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Refinement of the Multiple Exostoses Locus (EXT2) to a 3-cM Interval on Chromosome 11

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Summary

Hereditary multiple exostoses (EXT) is an autosomal dominant skeletal disorder characterized by the formation of multiple exostoses on the long bones. EXT is genetically heterogeneous, with at least three loci involved: one (EXT1) in the Langer-Giedion region on 8q23-q24, a second (EXT2) in the pericentromeric region of chromosome 11, and a third (EXT3) on chromosome 19p. In this study, linkage analysis in seven extended EXT families, all linked to the EXT2 locus, refined the localization of the EXT2 gene to a 3-cM region flanked by D11S1355 and D11S1361/D11S554. This implies that the EXT2 gene is located at the short arm of chromosome 11, in band 11p11-p12. The refined localization of EXT2 excludes a number of putative candidate genes located in the pericentromeric region of chromosome 11 and facilitates the process of isolating the EXT2 gene.

Introduction

Hereditary multiple exostoses (EXT; MIM 133700) is an autosomal dominant disease with an estimated prevalence of 1/50,000 (Schmale et al. 1994). EXT affects enchondral bone formation and is characterized by the presence of multiple exostoses. These exostoses are most frequently located in the juxtaepiphyseal regions of long bones and are often accompanied by characteristic skeletal deformities and by short stature (Solomon 1961). Generally, exostoses continue to grow until closure of the growth plate. The presence of exostoses can cause pressure on neighboring tissues, resulting in a disturbed articular function (Hennekam 1991). In rare cases, spinal or cervical cord compression (Eder et al. 1993) and myelopathy (Wen et al. 1989) due to the presence of exostoses has been observed. In ~5% of the cases, malignant transformation of one of the exostoses occurs, resulting in the development of an osteosarcoma or chondrosarcoma (Jaffe 1943; Luckert Wicklund et al. 1995).

The formation of multiple exostoses is also one of the findings of tricho-rhino-phalangeal syndrome type II (TRP II), often referred to as "Langer-Giedion syndrome" (LGS). Besides multiple exostoses, LGS patients usually show mental retardation, unusual facies, and cone-shaped epiphyses (Langer et al. 1984). The latter two features are also found in patients with tricho-rhino-phalangeal syndrome type I (TRP I), but these patients are rarely mentally retarded and do not have multiple exostoses. TRP I is sometimes, and TRP II is often, associated with chromosomal deletions in the 8q23-q24 region (Bühler and Malik 1984). Linkage analysis in families with isolated EXT has provided evidence for an EXT locus on 8q24.1 (Cook et al. 1993). This supports the hypothesis that TRP II is a contiguous-gene syndrome caused by the deletion of at least two genes: one causing TRP I and a second causing multiple exostoses. However, in some of the EXT families analyzed by Cook et al. (1993), linkage to the TRP II region on 8q23-q24 was excluded, suggesting genetic heterogeneity of EXT. A genome search in two large EXT families unlinked to chromosome 8q24.1 resulted in the localization of a second EXT locus (EXT2) to the pericentromeric region of chromosome 11 (Wu et al. 1994). Recently, evidence was provided for a third EXT locus (EXT3) on 19p11-p13 (Le Merrer et al. 1994). We now report the refinement of the EXT2 candidate region on chromosome 11 by linkage analysis in seven large EXT pedigrees and provide further evidence for the localization of the EXT2 gene on the proximal short arm of chromosome 11.

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Subjects and Methods

Families

A total of 168 individuals, including 72 EXT patients from seven large EXT families, were included in this study. EXT families 1 and 2, of Belgian and Dutch origin, respectively, have been described elsewhere and were used to locate the EXT2 locus (Wu et al. 1994). Family 3 is of Dutch origin, while the remaining families, 4–7, originate from the United States. Families 6 and 7 have been described by Cook et al. (1993) (who have designated them as families 99 and 88, respectively), whereas families 4 and 5 have not been described elsewhere. The diagnosis of EXT in these families was based on physical and radiological examination, as described by Wu et al. (1994). TRP I or other symptoms were not present in these families.

Linkage Analysis

A set of polymorphic microsatellites from the pericentromeric region of chromosome 11, including D11S935, D11S905, D11S1355, D11S903, D11S1361, and D11S1313 (Gyapay et al. 1994), was analyzed. In addition, the non-Généthon marker D11S554 was analyzed, since this marker resulted in the highest positive LOD score in our previous study (Wu et al. 1994). Two-point LOD scores between EXT2 and these marker loci were calculated with the MLINK and ILINK programs of the LINKAGE package. Allele frequencies were set at $1/n$ for all markers. Penetrance of the disease was estimated at 95%, and the frequency of the mutation was set at .00002. To delineate key recombinational events in families 1–3, additional microsatellite markers D11S863, D11S1290, D11S2095, and D11S2099 were analyzed in relevant parts of these families.

Results

The markers D11S935, D11S905, D11S1355, D11S903, D11S1361, D11S554, and D11S1313 were initially typed in 22 EXT families. Families for which the maximum LOD score was not $>+2.0$ for at least one of these chromosome 11 markers were withdrawn from this study. In six of the remaining seven families a positive LOD score $>+2$ was reached for at least two of these markers. Family 5 showed LOD scores $>+2$ for only one marker (D11S905). Pairwise LOD scores between EXT2 and the tested markers are listed in table 1. Combined LOD scores $>+3$ were obtained for all markers, with the exception of D11S935 and D11S1361. This can be explained by the large distance ($\geq$10 cM) of D11S935 from the EXT2 locus and by lack of informativeness for D11S1361 in most families.

D11S903, highly informative in all families but one, is the only marker with a maximum LOD score at 0% recombination, indicating absence of recombinants with the putative EXT2 locus in our set of families. A recombination event between EXT2 and D11S905 was observed in family 3 (patient II3) (fig. 1). Since D11S903 cosegregates with the disease in this crossover, EXT2 can be mapped proximal to D11S905 (fig. 2). In patient II5 of family 5, D11S1313 recombines with the disease, mapping EXT2 distal to this marker (fig. 2). On the Généthon linkage map, D11S903 is flanked by D11S1355 on the distal side and by D11S1361 on the proximal side, at a distance of 1 and 2 cM, respectively. Segregation analysis in the various pedigrees revealed two key recombination events: between EXT2 and D11S1361 in family 1 (patient II7) and between EXT2 and D11S1355 in family 2 (patient IV8). Both recombinations are confirmed by at least one additional marker (fig. 2). Unfortunately, D11S903 showed no recombination with the disease in family 1, whereas D11S903 was not informative in family 2, preventing further mapping of the disease relative to this marker.

On the basis of the radiation hybrid map (James et al. 1994), six additional microsatellite markers may be located in the EXT2 candidate region: D11S863, D11S1290, D11S2095, D11S1785, D11S1763, and D11S2099. Preliminary physical mapping data indicate that D11S1785 and D11S1763 are located distal to D11S1355, outside the EXT2 region (data not shown). The remaining four markers were analyzed in the key recombinants in families 1–3 to delineate further the EXT2 candidate region. The recombinant in family 5 was not analyzed with these markers, since it cannot reduce the EXT2 candidate region any further (fig. 2). In patient II7 from family 1, D11S863 and D11S2099 also recombined with EXT2, mapping EXT2 distal to these markers. However, the relative position of these markers in reference to D11S903 and D11S1361 is not well established. If D11S863 and/or D11S2099 maps distal to D11S1361, the EXT2 candidate region will be reduced. At the moment, we are constructing a contig of YACs and cosmids to map these markers more precisely. D11S1290 and D11S2095 were not informative in this meiosis. In patient IV8 from family 2, D11S863, D11S1290, and D11S2099 did not recombine with EXT2, whereas D11S2095 was not informative. In the recombination event in family 3, only D11S2095 was informative, but it did not recombine with EXT2. In conclusion, analysis of these markers in the key recombination events provides, at this time, no additional information with regard to the position of EXT2. Therefore, at present, we can conclude that the EXT2 gene resides within a 3-cM interval between D11S1355 and D11S1361 (fig. 2).

Of all markers analyzed, D11S554 has the highest LOD score (20.885 at $\theta = .013$). Unfortunately, this marker is not integrated into the Généthon linkage map. However, on the radiation hybrid map (James et al.

Table 1

Pairwise LOD Scores between the EXT2 Locus and Chromosome 11 Markers

Marker and Family	LOD Score at θ = .00	.01	.05	.10	.20	.30	.40
D11S935:							
1	−9.007	−1.219	.589	1.115	1.217	.893	.419
2	−5.250	−1.219	−.397	−.037	.230	.239	.122
3	−11.461	−6.318	−5.398	−4.383	−2.297	−1.110	−.389
4	.861	.848	.796	.728	.581	.414	.224
5	−6.279	−.015	.595	.742	.719	.550	.306
6	−4.024	−1.336	−.629	−.338	−.098	−.016	.002
7	−4.451	−.456	.291	.555	.647	.527	.303
Total	−39.610	−9.715	−4.153	−1.617	.998	1.498	.987
D11S905:							
1	−1.912	3.237	3.584	3.427	2.743	1.837	.800
2	−1.581	2.334	2.748	2.680	2.182	1.467	.630
3	−8.961	−3.735	−3.347	−3.435	−2.523	−1.257	−.470
4	3.523	3.464	3.224	2.910	2.234	1.490	.708
5	2.044	2.009	1.869	1.685	1.287	.849	.396
6	2.020	1.982	1.827	1.628	1.205	.749	.278
7	1.009	.987	.898	.787	.570	.364	.173
Total	−3.859	10.278	10.803	9.682	7.699	5.499	2.515
D11S1355:							
1	2.365	2.360	2.301	2.167	1.778	1.278	.683
2	−4.189	−.257	.328	.482	.481	.350	.177
3	−.160	−.140	−.090	−.040	.000	.000	.000
4	.000	.000	.000	.000	.000	.000	.000
5	1.389	1.363	1.259	1.122	.828	.512	.209
6	2.028	1.990	1.835	1.634	1.210	.753	.280
7	1.318	1.292	1.185	1.051	.784	.518	.258
Total	2.751	6.608	6.818	6.416	5.081	3.411	1.607
D11S903:							
1	3.038	2.987	2.777	2.503	1.913	1.267	.595
2	3.421	3.361	3.118	2.799	2.109	1.345	.539
3	4.143	4.070	3.774	3.387	2.546	1.610	.612
4	.000	.000	.000	.000	.000	.000	.000
5	.581	.572	.537	.492	.392	.280	.151
6	1.714	1.681	1.544	1.369	.998	.604	.211
7	4.986	4.898	4.539	4.073	3.082	2.018	.923
Total	17.883	17.570	16.289	14.623	11.040	7.124	3.032
D11S1361:							
1	−5.792	.345	.877	.965	.826	.560	.269
2	−.296	−.292	−.270	−.230	−.138	−.063	−.016
3	.413	.423	.444	.443	.385	.282	.152
4	.000	.000	.000	.000	.000	.000	.000
5	.000	.000	.000	.000	.000	.000	.000
6	.193	.188	.166	.138	.084	.038	.009
7	.000	.000	.000	.000	.000	.000	.000
Total	−5.482	.663	1.217	1.316	1.157	.817	.414
D11S554:							
1	−3.678	2.349	2.870	2.880	2.458	1.779	.947
2	4.183	4.235	4.258	4.073	3.369	2.390	1.193
3	4.018	3.948	3.661	3.286	2.476	1.581	.632
4	2.754	2.770	2.739	2.591	2.114	1.478	.730
5	1.743	1.708	1.568	1.385	.995	.590	.233
6	2.300	2.258	2.086	1.864	1.393	.884	.350
7	3.513	3.454	3.214	2.904	2.250	1.547	.793
Total	14.832	20.722	20.396	18.983	15.054	10.249	4.878
D11S1313:							
1	−2.519	4.085	4.381	4.158	3.341	2.301	1.100
2	3.327	3.336	3.281	3.108	2.567	1.819	.882
3	−2.220	−2.292	−2.590	−2.970	−2.381	−1.202	−.458
4	1.565	1.537	1.424	1.278	.972	.651	.324
5	−6.279	.015	.595	.742	.719	.550	.306
6	.475	.466	.428	.377	.274	.173	.082
7	5.266	5.174	4.798	4.309	3.269	2.146	.969
Total	−.382	12.321	12.316	11.002	8.761	6.438	3.206

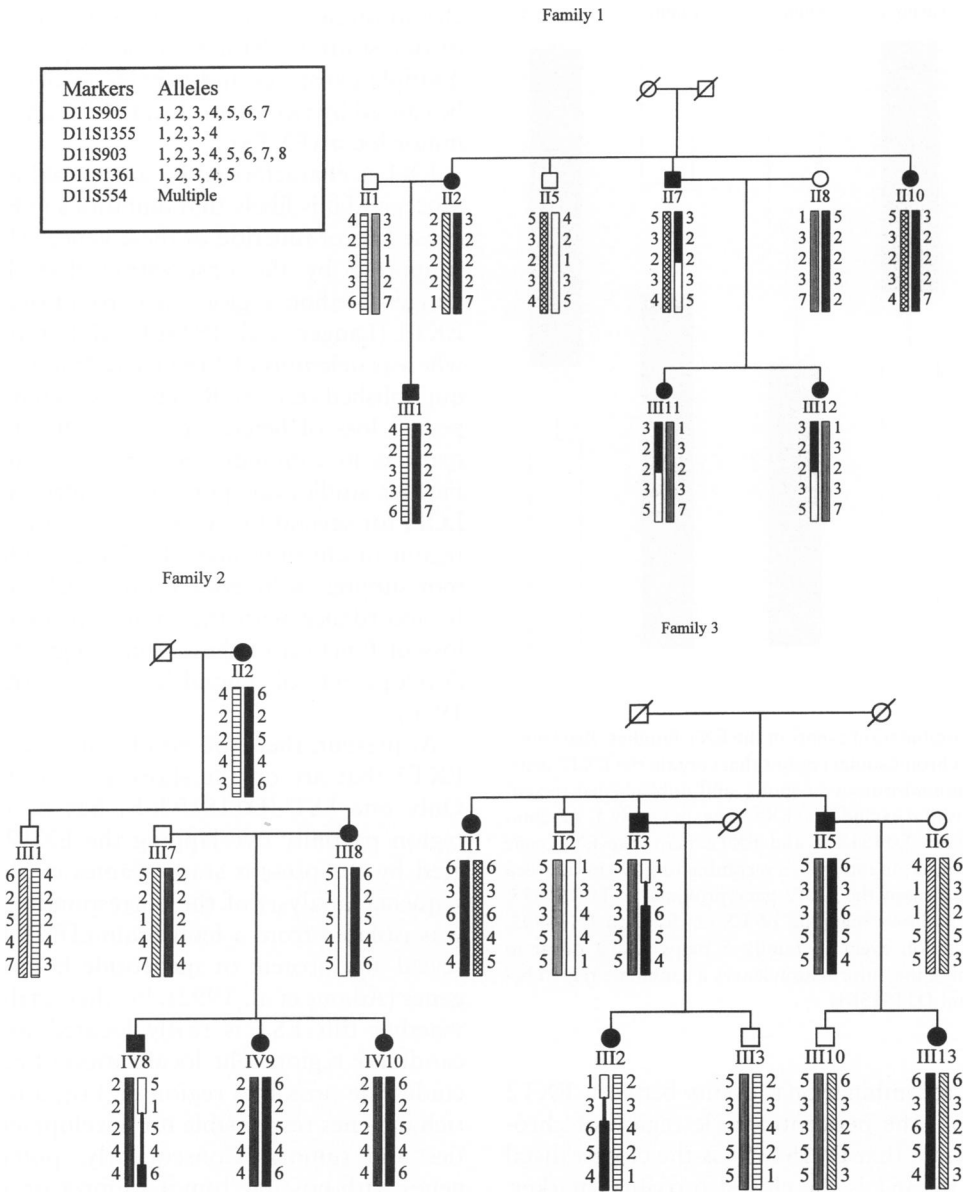

Figure 1 Representation of three key recombinational events. Haplotypes for five markers are shown in parts of the pedigrees. Affected chromosomes are indicated by blackened rectangles. In family 1, individual II7, there is recombination between D11S903 and D11S1361; in family 2, individual IV8, there is recombination between D11S1355 and D11S1361; and in family 3, individual II3, there is recombination between D11S905 and D11S903.

1994) this marker is located proximal to D11S1361. In an attempt to obtain a more detailed localization for D11S554, the cosmid cCI11-388 containing D11S554 (Tokino et al. 1991) was tested for the presence of Généthon markers by PCR screening. This revealed that D11S1361 is also contained in this 42-kb cosmid, suggesting a maximum distance of ~42 kb between D11S554 and D11S1361. Since cosmid cCI11-388 has been assigned to the chromosomal region 11p11-p12 by somatic cell hybrid mapping (Tokino et al. 1991), both D11S554 and D11S1361 must be located in 11p11-p12. In addition, D11S905, which is located 2 cM distal to D11S1355, maps to 11p12 (Couillin et al. 1994). Since D11S554/D11S1361 and D11S1355 flank EXT2, the disease gene must be located on the short arm of chromosome 11, in bands 11p11-p12.

Discussion

Using linkage analysis with polymorphic chromosome 11 markers, we were able to localize the EXT2 gene on the short arm of chromosome 11, in bands 11p11-p12.

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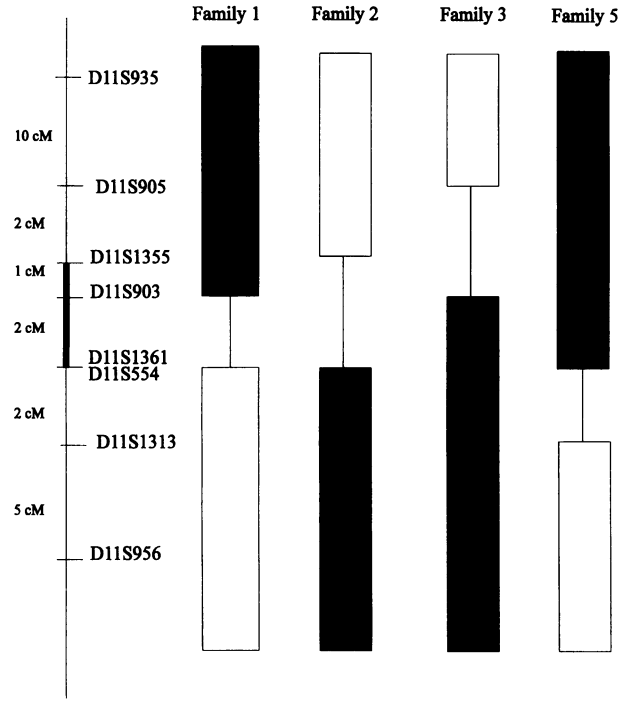

Figure 2 Recombination events in the EXT families. Blackened rectangles represent chromosomal regions that contain the EXT2 gene; single lines denote noninformative regions, and unblackened rectangles represent regions that exclude the EXT gene. In family 1, a recombination event between D11S1361 and EXT2 maps the EXT gene distal to the latter marker. In family 2, a recombination event between D11S1355 and EXT2 maps the EXT2 gene proximal to D11S1355. Analysis of family 3 allows mapping of EXT2 distal to D11S905, whereas a recombination event in family 5 maps EXT2 distal to D11S1313. Combining this information leaves a candidate region between D11S1355 and D11S1361.

Analysis of key recombinational events between EXT2 and markers from the pericentromeric region of chromosome 11 indicate that D11S1355 is the closest distal marker and D11S1361 is the closest proximal marker. These results were obtained in seven extended EXT families with clear evidence of linkage to chromosome 11 markers. Since EXT can be present in a very mild form, which might be difficult to detect, the refinement of the EXT2 candidate region was based on the recombination events in affected patients only. These recombinants reduce the candidate region for the EXT2 gene, from 17 cM, as reported in our original study (Wu et al. 1994), to 3 cM, in the present study.

At present, three EXT loci have been identified: EXT1 on chromosome 8, EXT2 on chromosome 11, and EXT3 on chromosome 19. No evidence of linkage to EXT3 on chromosome 19 was obtained in any of the families in thhis study. Further, in the original paper describing the EXT3 locus (Le Merrer et al. 1994), none of the families showed definite proof of linkage to chromosome 19, since no LOD score $>+3$ was obtained for any chromosome 19 marker in a single family. The results of our study underline the heterogeneous character of multiple exostoses and provide evidence that EXT can be caused by two major loci (EXT1 and EXT2) and one minor locus (EXT3).

EXT is characterized by autosomal dominant inheritance, and it is likely that mutations in EXT1 and EXT2 cause loss of function of these genes. This hypothesis is supported by the observation that deletions of the Langer-Giedion region on chromosome 8q24 lead to EXT1 (Langer et al. 1984; Goldblatt and Smart 1986), whereas deletions of 11p11-p12 lead to EXT2 (authors' unpublished results). Recently, Raskind et al. (1994) reported loss of heterozygosity (LOH) for several 8q24 markers in a chondrosarcoma of a patient with EXT. Further studies on sporadic chondrosarcomas revealed LOH for several loci in 8q24 or in the pericentromeric region of chromosome 11. These results suggest a tumor-suppressor function for the EXT1 and EXT2 genes. In accordance with the multistep theory of Knudson, loss of function of these genes might be related to the development of chondrosarcomas (Knudson et al. 1976).

At present, there are no obvious candidate genes for EXT2 that are on the short arm of chromosome 11. Only one EST (D11S1936E) has been mapped to a region partially overlapping the EXT2 region delineated by the present study (James et al. 1994). Partial sequence analysis of the corresponding cDNA, which was isolated from a fetal brain cDNA library, did not reveal any protein or nucleotide homology to known genes (Adams et al. 1992). Further analysis must reveal whether this EST is really located within the EXT2 candidate region. The localization of EXT2 to 11p excludes the proximal region of 11q, a region extremely rich in genes responsible for developmental abnormalities and tumors. Consequently, potential candidate genes with possible tumor-suppressor activity, such as MEN1 and FAU, that are located in this region are therefore excluded. Only a limited number of genes are mapped to the proximal part of 11p: the lysosomal acid phosphatase (ACP) gene (Pohlmann et al. 1988), the m4 muscarinic receptor (CHRM4) gene (Grewal et al. 1992), and the oncogene SPI 1 (Van Cong et al. 1990). However, these three genes have been mapped between D11S1344 and D11S1326, proximal to the EXT2 candidate region (James et al. 1994), excluding the possibility that one of them is the EXT2 gene. Since no candidate genes are located in the current candidate region, the identification of EXT2 will first require the isolation of expressed sequences from the candidate region. Therefore, the refinement of the EXT2 candidate region on chromosome 11 as presented here is an essential step toward this identification and characterization of the EXT2 gene.

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