Genotype-phenotype correlation in hereditary multiple exostoses

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Abstract

Hereditary multiple exostoses (HME) is a genetically heterogeneous autosomal dominant disorder characterised by the development of bony protuberances mainly located on the long bones. Three HME loci have been mapped to chromosomes 8q24 (*EXT1*), 11p11-13 (*EXT2*), and 19p (*EXT3*). The *EXT1* and *EXT2* genes encode glycosyltransferases involved in biosynthesis of heparan sulphate proteoglycans. Here we report on a clinical survey and mutation analysis of 42 HME French families and show that *EXT1* and *EXT2* accounted for more than 90% of HME cases in our series. Among them, 27/42 cases were accounted for by *EXT1* (64%, four nonsense, 19 frameshift, three missense, and one splice site mutations) and 9/42 cases were accounted for by *EXT2* (21%, four nonsense, two frameshift, two missense, and one splice site mutation). Overall, 31/36 mutations were expected to cause loss of protein function (86%). The most severe forms of the disease and malignant transformation of exostoses to chondrosarcomas were associated with *EXT1* mutations. These findings provide the first genotype-phenotype correlation in HME and will, it is hoped, facilitate the clinical management of these patients.

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Hereditary multiple exostoses (HME, MIM 133700) is an autosomal dominant condition characterised by the development of multiple osteochondromas (exostoses) from the meta-epiphyseal areas of the long bones.¹ The exostoses are either sessile (broad based) or pedunculated and they vary widely in size and number. They can be present at birth and continue to appear and grow throughout childhood and into puberty. Exostoses are usually located in the metaphyseal region of the proximal humerus, distal radius and ulna, proximal tibia, and distal femur, but can also be found

on the pelvis and scapula. They can cause multiple and severe complications including pain, restricted range of joint movement, deformities and shortening of the long bones, and nerve or blood vessel compression. The most severe complication is malignant transformation of an exostosis into a chondrosarcoma, which occurs in 0.5–2% of cases.^{2–4} In addition, intra- and interfamilial clinical variability of HME has long been recognised.^{2–3}

HME is genetically heterogeneous as three *EXT* loci have been identified so far. *EXT1* (MIM 133700) has been mapped to chromosome 8q23-24,⁵ *EXT2* (MIM 133701) to chromosome 11p11-p12,⁶ and *EXT3* (MIM 600209) to chromosome 19p.⁷ Linkage studies have identified *EXT1* and *EXT2* as the two major disease loci in HME families, while *EXT3* appears to be a minor locus.⁸ Three additional loci designated *EXTL1*, *EXTL2*, and *EXTL3* have been identified and mapped to chromosomes 1 (1p36, 1p11-p12) and 8 (8p12). However, no HME family has yet been linked to these loci.^{9–11} Loss of heterozygosity (LOH) at the *EXT1*, *EXT2*, and *EXT3* loci has been observed among patients with *EXT* related and unrelated chondrosarcomas suggesting that the *EXT* genes are tumour suppressors.^{12–15}

So far, only two *EXT* genes have been cloned, namely *EXT1* and *EXT2*.^{16–18} Both genes encode glycosyltransferases harbouring *N*-acetylglucosamine transferase (GlcNac-T) and D-glucuronic acid transferase (GlcA-T) activities that catalyse the biosynthesis of heparan sulphate proteoglycans (HSPG).^{19–20} Recent studies suggest that *EXT1* and *EXT2* may interact in vivo to form a functional oligomeric complex in the Golgi apparatus where the heparan sulphate polymerisation is thought to occur.²¹

A number of mutations have been reported in HME but the cause of its clinical variability remains unclear.^{22–23} Very few studies have concerned the correlation of genotype to phenotype.²⁴ We have carried out an extensive mutation analysis of the *EXT1* and *EXT2* genes in a large series of HME families and investigated whether specific clinical features could be associated with either locus. Here, we show that *EXT1* mutations caused the most severe

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Table 1 Evaluation of the functional rating

Rating	Pain	Deformity			
		Brachymetacarpus	Shortening of forearm (cm)	Varus-valgus angulation of knee (degrees)	Shortening of limb (cm)
Good	None/mild	–	None/<1	None	None/1
Fair*	Moderate/severe	+	>2	>10	>2

*At least one factor had to have had that rating.

forms of the disease and degeneration of exostosis into chondrosarcoma only occurred in *EXT1* patients.

Patients and methods

PATIENTS AND CLINICAL STUDY

A total of 42 HME French families (representing 217 affected subjects) were investigated. In order to document the severity of the disease and intrafamilial variability, a questionnaire was sent to each patient. Patients were asked to fill in the questionnaires with the help of their physician. In most cases clinical and x ray data were re-examined in collaboration with the physicians and orthopaedic surgeons to verify the accuracy of the answers. No discordance between the answers and our observations were noticed. For evaluating the functional rating, a modification of the scale established by the Musculoskeletal Tumor Society was used.^{2,25} The overall functional grade was based on five factors (including pain) and four categories of deformities, namely brachymetacarpus, bowing of the forearm, shortening of the forearm, varus or valgus angulation of the knees, and shortening of limbs. For an overall functional rating of "fair", at least one of the five factors had to have had that rating (table 1). For each patient, the severity of the disease was evaluated from five factors: age at onset, number of exostoses at the time of evaluation, location of the exostoses (vertebral), stature, and functional rating (table 2). For each family, the degree of severity was evaluated from the most affected members.

GENETIC STUDIES

Blood samples were obtained with the written consent of patients and unaffected relatives. The whole set of families was tested by using microsatellite DNA markers closely linked to *EXT1* (D8S199, D8S198, D8S85), *EXT2* (D11S905, D11S903, D11S544), and *EXT3* loci (D19S840, D19S413, D19S221).⁸ For mutation detection in probands, the whole coding sequence of the *EXT1* and *EXT2* genes was amplified²⁶ and analysed by single strand conformation polymorphism (SSCP) either on Hydrolink MDE gels or GeneGel Excel 12.5/24 gels. The Hoefer Automated Gel-stainer was used for gel silver staining (Pharmacia Biotech). Amplification products showing abnormal patterns of migration were

Table 3 Subgroups of the severe type

	No of exostoses	Vertebral location	Stature (centile)	Functional rating
Type IS	25	–	<10	Fair
Type IIS	25	–	<10	Fair
Type IIIS	10	+	<10	Fair
Type IVS	10	–	<3	Fair

reamplified and sequenced by using the fluorescent dideoxy terminator method on an automatic sequencer ABI.

STATISTICAL ANALYSIS

Fisher's exact test was used for the analyses; p values equal to or less than 0.05 were considered to be significant.

Results

GENETIC AND MOLECULAR STUDIES

Linkage analyses confirmed the existence of at least three *EXT* loci on chromosomes 8, 11, and 19. Among the 42 families, 29 showed strong linkage to *EXT1* (69%), nine to *EXT2* (21%), and one to *EXT3* (3%). Interestingly, several pedigrees (7%) gave negative lod scores with markers of chromosomes 8, 11, and 19. Taken together, these results suggest that *EXT1*, *EXT2*, and *EXT3* account for about 90% of cases but that at least one additional locus could account for HME. An abnormal pattern of migration and a mutant genotype was found in 36/38 patients. The mutations identified in *EXT1* and *EXT2* are summarised in table 3. No mutation was detected in two families linked to *EXT1* despite SSCP analysis and extensive sequencing of all *EXT1* exons.

Among the 36 mutant alleles reported here, 27 (75%) were found in *EXT1*. Mutations identified included four nonsense mutations, 19 frameshift deletions or insertions, three missense and one splice site mutation. These mutations were almost randomly distributed over the first nine exons. Most of them were novel, except for five previously reported mutations²³ (table 4). Ninety percent of them caused a premature termination of the *EXT1* protein. Among the nine mutations found in the *EXT2* gene, four were nonsense mutations, two were frameshift deletions or insertions, one altered a splice site, and two were missense mutations. Most of the *EXT2* mutations were novel mutations, randomly distributed over the first eight exons.

CLINICAL STUDY

Based on the phenotypic expression of the disease, two groups of families were recognised. The first group, which included families with severely affected members, was referred to as group S. In the second group (group M),

Table 2 Evaluation of the severity of the phenotype

	Age of onset (years)	No of exostoses	Vertebral location	Stature (centile)	Functional rating
Severe*	3	10	+	10	Fair
Moderate	>3	<10	–	>10	Good

*At least three factors had to have had that rating.

Table 4 Summary of *EXT* gene mutations, clinical data

Mutation						Clinical data										Classification
Gene	Exon	cDNA change	Protein change	Type of mutation	Mutation reported*	Proband†	Affected subjects	Onset (years)	No of exostoses	Vertebral location	Functional rating	Stature (centile)	Chondro sarcoma	Variable expressivity	Type	M/S
EXT1	Exon 1	42delG	G15	Frameshift	–	A1-1	2	8	6	+	Fair	3	–	+	III/IV S	
EXT1	Exon 1	248 insC	R83	Frameshift	1	A1-2	9	2	17	+	Fair	50	–	+	III S	
EXT1	Exon 1	C250T	Q84X	Nonsense	–	A1-3	5	1	40	+	Fair	3	–	+	II/III/IV S	
EXT1	Exon 1	352insC	V118	Frameshift	–	A1-4	6	2	10	–	Good	<3	–	+	IV S	
EXT1	Exon 1	458 delTC	L153	Frameshift	–	A1-5	4	3	12	–	Fair	3	+	+	IV S	
EXT1	Exon 1	460del2T	F154	Frameshift	–	A1-6	8	9	15	+	Fair	50	+	+	III S	
EXT1	Exon 1	477delTA	D160	Frameshift	–	A1-7	18	<1	>40	+	Fair	<3	+	+	II/III/IV S	
EXT1	Exon 1	515delA	H172	Frameshift	–	A1-8	6	1	20	–	Fair	25	–	+	I S	
EXT1	Exon 1	549delGT	S180	Frameshift	–	A-9	5	4	30	–	Fair	25	–	+	II S	
EXT1	Exon 1	679delC	R227	Frameshift	–	A1-10	9	2	20	–	Fair	25	–	+	I S	
EXT1	Exon 1	712delT	S238	Frameshift	–	A1-11	4	2	30	–	Fair	3	–	+	II/IV S	
EXT1	Exon 1	712delT	S238	Frameshift	–	A1-12	6	3	17	+	Fair	50	–	Anticip	III S	
EXT1	Exon 2	C1018T	R340C	Missense	7	A-13	7	5	17	+	Fair	75	–	+	III S	
EXT1	Exon 2	C1018T	R340C	Missense	+	A2-14	9	4	<10	–	Fair	50	–	–	M	
EXT1	Exon 2	C1018T	R340C	Missense	+	A2-15	2	5	10	+	Fair	50	+	+	III S	
EXT1	Exon 4	1370delT	T424	Frameshift	–	A4-16	5	3	20	–	Fair	75	–	+	I S	
EXT1	Intron 5	G1417A		Splice site	1	A5-17	7	1	12	+	Fair	75	+	–	IIIS	
EXT1	Exon 6	1431insT	S478	Frameshift	1	A6-18	7	1	12	+	Fair	50	–	+	III S	
EXT1	Exon 6	1469delT	L490	Frameshift	7	A6-19	3	1	22	–	Fair	50	–	Anticip	I S	
EXT1	Exon 6	1469 delT	L490	Frameshift	+	A6-20	2	6	10	–	Good	25	–	+	M	
EXT1	Exon 7	1568delT	L523	Frameshift	–	A7-21	12	2	15	–	Fair	25	–	+	I S	
EXT1	Exon 8	1642delA	S548	Frameshift	–	A8-22	2	<1	40	–	Fair	25	–	+	II S	
EXT1	Exon 9	G1745A	W582X	Nonsense	–	A9-23	13	<1	20	+	Fair	25	–	+	III S	
EXT1	Exon 9	G1744A	W582X	Nonsense	–	A9-24	12	<1	30	–	Fair	25	+	+	II S	
EXT1	Exon 9	1773delG	G591	Frameshift	–	A9-25	6	2	10	–	Fair	10	–	+	I S	
EXT1	Exon 9	C1776A	Y592X	Nonsense	–	A9-26	4	2	10	–	Fair	75	–	–	I S	
EXT1	Exon 9	1784delG/C	R595	Frameshift	–	A9-27	2	2	30	–	Fair	50	+	–	II S	
EXT1	NI					AX-28	9	6	30	–	Fair	25	–	+	II S	
EXT1	NI					AX-29	5	2	40	+	Fair	50	–	+	II/III S	
EXT2	Exon 2	C514T	Q172X	Nonsense	3	B2-30	5	3	10	–	Good	50	–	–	M	
EXT2	Exon 2	G537C	R180T	Missense	–	B2-31	5	8	10	–	Good	50	–	–	M	
EXT2	Exon 3	G580T	G193X	Nonsense	–	B3-32	10	10	30	+	Fair	75	–	Anticip	II/III S	
EXT2	Exon 3	624delC	D208	Frameshift	–	B3-33	2	2	20	–	Fair	50	–	Anticip	I S	
EXT2	Exon 4	G679A	D227N	Missense	2	B4-34	4	2	5	–	Good	50	–	–	M	
EXT2	Exon 5	C772T	Q258X	Nonsense	–	B5-35	6	?	?	?	?	?	?	?		
EXT2	Exon 7	1104insGA	E368	Frameshift	–	B7-36	6	5	9	–	Fair	75	–	–	M	
EXT2	Intron 7	1173+G	R360	Splice site	2	B7-37	6	1	12	–	Fair	50	–	–	I S	
EXT2	Exon 8	T1257A	Y419X	Nonsense	–	B8-38	5	<1	20	–	Fair	25	–	–	I S	
EXT3						C-39	8	4	8	–	Fair	50	–	+	M	
Un						D-40	2	1	10	–	Fair	10	–	–	I S	
Un						D-41	2	6	30	–	Fair	25	–	–	II S	
Un						D-42	10	?	?	?	?	?	?	?		

Un = unknown. NI = none identified.

*Mutations previously reported.²³†A = *EXT1* locus, B = *EXT2* locus, C = *EXT3* locus, D = unknown locus. The first number corresponds to exon and the second number refers to family.

affected members had a moderate phenotype. Group S could be subdivided into four distinct clinical subgroups (table 3). In type IS, the number of exostoses ranged from 10 to 25 with no vertebral location and a height above the 10th centile. Type IIS was associated with a larger number of exostoses (>25). Patients in the type IIIS group had vertebral exostoses and patients belonging to type IVS group had very short stature (equal to or below the 3rd centile).

Clinical data were available for 42 pedigrees and 110 questionnaires from affected subjects were collected (table 4). Among the 42 families, seven belonged to group M (type M). The 35 other families belonged to group S. Among them, 10 had the characteristics of type IS, five of type IIS, eight of type IIIS, two of type IVS, and six families could not be assigned a specific type, as the patients exhibited characteristics of several types. Interestingly, chondrosarcoma was observed in nine patients from seven families, which all belong to group S.

Intrafamilial variability was observed in most families, supporting the previously reported variable expressivity of HME.²⁸ In four families (A1-12, A6-19, B3-32, B3-33), clinical assessment of the affected subjects was in favour of anticipation. The disease showed a gradient of

severity ranging from a mild phenotype in the grandparents (one to two exostoses) to a severe form in the probands (17–30 exostoses).

GENOTYPE-PHENOTYPE CORRELATION IN HME PATIENTS

The relationship between genotypes and clinical features are shown in fig 1. Most of the families with a moderate phenotype (type M) were associated with *EXT2* mutations, while all type IIS, IIIS, or IVS families except one (B3-32) were associated with *EXT1* mutations (table 4). A statistical analysis of the data confirmed that type S (IS, IIS, IIIS, IVS) was significantly associated with *EXT1* mutations while type M was associated with *EXT2* mutations ($p=0.0129$, Fischer's exact test). Interestingly, the type IS phenotype was associated with either *EXT1* or *EXT2* mutations, without hotspots or a specific type of mutation in one or the other gene. No correlation was found between the type IIS or type IIIS phenotypes and location of the mutations in the *EXT1* gene. By contrast, mutations associated with type IVS (patients with very short stature) were consistently located in the first *EXT1* exon. It is worth noting that chondrosarcomas were restricted to patients carrying *EXT1* mutations.

The phenotypic variability of HME was noted in 2/3 of the familial forms caused by *EXT1* mutations. By contrast, members of families harbouring an *EXT2* mutation showed clinical homogeneity of the disease except for two families. The family linked to *EXT3* belonged to group M. Interestingly, incomplete penetrance was noted in that family, especially in females. This was confirmed by x ray analysis of all family members. No specific phenotype was observed in three families unlinked to the three *EXT* loci.

Discussion

In order to determine the respective contribution of the three *EXT* loci in HME, we investigated their distribution among 42 families. Thirty nine families (93%) proved to be linked to one of the three loci but three families showed no linkage to any of them. No linkage to one of the three *EXT-like* loci has been reported so far in HME and no *EXT-like* gene mutation has been identified.²⁷ All these observations support the existence of at least one additional (*EXT4*) locus in HME.

Mutation analysis showed that *EXT1* mutations were involved in 64% of HME families while *EXT2* mutations occurred in 21% of families. These findings differ from previous studies in white patients.²³ This may be because of a more efficient detection of mutations which allowed us to detect 95% of them. The failure to detect mutations in two *EXT1* linked families suggests that mutations might be located either within the promoter or elsewhere outside the coding region.

Mutations within the *EXT1* gene were found to be evenly distributed over the first nine exons, while the last exon, encoding part of the conserved carboxy-terminal domain, harboured significantly fewer mutations. Most *EXT2* mutations appeared to be novel and randomly distributed over the first eight exons. As observed in *EXT1*, no mutation was identified in the carboxy-terminal region of the protein. The *EXT* mutations reported here are expected to cause premature translation termination and loss of function as mutated gene products lack one of their catalytic domains putatively located at the C-terminal end of the protein.²¹

It is worth noting that most *EXT1* mutations reported here (19/27) were novel mutations, but the common *EXT1* mutations were also

detected. Hence, the 1 bp deletion (1469delT) previously detected in seven families²³ was also identified in two of our families. This deletion occurred at the end of a polycytosine tract, known to be a mutation hot spot. Similarly, the previously reported R340C mutation was found in three patients. This amino acid substitution is likely to alter proper functioning of the *EXT1* protein. Remarkably, the corresponding arginine residue was substituted for lysine in a Chinese hamster ovary (CHO) cell mutant lacking glycosyltransferase activity.²⁸ Furthermore, McCormick *et al*²¹ have shown that an *EXT1* construct containing the R340C mutation was enzymatically inactive in vitro.²¹

The genotype-phenotype analyses reported here suggested a strong correlation between *EXT1* mutations and the severity of HME phenotypes. Statistical analyses firmly established a significant correlation between *EXT1* mutations and the severe phenotypes (type S). Hence, large numbers of exostoses, short stature, and/or vertebral location were consistently associated with *EXT1* mutations. These particularly severe cases were not accounted for by a cluster of specific mutations. Moreover, malignant degeneration of exostoses into chondrosarcomas was also observed in families carrying *EXT1* mutations. In keeping with this, it has recently been shown that hereditary osteochondromas and secondary chondrosarcomas were associated with LOH at the *EXT1* locus.¹⁴ By contrast to a previous report,¹⁵ we did not observe loss of the second *EXT* allele in the somatic DNA of the benign osteochondromas that we have studied. According to the tumour suppressor model,²⁹ we speculate that single *EXT1* germline mutations would be the first hit causing multiple benign osteochondromas while inactivation of the second allele would trigger malignant transformation. Our preliminary results on somatic DNA from two secondary chondrosarcomas also indicate LOH at the *EXT1* locus (not shown).

Similarly, moderate phenotypes were frequently associated with *EXT2* mutations, while the group of severe type IS phenotype resulted from mutations in either gene. The intrafamilial clinical variability that we have observed remains unclear at present. One could speculate that mutations or polymorphisms in a modifier gene may modulate the disease phenotype.

Recent studies have shown that the *EXT1* and *EXT2* gene products exert both GlcA-T and GlcNAc-T activities in vitro¹⁹ and biochemical analyses have shown that the two *EXT* proteins can interact to form homo/hetero-oligomers in vivo.³⁰ Indeed, formation of a stable *EXT1-EXT2* complex in vitro is required to elicit maximal GlcA-T activity.²¹ Recent experiments indicate that *EXT1* and *EXT2* cannot substitute for each other in transfected cells. However, while *EXT1* alone exhibited significant Glc-T and GlcNAc-T activity in transfected cells, *EXT2* alone had no glycosyltransferase activity and was unable to compensate for the abrogation of GlcA-T activity caused by a R340C mutation in *EXT1*.

Figure 1 Genotype-phenotype correlation. Distribution of *EXT1* and *EXT2* mutations among moderate (M) and severe (IS, IIS, IIIS, IVS) types of HME (data from only one person (proband) in each family were used).

Whether a functional EXT1 protein could partially restore GlcA-T activity in EXT2 mutant cells is still unknown. Such difference in the glycosyltransferase activities of the two EXT enzymes could explain the lesser severity of HME phenotypes caused by *EXT2* mutations. Alternatively, *EXT2* may play a non-essential role in heparan sulphate synthesis, acting as a chaperone for EXT1.²⁸

In conclusion, our study provides what we believe to be the first evidence of genotype-phenotype correlation in HME, as *EXT1* mutations are more likely to trigger malignant degeneration of exostoses. It is hoped that this study will improve the management of patients.

Accession numbers and URLs for data in this article are as follows: GenBank, <http://www.ncbi.nlm.nih.gov/index.html> for *EXT1* genomic sequence (135994) and for *EXT2* genomic sequence (344921). Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/omim> for *EXT1* (MIM 133700), *EXT2* (MIM 133701), and *EXT3* (MIM 600209). We are deeply grateful to the HME patients and their families for participation in this study. We would like to thank the following physicians for collaboration: P Calvas, M P Cordier, V Cormier-Daire, M M Freund, D Gaillard, B Gilbert, A Joannard, D Lacombe, B Leheup, J F Mallet, P Maroteaux, A Nivelon, S Odent, M O Peter, N Philip, H Plauchu, G Plessis, J C Pouliquen, and D Rosenberg. The authors are grateful to P Margaritte-Jeannin for statistical analyses and W Van Hul for sending EXT primers. This work was supported by a grant from ARC (Association pour la Recherche sur le Cancer).

- Solomon L. Hereditary multiple exostosis. *J Bone Joint Surg Br* 1963;45:292-304.
- Schmale GA, Conrad EU, Raskind WH. The natural history of hereditary multiple exostoses. *J Bone Joint Surg Am* 1994;76:986-92.
- Wicklund LC, Pauli RM, Johnston D, Hecht JT. Natural history study of hereditary multiple exostoses. *Am J Med Genet* 1995;55:43-6.
- Legeai Mallet L, Munnich A, Maroteaux P, Le Merrer M. Incomplete penetrance and expressivity skewing in hereditary multiple exostoses. *Clin Genet* 1997;52:12-16.
- Cook A, Raskind W, Blanton SH, Pa RM, Gregg RG, Francomano CA, Puffenberger E, Conrad EJ, Schmale G, Schellenberg G, Wijsman E, Hecht JT, Wells D, Wagner MJ. Genetic heterogeneity in families with hereditary multiple exostoses. *Am J Hum Genet* 1993;53:71-9.
- Wu YQ, Heutink P, De Vries BBA, Sandkuijl LA, Van Den Ouweland AMW, Niermeijer MF, Galijard, Reyniers E, Willems PJ, Halley DJJ. Assignment of a second locus for multiple exostoses to the pericentromeric region of chromosome 11. *Hum Mol Genet* 1994;3:167-71.
- Le Merrer M, Legeai-Mallet L, Jeannin P, Horsthemke B, Schinzel A, Plauchu H, Toutain A, Achard F, Munnich A, Maroteaux P. A gene for hereditary multiple exostoses maps to chromosome 19 p. *Hum Mol Genet* 1994;5:717-22.
- Legeai-Mallet L, Margaritte-Jeannin P, Lemdani M, Le Merrer M, Plauchu H, Maroteaux P, Munnich A, Clerget-Darpoux F. An extension of the admixture test for the study of genetic heterogeneity in hereditary multiple exostoses. *Hum Genet* 1997;99:298-302.
- Wise CA, Clines GA, Massa H, Trask BJ, Lovett M. Identification and localization of the gene for EXT1, a third member of the multiple exostoses gene family. *Genome Res* 1997;7:10-16.
- Wuyts W, Van Hul W, Hendrickx J, Speleman F, Wauters J, De Boule K, Van Roy N, Van Agtmael T, Bossuyts P, Willems PJ. Identification and characterization of a novel member of the EXT gene family, EXT2. *Eur J Hum Genet* 1997;5:382-9.
- Van Hul W, Wuyts W, Hendrickx J, Speleman F, Wauters J, De Boule K, Van Roy N, Bossuyts P, Willems PJ. Identification of the third EXT-like gene (EXT3) belonging to the EXT gene family. *Genomics* 1998;47:230-7.
- Hecht JT, Hogue D, Strong LC, Hansen MF, Blanton SH, Wagner M. Hereditary multiple exostosis and chondrosarcoma: linkage to chromosome 11 and loss of heterozygosity for EXT-linked markers on chromosomes 11 and 8. *Am J Hum Genet* 1995;56:1125-31.
- Hecht JT, Hogue D, Wang Y, Blanton SH, Wagner M, Strong LC, Raskind W, Hansen MF, Wells D. Hereditary multiple exostoses (EXT): mutational studies of familial EXT1 cases and EXT-associated malignancies. *Am J Hum Genet* 1997;60:80-6.
- Raskind WH, Conrad EU, Chansky H, Matsushita M. Loss of heterozygosity in chondrosarcomas for markers linked to hereditary multiple exostoses. *Am J Hum Genet* 1995;56:1132-9.
- Bovée J, Cleton-Jansen A, Wuyts W, Caethoven G, Taminiau A. EXT mutation analysis and loss of heterozygosity in sporadic and hereditary osteochondrosarcomas and secondary chondrosarcomas. *Am J Hum Genet* 1999;65:689-98.
- Ahn I, Ludecke HJ, Lindow S, Horton WA, Lee B, Wagner MJ, Horsthemke B, Wells DE. Cloning of the putative tumour suppressor gene for hereditary multiple exostoses (EXT1). *Nat Genet* 1995;11:137-43.
- Stickens D, Clines G, Burbee D, Ramos P, Thomas S, Hogue D, Hecht JT, Lovett M, Evans GA. The EXT2 multiple exostoses gene defines a family of putative tumor suppressor genes. *Nat Genet* 1996;14:25-32.
- Wuyts W, Van Hul W, Wauters J, Nemtsova M, Reyniers E, Van Hul E, De Boule C, De Vries B, Hendrickx J, Herrygers I, Bossuyt P, Bailemans W, Fransen E, Vits L, Coucke P, Nowak NJ, Shows T, Mallet L, Van Der Ouweland AMW, McGaughan J, Halley DJJ, Willems PJ. Positional cloning of a gene involved in hereditary multiple exostoses. *Hum Mol Genet* 1996;10:1547-57.
- Lind T, Tufaro F, McCormick C, Lindahl U, Lidholt K. The putative tumor suppressors EXT1 and EXT2 are glycosyltransferases required for the biosynthesis of heparan sulfate. *J Biol Chem* 1998;273:26265-8.
- McCormick C, Leduc Y, Martindale D, Mattison K, Esford LE, Dyer AP, Tufaro F. The putative tumor suppressor EXT1 alters the expression of cell-surface heparan sulfate. *Nat Genet* 1998;19:158-61.
- McCormick C, Duncan G, Goutsos KT, Tufaro F. The putative tumor suppressors EXT1 and EXT2 form a stable complex that accumulates in the Golgi apparatus and catalyzes the synthesis of heparan sulfate. *Proc Natl Acad Sci USA* 2000;97:668-73.
- Philippe C, Porter PE, Emerton ME, Wells DE, Simpson A, Monaco AP. Mutation screening of the EXT1 and EXT2 genes in patients with hereditary multiple exostoses. *Am J Hum Genet* 1997;61:520-8.
- Wuyts W, Van Hul W. Molecular basis of multiple exostoses: mutations in the EXT1 and EXT2 genes. *Hum Mutat* 2000;15:220-7.
- Carroll K, Yandow SM, Ward K, Carey JC. Clinical correlation to genetic variations of hereditary multiple exostosis. *J Pediatr Orthop* 1999;19:785-91.
- Enneking WE. Modification of the system for functional evaluation in the surgical management of musculoskeletal tumors. In: Enneking WF, ed. *Limb salvage in musculoskeletal oncology*. Bristol-Myers orthopedic symposium. New York: Churchill Livingstone, 1987:626-39.
- Wuyts W, Van Hul W, De Boule K, Hendrickx J, Bakker E, Vanhoenacker F, Mollica F, Ludecke HJ, Sayli BS, Pazzaglia UE, Mortier G, Hamel B, Conrad EU, Matsushita M, Raskind WH, Willems PJ. Mutations in the EXT1 and the EXT2 genes in hereditary multiple exostoses. *Am J Hum Genet* 1998;62:346-54.
- Xu L, Xia J, Jiang H, Zhou J, Li H, Wang D, Pan Q, Long Z, Fan C, Deng HX. Mutation analysis of hereditary multiple exostoses in the Chinese. *Hum Genet* 1999;105:45-50.
- Wei G, Bai X, Gabb M, Bame KJ, Koshy TI, Spear PG, Esko JD. Location of the glucuronyltransferase domain in the heparan sulfate copolymerase EXT1 by analysis of Chinese hamster ovary cell mutants. *J Biol Chem* 2000;275:27733-40.
- Knudson AG. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci USA* 1971;68:820-3.
- Kobayashi S, Morimoto K, Shimizu T, Takahashi M, Kurosawa H, Shirasawa T. Association of EXT1 and EXT2, hereditary multiple exostose gene products, in Golgi apparatus. *Biochem Biophys Res Commun* 2000;268:860-7.