem Repeats (STRs; HUMTH01 and MIT-MH26), both parents proved to be perfectly compatible. A high probability of paternity was in fact deduced (99.9999).
This strongly supported the hypothesis that a mutation could have occurred at either the maternal or paternal gamete. Therefore, a closer analysis of COL2A1 fragments of this family was carried out.

BAND CLONING AND SEQUENCE ANALYSIS

Amplified products of the trio were run on agarose, then each individual band was excised, electroeluted and separately reamplified. Reamplified alleles were passed through a Centricon 100 filter (Amicon), cloned in Bluescript vector and sequenced by deoxy chain termination (Sequenase, USB).
Sequence analysis supplied the following indications:
1) COL2A1 12 of the mother was identical to the child's allele;
2) COL2A1 14 of the child showed an unusual base transition (C - T) 467; the same mutation was present in COL2A1 12 of the father.
3) COL2A1 14 of the child exhibited a base insertion (A244),

which was also present in COL2A1 8 of the father.
Both alterations of the basic repeat were unlikely to be observed by chance in any other repeat of the three individuals. It was therefore deduced that COL2A1 14 was a mutant allele deriving from the paternal line of inheritance, by unequal crossing over.

Several questions remain to be answered, concerning:
1) the position at which recombination took place;
2) the possible role of the sequence alterations in triggering crossing over;
3) the true frequency of mutational events at COL2A1 locus;
4) the precautions to adopt in using this locus for paternity investigations.

REFERENCES

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