Letter to the Editor

Re. Review Article entitled 'The neoplastic pathogenesis of solitary and multiple osteochondromas'

In a recent Review Article, Porter and Simpson summarize the many theories proposed over the years to explain the pathogenesis of osteochondroma and speculate about the underlying genetic changes [1]. Based on progress that we have recently made in understanding the genetics of the neoplastic pathogenesis of osteochondroma, and the multistep model towards secondary chondrosarcoma, we would like to supplement their article with some very recent data.

Based on the available literature, the authors correctly conclude that a neoplastic pathogenesis for osteochondroma should be suspected. In our opinion, the most important support for a clonal origin of osteochondroma lies in the clonal karyotypic abnormalities described [2,3]. In support of this, we have recently obtained data demonstrating loss of heterozygosity (LOH) in the cartilaginous cap of 6 of 14 osteochondromas and DNA aneuploidy in 4 of 10 osteochondromas [4], strongly pointing towards a clonal, and thus neoplastic, origin for the cartilaginous tissue of osteochondroma. Although we understand the temptation of Porter and Simpson to speculate that the osteal part of osteochondroma may be considered reactive tumour stroma, there are no data from molecular or immunohistochemical studies available from the literature to support this view. We feel that further molecular genetic studies are required to confirm their statement.

To date, it is unclear whether complete inactivation of an *EXT* gene (according to the Knudson tumour suppressor model) is required for osteochondroma development, or whether a single *EXT* germline mutation acts in a dominant negative way, resulting in multiple benign osteochondromas. In the latter, inactivation of the remaining allele would be a prerequisite for malignant transformation. The authors propose five speculative and, to us, unconvincing arguments for a 'dominant negative' effect of the *EXT* tumour suppressor genes in hereditary multiple exostoses. We describe two patients with multiple osteochondromas demonstrating a germline *EXT1* mutation [4]. This is combined with loss of the remaining wild-type allele in three osteochondromas, indicating that, in cartilaginous cells of the growth plate, inactivation of both copies of the *EXT1* gene is required for osteochondroma formation in hereditary cases [4]. This was also suggested by others [5] and excludes a dominant negative effect of the *EXT1* gene in hereditary osteochondroma.

Their comparison of the 'stepwise' process of oncogenesis for the osteochondroma–chondrosarcoma sequence to the colorectal adenoma–carcinoma sequence (Table II [1]) is interesting, but requires further comment. We do not believe that it is justified to subdivide osteochondroma into pre-neoplastic and neoplastic categories, since there is now sufficient evidence that at least the cartilaginous cap of osteochondroma is a true neoplasm. They correctly speculate that both copies of an *EXT* gene are inactivated, which indicates neoplastic growth. Furthermore, there is no indication that additional genetic alterations in osteochondroma occur, since both cytogenetic studies [2,3] and our own study [4] show alterations mainly restricted to the *EXT* loci, especially *EXT1*.

We agree with the authors that in sporadic osteochondroma, somatic inactivation of both copies of an *EXT* gene could be expected. So far, only one somatic mutation in the *EXT1* gene has been described in a sporadic chondrosarcoma [6]. We did not find any somatic *EXT1* cDNA alterations in eight sporadic osteochondromas and 14 sporadic chondrosarcomas. Future studies on a larger panel of tumours should reveal whether the situation is similar to the *BRCA1* gene, for which only germline mutations in hereditary breast cancer are described [7].

The authors' suggestion that progression to a low-grade chondrosarcoma is accompanied by chromosome 3q and 10q deletion may be an oversimplification. We compared LOH patterns of peripheral chondrosarcomas secondary to osteochondromas with those of primary central chondrosarcomas [8,9]. Nineteen of 20 peripheral chondrosarcomas showed LOH at all loci tested (*EXT* genes, *EXT*-like genes, and at 9p21, 13q14, 17p13, and chromosome 10), while only 3 of 12 central chondrosarcomas exhibited LOH, restricted to 9p21, 10, 13q14, and 17p13. DNA flow cytometry demonstrated a wide variation in the ploidy status in peripheral chondrosarcomas (DNA indices 0.56–2.01), whereas central chondrosarcomas were predominantly peridiploid. Remarkably, near-haploidy was found in peripheral chondrosarcomas, which could explain some of the high LOH percentages. Also, polyploidization of a near-haploid clone had occurred in two high-grade peripheral chondrosarcomas. In all studies reported in the literature so far, no separation has been made between central and peripheral chondrosarcoma; LOH data presented in this way should therefore be interpreted with caution [8,9].

In conclusion, it seems to be more appropriate to propose a genetic progression model for peripheral cartilaginous tumourigenesis based on recently available data. First, inactivation of both copies of the *EXT1* gene in cartilaginous cells of the growth plate is required for osteochondroma formation, as demon-

strated by loss of the remaining wild-type allele in hereditary osteochondromas [4]. Whether complete inactivation occurs in sporadic cases remains to be investigated. One or more additional genetic alterations may then be required for a peripheral chondrosarcoma to arise within its benign precursor. The process of malignant transformation is genetically represented by chromosomal instability with severe aneuploidy and a high LOH incidence in peripheral chondrosarcoma [8,9].

Finally, in their discussion of the physiological function of the *EXT* gene family, the authors omit the important finding that an *EXT1* homologue in Drosophila (*tout-velu*) was demonstrated to be required for diffusion of the morphogen Hedgehog [10]. Remarkably, in humans, Indian Hedgehog is normally expressed in the growth zone and pre-hypertrophic chondrocytes of cartilage [11]. Furthermore, not only the *EXT1* but also the *EXT2* gene products were shown to be glycosyltransferases required for the biosynthesis of heparan sulphate [12,13]. The role of the resulting altered heparan sulphate expression on the cell surface and the abnormal diffusion of Hedgehog within chondrocytes of the growth plate caused by *EXT* mutations will be an interesting field of study in the future, further revealing the pathogenesis of osteochondroma.

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Authors' reply

We are grateful for the comments on our review from Drs Bovée and Hogendoorn.

Our suggestion that the 'osteal' part of the osteochondroma may be reactive or supportive, rather than truly neoplastic, reflects the surgical fact that ablation of the cartilage cap alone will ablate further growth of the osteochondroma.

We agree that it is unclear whether complete inactivation of the *EXT* gene is required for osteochondroma development. Despite this, our review emphasizes that no research to date indicates any features of *EXT* activity other than those which suggest a recessive tumour suppressor gene function. Unfortunately, due to an oversight in manuscript preparation for which we apologize, we on one occasion describe the function of the *EXT* gene as 'dominant negative'. The five features which we attribute to *EXT* behaviour do, of course, suppose an entirely 'recessive' and not 'dominant negative' action of the gene.

Our review deals at length with the problems of defining the cartilage cap of an osteochondroma as a neoplasm on the traditional model. We justify our classification of the childhood osteochondroma, which ossifies at the end of skeletal growth, as 'pre-neoplastic' to overcome these traditional objections. We believe that this is entirely consistent with the molecular data.

We agree that non-*EXT* gene changes have not been identified in osteochondromas in any significant number. Major genetic abnormalities would not be expected to occur in simple 'pre-neoplastic' childhood osteochondromas. We suggest that reactivated, adult-type osteochondromas, which are much less common, would be the best candidates to investigate the incidence of non-*EXT* genetic changes in accordance with a step-wise model of osteochondroma–chondrosarcoma carcinogenesis.

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