The gene for hereditary multiple exostoses does not map to the Langer-Giedion region (8q23-q24)

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Abstract
Hereditary multiple exostoses is a dominantly inherited skeletal disorder which alters enchondral bone during growth and is characterised by exostoses of the juxtaepiphyseal regions. Using polymorphic DNA probes, we have been able to exclude the disease gene from close proximity to the 8q24.1 region where a dominant syndrome with multiple exostoses, the trichorhinophalangeal syndrome type II (TRP II, Langer-Giedion syndrome, MIM 15025), has been previously localised (pairwise linkage Z = -8.96 at \( \theta = 0 \) with probe L48 at locus D8S51). Multipoint linkage analysis using probes L48, L24, and L1 consistently excluded the HME gene from a large area of the distal long arm of chromosome 8, spanning the smallest region of overlap assigned to the TRP II gene. These studies support the clinical view that HME and TRP II are distinct entities.

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Hereditary multiple exostoses (HME, MIM 13370) is an autosomal dominant skeletal disorder with altered enchondral bone development. It is characterised by exostoses usually arising in the juxtaepiphyseal region of the long bones and sometimes the pectoral and pelvic girdles, ribs, and rarely the vertebrae.1 Interestingly, a strikingly different autosomal dominant disease, trichorhinophalangeal syndrome type II (Langer-Giedion syndrome, TRP II syndrome, MIM 15025), is associated with multiple exostoses. The syndrome also includes characteristic facial features, namely pear shaped nose, tented alae, sparse hair, cone shaped epiphyses, mild microcephaly, and mental retardation. In contrast, no exostoses are present in TRP I syndrome (MIM 19035).

Considering the phenotypic overlap between the three syndromes, Bühler and Malik2 suggested that HME, TRP I, and TRP II might either be expressed by contiguous genes or represent various allelic forms of one single genetic entity, especially as deletions of the long arm of chromosome 8 (8q23-8q24.1) have been reported in both TRP II and TRP I syndromes.34

Along the same lines, Ogle et al5 recently described a balanced reciprocal translocation t(8;11)(q24.1;p15.5) in a woman presenting with clinical features of HME. This interesting observation provides additional support for the view that the gene for HME might map on the long arm of chromosome 8 as well. In this study we tested six large HME pedigrees for linkage to the TRP II locus, using polymorphic DNA probes derived from the microdissected q23-q24 region of chromosome 8,11 and provide evidence for exclusion of the HME gene from close proximity to the TRP II locus.

Patients and methods
In recent years, 166 new cases of HME have been observed in the Hôpital des Enfants Malades. Out of the 28 families with at least three affected subjects, six families were selected for their informativeness (47 subjects including 25 affected) (fig 1).

The criterion for diagnosis of HME syndrome was the observation of at least two exostoses of the juxtaepiphyseal regions of the long bones. Exostoses associated with metaphy- dromatosis (MIM 15625) were excluded from the study.

Owing to the existence of latent forms of HME, family studies usually included radiography of the knees. Children under 3 years were excluded because of delayed expression of the disease. A 20 ml EDTA blood sample was collected from each of the 47 subjects. DNA was extracted from lymphocyte pellets by SDS lysis, proteinase K digestion, phenol/chloroform extraction, ethanol precipitation, and Tris-EDTA resuspension. Restriction digest of genomic DNA were performed for four hours in buffer supplied by Boehringer. DNA fragments were separated by horizontal gel electrophoresis in TEA buffer, stained with ethidium bromide for visual inspection, and denatured in situ with 0.25 mol/l HCl. The denatured DNA fragments were transferred onto a nylon membrane (Zetabind, Fico Cuno) using the technique of Southern. The DNA probe was labelled with \(^{32}\)P-dCTP by nick translation and purified on a Sephadex G-50 column to remove unincorporated nucleotides. The labelled plasmid was then competitively hybridised to sheared total human DNA to reduce lane background caused by common repeats in the probe.12 The mix was then added to 25 ml of the hybridisation solution. Membranes were hybridised in polythene bags overnight at 65°C. They were washed twice at room temperature in 2 x SSEP and 0.1% SDS, then washed at 65°C in 1 x SSEP and 0.1% SDS.
Results

Pairwise linkage showed negative lod score values with probe L48 \((Z = -8.96 \text{ at } \theta = 0)\), excluding the HME gene from close proximity to the \(D8S51\) locus (table). Multipoint analysis using probes L48, L24, and L1 consistently excluded the HME gene from a relatively large area of the distal long arm of chromosome 8, spanning the smallest region of overlap assigned to the TRP II gene (fig 2). The other probes tested were not informative. However, using probe MCT128, we were able to exclude the HME gene from close proximity to the \(D8S39\) locus, which maps 50\,cM distal to the TRP II gene.

Discussion

In the last few years, HME, TRP I, and TRP II have been regarded as closely related diseases and the hypothesis of either contiguous genes or allelism at a single locus has been discussed.\(^2\)\(^\text{10}\) Here, we exclude the HME gene from close proximity to the TRP II locus in six autosomal dominant families with multiple exostoses. The present data tend to favour the view that HME and TRP are clearly distinct entities. These results are not really surprising as a number of clinical, radiological, and laboratory features allow differentiation of the three conditions.

First, neither facial dysmorphism nor ectodermal dysplasia are present in HME, while those are consistent features in TRP syndrome. Similarly, the clinical progression of exostoses stops at the end of growth in HME while it may continue throughout adult life in TRP II, resulting in severe orthopaedic complications. Second, radiological features are also different in the two conditions. Metaphyseal exostoses, for instance, are pediculated in HME, while in TRP II they are more sessile. Whatever the type, the association of exostoses with shortness of the ulna, fibula, and metacarpals is more specific of HME disease, while cone shaped phalangeal epiphyses are characteristic of TRP II.\(^1^\)\(^9\) Finally, the histopathological aspects of the cartilage are strikingly different.\(^1^\)\(^9\) In HME, the exostoses are covered by thickened periosteum that closely adheres to the cartilaginous cap. It arises in contact with the bone.

Autoradiographs were obtained by exposure to Kodak X/OMAT films for one to four days at \(-80\,^\circ\text{C}\). Polymorphic DNA probes were generated by Hortschek and Lüdek\(e\)\(^1\)\(^1\) from a 8q24.1 specific microdissection library of the TRP II deletion: L48 (\(D8S51\)), L24 (\(D8S45\)), XmnI, and L1 (\(D8S42\)) Taql. Five additional probes located on both sides of the deletion on the long arm of chromosome 8 were also used, namely: PYNM3 at locus \(D8S17\),\(^1\)\(^3\) LT II at locus \(D8S5\),\(^1\)\(^4\) R370 at locus \(D8S25\),\(^1\)\(^5\) L1251 at locus \(D8S29\),\(^1\)\(^5\) and MCT128 at locus \(D8S39\).\(^1\)\(^6\) All these DNA markers showed biallelic RFLPs.

The most likely order of markers was: cen-PYNM3-LT11-L1-L48-L24-Myc-R370-L1251-MCT128-tel. Considering the uncertainty of the exact age of onset during growth, a penetrance of 0-90 was arbitrarily assigned. Linkage analysis was performed using the MLINK and LINKMAP options of version 5.1 of the LINKAGE package.\(^1\)\(^7\)
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with the columns of growth cartilage, which are in abnormal orientation at the periphery (fig 3). In contrast, in TRP II, the exostoses seem to arise as nodules linked to the cartilage of the epiphysis by a pedicle and the cells are voluminous, rich in glycogen, and arranged in ovoid groups (fig 4). Ultrastructural studies show inclusions in chondrocytes and numerous vesicles in the cytoplasm of the osteoblasts.

In conclusion, the genetic data presented here support the clinical view that HME and TRP are probably distinct entities, especially as no deletion of chromosome 8q has been hitherto reported in cells cultured from exostoses. Nevertheless, the observation of Ogle et al suggests that this condition might be heterogeneous and future linkage studies will hopefully contribute to the mapping of the gene(s) responsible for hereditary multiple exostoses.

Addendum
Three new families with HME were tested by D8S51 (L48). One family was not informative and in the other two a recombination in each was found. Pairwise linkage analysis showed a negative lod score with probe L48 at Z = -18:32 at θ = 0.

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Figure 3 Hereditary multiple exostoses (frozen section 7μ, Azure A pH 2 staining). The growth cartilage columns have an abnormal orientation at the periphery of the cartilage.

Figure 4 TRP II syndrome (frozen section 7μ, PAS staining). Cartilage nodule containing groups of large cells, situated under the perichondrium of the epiphysis.
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