

# EXT-MUTATION ANALYSIS IN SPORADIC AND HEREDITARY OSTEOCHONDROMA, SECONDARY CHONDROSARCOMA TISSUES AND PERIPHERAL Lymphocytes

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## Introduction

Osteochondroma is the most common benign bone tumor which occurs as sporadic (solitary, SO) or, in the hereditary multiple exostoses (HME), as multiple lesions (MO). Both conditions have been associated with genetic alterations in either EXT1 (8q24.1) or EXT2 (11p12-11) gene. Malignant transformation of osteochondroma in peripheral secondary chondrosarcoma (CS) occurs in 1-5% of the patients. Aim of this study is to analyse the mutational status of EXT1/EXT2 genes related to the development of osteochondroma and the sarcomatous transformation of an osteochondroma into a chondrosarcoma.

## Samples analysed

- 17 osteochondromas (5 SO and 12 MO)
- 7 peripheral CS (3 derived from SO and 4 from MO).

Excluding SO tissues, in the rest of the cases also the DNA extracted from the peripheral lymphocytes was analysed.

## Methods

- Extraction of DNA from fresh frozen tissues and from peripheral lymphocytes
- Analysis of the exons and splice-site junctions of EXT1/EXT2 genes with DHPLC-based mutation screening and subsequent direct sequencing of the samples with abnormal elution profile.

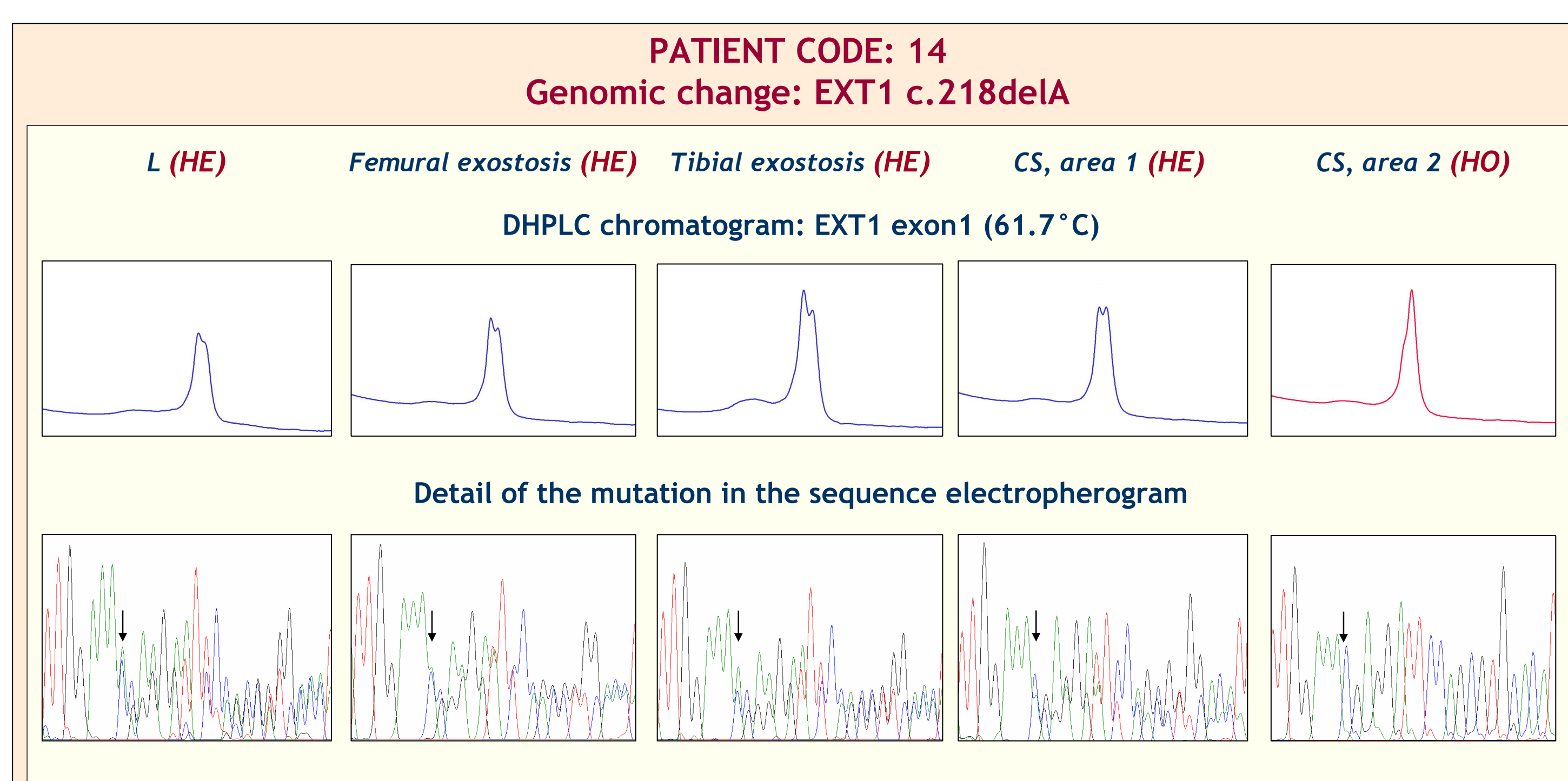
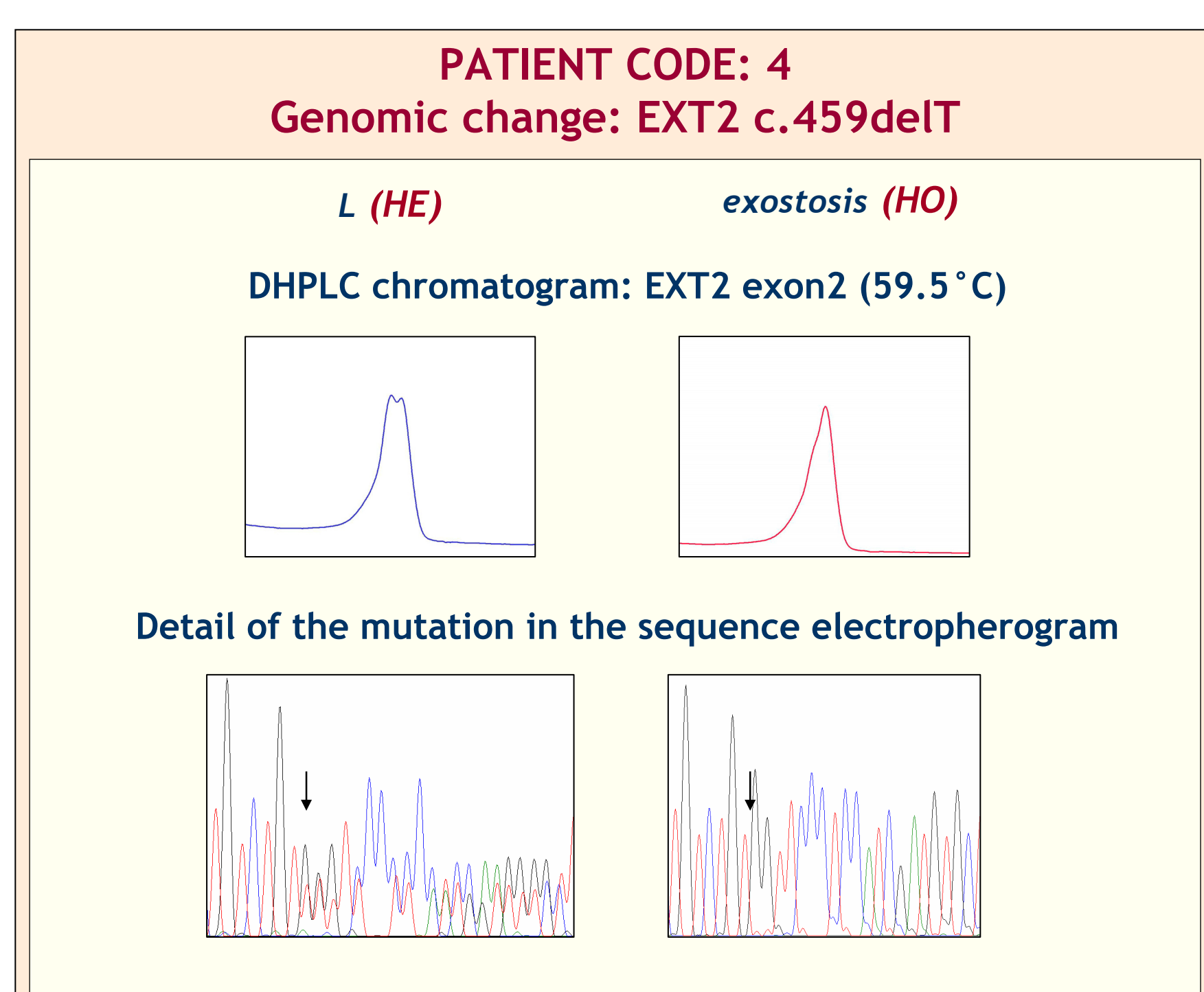
**Abbreviations used in the table:** L, peripheral lymphocytes; SO, solitary osteochondroma; MO, multiple osteochondroma; CS, chondrosarcoma; HE, heterozygosity; HO, homozygosity.

## Results

### Mutations in EXT1/EXT2 genes identified in the tissues analysed

PATIENT CODE	GENOMIC CHANGE	GENE (Exon/intron)	TYPE OF MUTATION	TISSUES ANALYSED	STATUS
1	c.(1285-2)_1286del AGAT	EXT2 (Exon 5)	Frameshift	SO	HE
2	c.659G>A	EXT2 (Exon 2)	Nonsense	L / MO	HE
3	c. 599G>A	EXT1 (Exon 1)	Nonsense	L / MO	HE
4	c.459delT	EXT2 (Exon 2)	Frameshift	L	HE
				MO	HO
5	c.1469delT	EXT1 (Exon 6)	Frameshift	L / MO	HE
6	c.1468_1469insC	EXT1 (Exon 6)	Frameshift	L / MO	HE
7	G151T	EXT2 (Exon 2)	Nonsense	L / MO	HE
8	c.(1633-2)A>G	EXT1 (Intron 7)	Splice	L / MO	HE
9	c.1720G>T	EXT1 (Exon 8)	Nonsense	L / MO	HE
10	c.1720G>T	EXT1 (Exon 8)	Nonsense	L / MO	HE
11	c.772C>T	EXT2 (Exon 5)	Nonsense	L / CS from MO	HE
				CS recurrence	HO
12	c.2038G>T	EXT1 (Exon 10)	Nonsense	L / MO / CS from MO	HE
13	c.124delA	EXT2 (Exon 2)	Frameshift	L	HE
				CS from MO	HE / HO
14	c.218delA	EXT1 (Exon 1)	Frameshift	L / MO	HE
				CS from MO	HE / HO

### Details of two frameshift mutations detected in the tissues of two different patients both in homo- and heterozygosity status



A part from one negative case, in all the tissues of MO and CS from MO screened we confirmed the presence of the same mutation found in the related peripheral lymphocytes. In one case of MO tissue (patient code 4), the heterozygous mutation detected in the lymphocytes was found in homozygosity status. In two cases of CS derived from MO (patient code 13 and 14) the heterozygous mutation detected in the lymphocytes was found both in HO and in HE in different areas of the same tumoral resection. In one case of CS derived from MO (patient code 11) the heterozygous mutation detected in the lymphocytes and in the primary tumor was found in HO in the recurrence of the CS. Excluding one case of SO (patient code 1), no mutation in EXT1 / EXT2 was found in tissues of SO and CS derived from SO.

## Conclusions

Results of this pilot study suggest that, in the tissues of affected patients, a progressive variation of EXT1/EXT2 genes from wild-type genetic status could be involved in the development of osteochondroma and in the sarcomatous transformation of an osteochondroma into a chondrosarcoma. In two samples histological features of peripheral secondary CS correlate with the presence of EXT genes mutations at the HO level; nevertheless, in both samples, the HE status was also detected in different areas from the same resection specimens. This result suggest a progression of malignancy in certain tumoral areas. In one MO tissue sample HO status for an EXT2 mutation was also detected.

Taken together, these results suggest that, at least at a genetic level, the boundary line between osteochondroma and peripheral secondary CS is not so well defined. It is also possible that other mechanisms, different from the "two-hit mutational model" (where a germline mutation, coupled with a somatic mutation, results in the loss of EXT1/EXT2 function and subsequent tumor formation), could be involved in the development of peripheral CS.