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Investigations in generalized osteoarthritis. Part 2: Special histological features in generalized osteoarthritis (histological investigations in Heberden's nodes using a histological score)[1]

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Summary

Objective: In accordance with the literature, our previous epidemiological, clinical and genetical investigations have confirmed a correlation between generalized osteoarthritis (GOA) and Heberden's nodes. Heberden's nodes can be considered as genetic markers for the existence of a generalized osteoarthritic predisposition. The present study's concern was to establish whether there are special histological features in this disease.

Methods: Layered sections of 218 distal finger joints from 56 deceased persons were investigated using a histological–histochemical score modified by Mankin.

Results: In Heberden's nodes, we found all the typical degradative sequences of the osteoarthritic process but also some specific modifications. The osteoarthritis (OA) starts with a subchondral ossification and manifests a reactive tidemark flaking. At this time, the surface of the cartilage is not yet destroyed. Later on, there is progression of general degradation. Significant differentiation from the control group is possible using a histological score.

Conclusions: In patients with Heberden's nodes, the OA starts with the subchondral ossification. Heberden's nodes are the specific manifestation of GOA in the distal finger joints. Further studies are therefore required to assess whether the same pathogenetic mechanism can be seen in OA of the large joints in GOA.

Key words: Heberden's nodes, Heberden's arthritis, Generalized osteoarthritis GOA, Tidemark, Tidemark flaking, Calcified cartilage.

Introduction

Generalized osteoarthritis (GOA) is a term that was already introduced by Kellgren and Moore, Lawrence and Peyron to define a discrete entity[1–4]. Nevertheless, for reasons that are contradictory and unclear the definition of the clinical picture is not yet clear in the literature so that GOA is not often diagnosed in daily clinical routine. A few authors have investigated only the smaller hand and foot joints, whereas others investigated hip and knee joints and also the spine. If and in which combination the single joints are affected is therefore described differently in the literature[5–13]. Moreover, "erosive osteoarthritis" must be distinguished[14].

It is also interesting that numerous authors mention a connection between Heberden's nodes and the GOA[15–17]. Loughlin *et al.* define GOA on the basis of the incidence of Heberden's nodes before the age of 46 and the involvement of at least three more joint groups[18]. On the one hand, osteophytes are mentioned as Heberden's nodes in the literature. On the other hand, "hyaluronic acid" cysts are mentioned which may be observed in young adults[19–22]. In our investigation, we considered only patients with manifest osteophytes.

Contradictory statements regarding the epidemiology and clinical features of GOA in the literature were checked with our own epidemiological investigation in different geriatric centers on 1997 pensioners from an urban and a rural population and in a clinical study on 106 patients with Heberden's arthritis and 109 control persons[23,24]. In both studies, we found a clear indication for predisposition to GOA with polyarthritis of the fingers. For numerous functional and radiological parameters in the hip, knee, shoulder and finger joints and in the cervical, thoracic and lumbar spine, the signs of degradation were greater in patients with Heberden's arthritis than in the equivalent control groups. Depending on the population, the maximum incidence was nearly 30%. The generalized character was shown especially by the fact that different finger joint levels were affected (Heberden's arthritis, Bouchard's arthritis, and rhizarthritis).

Different surveys suggest but do not yet prove that a mutation in a gene of the cartilaginous matrix (COL 2 A1) mainly entails the manifestation of Heberden's nodes or GOA[25–29]. On the other hand, no correlation was found between Heberden's nodes or GOA and the three cartilaginous matrix genes investigated[18]. Other authors reported a higher level of various metalloproteinases in GOA than

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in knee osteoarthritis (OA) and concluded that they may be an indicator for whole-joint degeneration[30].

Before the current investigation, a genetic predisposition was postulated for Heberden's nodes on the basis of an autosomal-monogenic inheritance. Accordingly, the illness already becomes manifest in the heterozygous condition in women owing to a dominant inheritance and only in the homozygous condition in men on the basis of a recessive inheritance. All references in the literature are based on the work of Stecher, who investigated 74 families from 1940 to 1950[31–35]. These statements are cited right into the 1970s and 1980s. In the current literature, we found only references to secondary papers. In our own genealogic examination of 88 families with 931 family members, these statements could be confirmed[36]. In another investigation, they found a significant genetic contribution with evidence for a major recessive gene and a multifactorial component, representing either polygenic or environmental factors[37].

Accordingly, Heberden's nodes can be considered as a genetic marker for a predisposition to general arthritis. We therefore conducted a histological investigation of the finger joints of patients with Heberden's arthritis; first to reexamine the well-known sequences of the osteoarthritic process (especially the early stages) and secondly in order to reveal specific features within the framework of GOA[38].

Materials and methods

The consecutive autopsy material available from the Institute of Pathological Anatomy at the University of Leipzig comprised 224 distal joints of the second to the fifth finger taken from 56 deceased persons (30 men and 26 women). Of these distal joints, 218 could be processed for the histological investigations.

From all joints, the heads of the middle phalange were worked up with layered sections (30 cuts at intervals of 300 μm). The specimens were stained with hematoxylin-eosin, Crossmon, Safranin-O, Ritter–Oleson, Alcian with pH 4.1 and 2.63 and silver impregnation according to Gomori[39,40]. Four differently defined areas of each single joint (Fig. 1) were investigated in order to obtain a comprehensive assessment.

The samples were histomorphologically graded using the Mankin score (Table I) modified in the light of our special questions[41,42]. So we performed out a grading of the roentgenologic and macroscopic visible degradation as well as a staging of the osteophyte addition. The most serious characteristic feature was always evaluated. This study was done as a blinded test. The investigator had access only to a registration number of the preparations but not to the diagnosis or the name of the deceased. Apart from age-dependent changes and other questions under investigations, we were especially interested in comparing the people with manifest arthritis of the finger joints (Heberden's, Bouchard's or rhizarthritis in different combinations, whereas Heberden's arthritis was always present = polyarthritis group) with a control group. The categorization in the polyarthritis group was on the basis of the clinical criteria "Heberden's nodes" if only one or more joints showed clinically manifest Heberden's nodes.

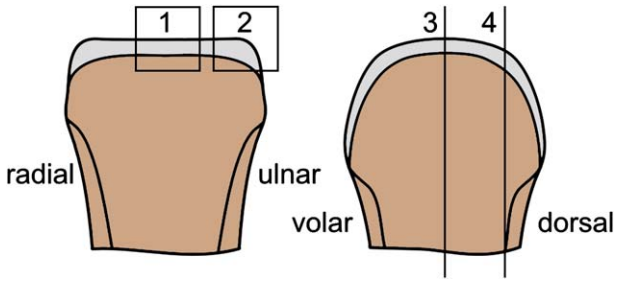


Fig. 1. Presentation of the four joint sections assessed (1 = middle area, 2 = marginal area, 3 = median area, and 4 = dorsal area).

Table I
Original Mankin score[41]: histological–histochemical grading system for osteoarthritic articular cartilage (we used a modified score, enlarged of many parameters)

	Grade
I. Structure	
A. Normal	0
B. Surface irregularities	1
C. Pannus and surface irregularities	2
D. Clefts to transitional zone	3
E. Clefts to radial zone	4
F. Clefts to calcified zone	5
G. Complete disorganization	6
II. Cells	
A. Normal	0
B. Diffuse hypercellularity	1
C. Cloning	2
D. Hypocellularity	3
III. Safranin-O staining	
A. Normal	0
B. Slight reduction	1
C. Moderate reduction	2
D. Severe reduction	3
E. No dye noted	4
IV. "Tidemark" integrity	
A. Intact	0
B. Crossed by blood vessels	1

Results

The osteoarthritic process reveals all the sequences of the osteoarthritic process within the framework of the generally known formal pathogenesis. A few characteristic pathological-anatomic features will also be considered below.

The OA starts with a subchondral ossification. In early adulthood, processes of remodeling of the osteochondral transition interface in the area of the cartilage–capsule–periosteal junction are already visible. The subchondral bone is rebuilt and reduced to a thin lateral compacta which is coated with fibrocartilage in the margin area (Fig. 3). Large bone lacunae can already appear at this stage which are especially distinctive changes compared to the normal histology (Fig. 2).

The changes extend to the tidemark, the borderline between calcified and noncalcified cartilage, through which the tidemark advances in the direction of the joint surface (Fig. 4). It becomes thicker and the number of lines increases (we saw a maximum of 13 lines). This reaction is locally restricted and it is associated with an expansion of the basal calcified layer. At this time, the surface of the cartilage is not destroyed. There is a general loss of proteoglycans over the advanced tidemark.

Interruptions of continuity which start at the top of the tidemark curvature are then manifested (tidemark flaking,

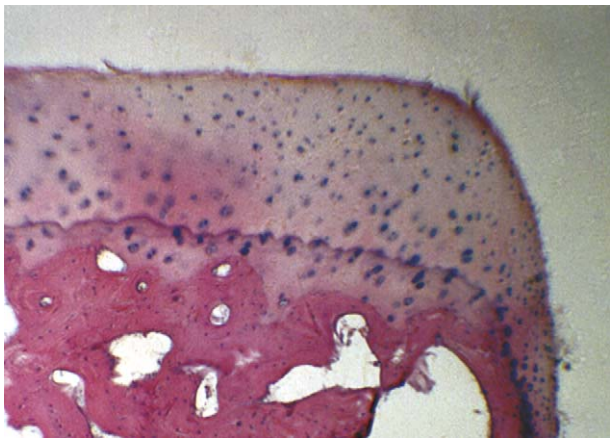


Fig. 2. Normal cartilage within the margin area. Intact cartilage surface, normal configuration of the tidemark and the bone lamella. HE, 16 times.

Fig. 4). These defects can become connected to surface lacerations (Fig. 5). In this case, necrotic material can be seen in the gaps. These defects can cause the detachment of entire cartilage regions. The tidemark then lies free on the surface (Fig. 6).

This arthritic process initiated in this way initially shows a predominance of repair mechanisms. From the area of the osteocartilaginous junction and the insertion point of the synovialis, fibrocartilage grows over the defect. Partly vascularized granulation tissue grows in from subchondral defects situated more medially and can connect to the laterally infiltrating fibrocartilage (Fig. 7). Via a chondral metaplasia, the whole margin area can ultimately be covered with fibrocartilage. The tissues mentioned close the defect. However, at the same time they are located under an endochondral ossification which leads to an enlargement of the marginal osteophytes. Under special conditions, the repair mechanisms described are not sufficient. The progressing osteoarthritic process then leads to a complete cartilage abrasion via the uncovered tidemark – eburnation.

In the middle part of the joints, all the degenerative processes occur more rarely and not as strongly

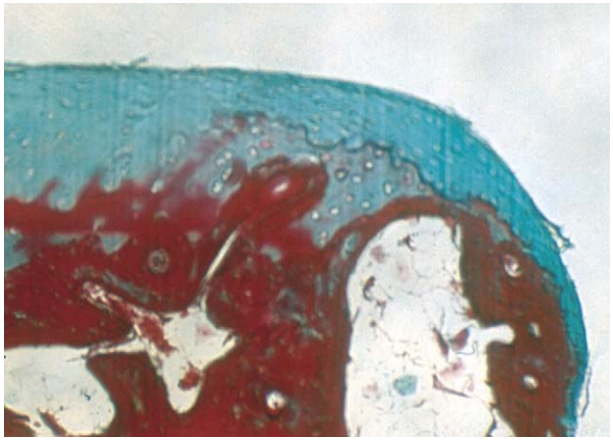
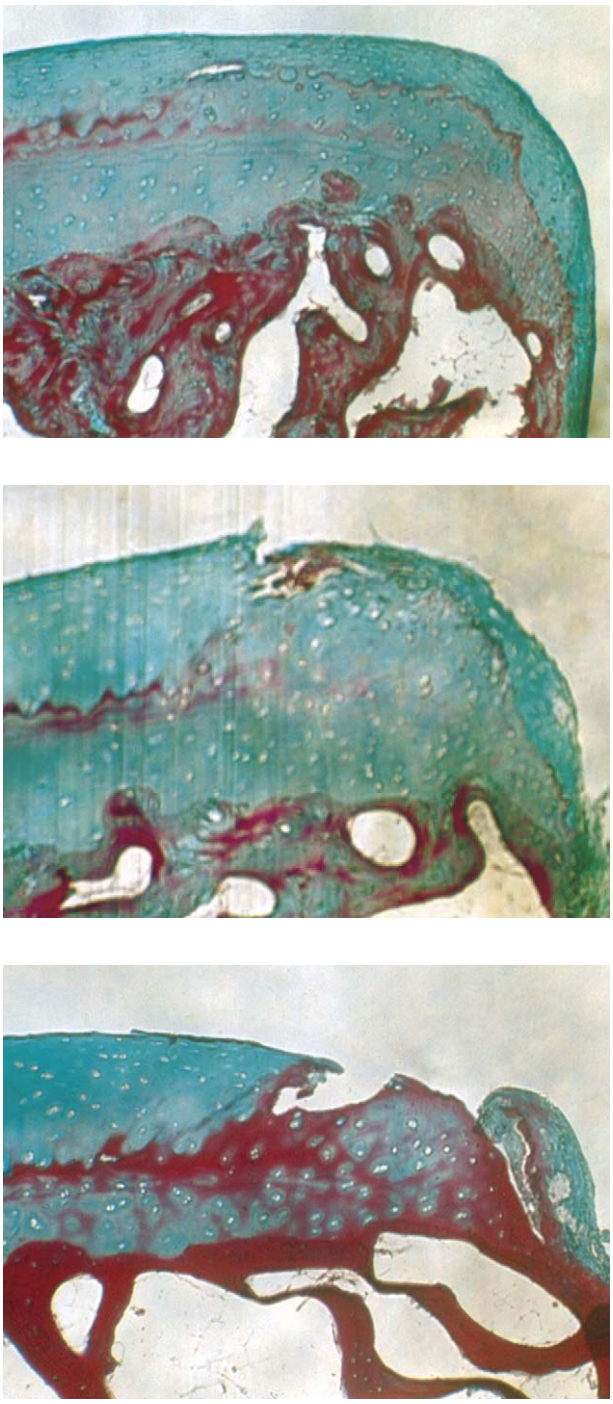


Fig. 3. The rebuilding process in the area of the osteocartilaginar interface leads to a huge lacuna, thinning of the bone lamella, vessel penetration in the calcified cartilage and to a displaced tidemark at a nearly intact surface of the cartilage. Crossmon, 16 times.



Figs. 4–6. From Figs. 4 to 6 processing tidemark flaking, first beginning over the top of the curvature in intact surface, later with connection to the surface with embedded necrotic material, and finally with a free lying tidemark on the surface. Crossmon, 16 times.

developed as in the margin area. In principle, the same changes can be seen starting from the vessel penetration (Fig. 8) over the endochondral ossification and the tidemark flaking up to the ingrowth of the subchondral granulation tissue.

For the comparison between the polyarthritis group (Heberden's nodes) and the control group, only specimens

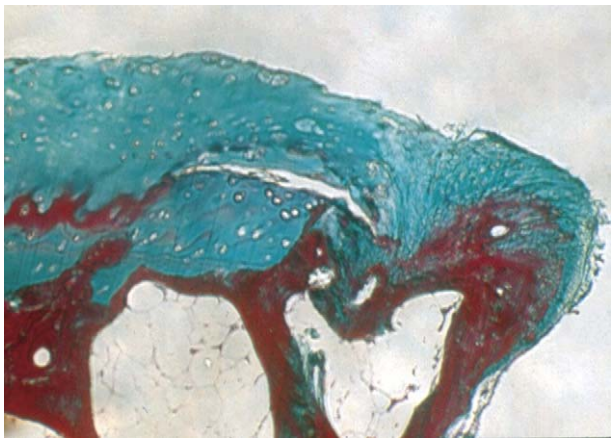


Fig. 7. Large osteophyte covered with fibrocartilage. It comes from both the lateral area of the osteocartilaginous junction and also from a medial defect of the subchondral bone lamella. Crossmon, 16 times.

from patients who were older than 56 years are used to establish the score (Tables II and III, "HEB 3"). An examination of patients of nearly the same average age was therefore possible. Altogether, there were 54 parameters used which were separately assessed from every single part of the four joint sections (Fig. 1). Additionally, we implemented an index of "total joint" values, which comprised the sum of the histological–histochemical values of the four sections mentioned. Table IV presents the average values of the parameters which differ significantly and also a few more interesting values. It is noteworthy that the differences are nearly all confined to the "median cut – margin area", whereas in the other areas significant differences occur only sporadically. The findings described are confirmed in morphometric examinations not cited in the present paper.

The corresponding changes with age were investigated using the parameter "total joint". The rank correlation after Kendall reveals a strict dependency with one correlation coefficient $r = 0.5618$ with a statistical significance of $P = 0.001$ ($n = 214$).

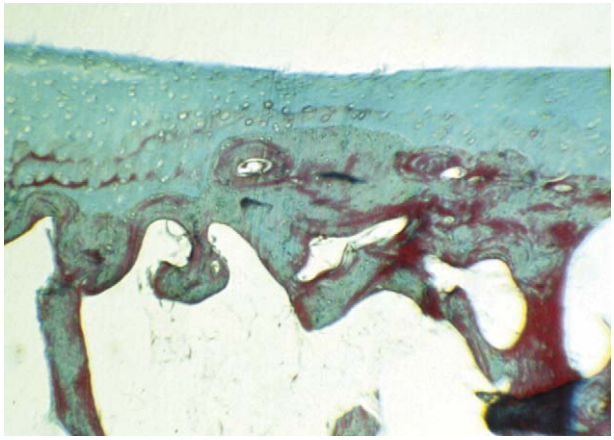


Fig. 8. Median section of the middle area. Above the vessel penetration, an advanced tidemark is to be seen. Crossmon, 12 times.

Table II
Age distribution of the patients, n = 56

	Age distribution	Standard deviation/s	Minimum	Maximum	*n*
1. Total	59.9	18.2	22	87	56
2. Heberden's nodes, total (HEB 1)	68.7	12.4	43	85	23
3. No Heberden's nodes, total (HEB 2)	53.0	19.1	22	87	33
4. No Heberden's nodes, from 56 years of age (HEB 3)	69.4	10.1	56	87	15

Discussion

Heberden's arthritis (Heberden's nodes) contrasts with the generally known sequences of the osteoarthritic process. They do not reveal superficial fibrillation or irregularities of the coloring of the ground substance, but an increased ossification within the margin area of the subchondral bone. These changes cause an advancement and also an increase in the thickness of the tidemark. Lastly, this leads to irregularities and horizontal ruptures at the interface between the calcified and noncalcified layer although the cartilage surface is completely intact. These ruptures start to increase and they lead to a disruption of the hyaline cartilage. These changes are so characteristic that a significant differentiation was possible with the help of numerous parameters.

One paper concluded on the basis of analogous observations that tidemark advancement and horizontal cartilage ruptures are the primary mechanisms within the progressive cartilage degradation[43]. At the top of the curved area where the tidemark is advanced, the shearing forces obviously reach a limit that is no longer tolerable. This then causes a fracture of the collagen fibers with subsequent tidemark flaking[44]. Others observed horizontal cartilage ruptures at the patellar cartilage and point to the vulnerable behavior of the "line of resistance", which could also lead to cartilage removal in case of trauma[45,46]. Similar tidemark changes with the spontaneous OA of the knee of mice from the breed C57 black were found[47,48], and six tidemark lines above a margin osteophyte were also observed in another study[39]. Others describe tidemark reduplication over an osteophyte with OA of the hip joint[49]. The increase of the tidemark lines

Table III
Age distribution of joints, n = 218

	Age distribution	Standard deviation/s	Minimum	Maximum	*n*
1. Total	59.3	17.9	22	87	218
2. Heberden's nodes, total (HEB 1)	68.5	12.1	43	85	91
3. No Heberden's nodes, total (HEB 2)	51.9	18.4	22	87	127
4. No Heberden's nodes, from 56 years of age (HEB 3)	68.4	9.5	56	87	55

Table IV
Comparison of the group of Heberden's nodes (n = 91) with the control group (n = 55) using a modified Mankin score[41,42]

	Heberden's nodes/control group (HEB 1/HEB 3) (*P*)		
Macroscopic grading	2.71	+++	2.03
X-ray-grading	2.09	+	1.63
Pannus	62.80	++	37.20
Osteophyte	61.30	(+)	38.70
Dorsal area, middle			
Fibrous cartilage	1.11	(+)	0.34
Median area, margin			
Cell structure	5.83	+++	4.76
Hyaline cartilage	5.80	+++	4.52
Tidemark	4.81	+	4.43
Fibrous cartilage	4.63	++	3.12
Bone	7.61	−	7.63
Safranin-O	4.38	++	4.02
Ritter−Oleson	4.33	−	4.15
Alcian	4.33	++	4.05
Histological value	28.78	++	24.49
Histochemical value	12.95	++	11.89
Histological−histochemical value	41.68	++	36.23
Dorsal area, margin			
Histochemical value	12.80	(+)	12.28
Age	68.52	−	68.40

Only the most important correlations are mentioned (*u*-test; for the parameters "pannus" and "osteophyte" Chi^2-test; +++ = statistical significance less than 0.001; ++ = under 0.01; + = under 0.05; and (+) = under 0.1 which means not significant).

was also assessed as a procedure for keeping a "steady state" under changing conditions of strain at the osteochondral junction[50]. Other authors also discuss whether the subchondral rebuilding processes might be a cause and not the consequence of OA[51−53]. This is identical with other observations of the tidemark being forced aside and a thinning of the hyaline cartilage as a reaction to vessel penetration inside the calcified cartilage[54−56]. Other authors found that chondrocytes in osteophytes release a vascular endothelial growth factor and this can promote vascular infiltration of cartilage[57].

In general, osteophytes are seen as a secondary late sequel or repair mechanism with OA[58−62]. Another author observed an increase of osteophytes before the cartilage was damaged[63]. According to other studies, the increased bone density causes a mechanical strain of the cartilage[49,64−68]. For this reason, patients with above-average bone mineral content seem to develop OA of the hip joint more often[69]. Changes in the mineral content and thickness of the calcified cartilage seem likely to play a greater role in the pathogenesis of OA than had been realized[70]. In another investigation, polymorphic tidemark changes were found as a sign of an increase of the tidemark front and were considered to be a reaction to a higher cartilage strain caused by a decreased ability of the subchondral bone for shock absorption[71].

Our results are confirmed by investigations that found an advancement in the calcified cartilage only at the convex articular surfaces[72]. This suggests that factors associated with vascular changes and related to subchondral bone remodeling are responsible. Transformation from fibrous cartilage tissue to neo-cartilage above osteophytes such as seen in our specimen has been described[73]. Pannus-like tissue was also found, preferentially in the marginal zone, which strongly suggests that it contributes to cartilage degradation[74].

The significance of the basal cartilage layer has been documented by an experiment on animals[46]. It was proved that all sequences of OA at the patellar cartilage could be triggered by inducing ischemia in the subchondral bone. All the tidemark changes then observed were highlighted by the authors in a new concept for the etiopathogenesis of chondromalacia.

The margin areas can also be places of highest stress. Then the growth would be provoked by the forces that are led into the bone via cartilage, because a higher strain is associated with an increase of remodeling[52,59,75,76]. On the other hand, there are the significant differences between polyarthritis and control groups in respect of the subchondral bone lamella which we observed in our examination. This phenomenon is explained in terms of the higher stress, because this would be seen in both spot checks. A higher strain would only occur if cartilage with genetically determined Heberden's arthritis underwent a change in its mechanical features that resulted in decreased stress absorption. If there is no external cause for the osteophyte growth, the genetic moment of Heberden's nodes may be a possible reason. Other investigators found the strongest tidemark irregularities inside the peripheral unloaded zone[77,78].

The Heberden's polyarthritis group in our study comprises a mixture of different degenerative changes. All joints of one person were examined even if only one joint showed clinically manifest Heberden's nodes. In case of a more homogeny composition of the polyarthritis group is therefore a possibility of an even higher distinction.

In summary, the osteoarthritic process in the case of Heberden's nodes starts with a subchondral remodeling that entails a subchondral ossification. Accordingly, the tidemark is advanced and a tidemark flaking marks the beginning of the general degradation while the surface is not destroyed at this point. A significant differentiation from a control group is possible on the basis of a histological score. Like another study[49], these results demonstrate that OA is a disorder of the entire joint end on the basis of a reaction of growth factors in the mineralized part of the joint cartilage and not a simple degenerative disease of the cartilage with secondary bone changes.

According to the results of our genetic and clinical investigations, Heberden's arthritis is not an isolated clinical picture but a special manifestation of OA of the distal finger joints within the framework of GOA. Heberden's nodes (the osteophytes) are therefore considered to be a genetic marker for the existence of a predisposition to GOA. Further investigations are required to clarify whether the start of the osteoarthritic process with a subchondral ossification and a reactive tidemark flaking can be generalized and extended to the larger joints such as hip or knee joints in the case of GOA.

References

1. Kellgren JH, Moore R. Generalized osteoarthrosis and Heberden's nodes. Br Med J 1952;1:181−7.
2. Lawrence JS, de Graaf R, Laine VAI. Degenerative Joint Disease in Random Samples and Occupational Groups. In: Kellgren JH, Ed. The Epidemiology of Chronic Rheumatism. Oxford: Blackwell Scientific Publications 1963;Volume I:98−121.
3. Lawrence JS. Generalized osteoarthritis in a population sample. Am J Epidemiol 1969;90:381−9.

4. Peyron JG. Epidemiologic and etiologic approach of osteoarthritis. Semin Arthritis Rheum 1979;4:288–306.
5. Abe M, Takahashi M, Naitou K, Ohmura K, Nagano A. Investigation of generalized osteoarthritis by combining x-ray-grading of the knee, spine and hand using biochemical markers for arthritis in patients with knee osteoarthritis. Clin Rheumatol 2003;22:425–31.
6. Croft P, Cooper C, Wickham C, Coggon D. Is the hip involved in generalized osteoarthritis? Br J Rheumatol 1992;31:325–8.
7. Dougados M, Nakache JP, Gueguen A. Criteria for generalized and focal osteoarthritis. Rev Rhum Engl Ed 1996;63:569–75.
8. Günther KP, Stürmer T, Sauerland S, Zeissig I, Sun Y, Kessler S, *et al*. Prevalence of generalised osteoarthritis in patients with advanced hip and knee osteoarthritis: The Ulm Osteoarthritis Study. Ann Rheum Dis 1998;57:717–23.
9. Lagier R, Mac Gee W. Erosive intervertebral osteochondrosis in association with generalized osteoarthritis and chondrocalcinosis; anatomico-radiological study of a case. Z Rheumatol 1979;38:405–14.
10. Leistner K. Epidemiologische Aspekte der Osteoarthrose und Bandscheibenschäden (in German). Beitr Orthop Traumatol 1980;27:11–21.
11. Vignon E. Hand osteoarthritis and generalized osteoarthritis: a need for clarification. Osteoarthritis Cartilage 2000;8:22–4.
12. Villiaumey J. Is the hip involved in generalized arthritis? Br J Rheumatol 1993;32:85–6.
13. Yazici H, Saville PD, Salvati EA, Bohne WHO, Wilson PD Jr. Primary osteoarthrosis of the knee or hip. Prevalence of Heberden nodes in relation to age and sex. J Am Med Assoc 1975;231:1256–60.
14. Punzi L, Ramonda R, Sfriso P. Erosive osteoarthritis. Best Pract Res Clin Rheumatol 2004;18:739–58.
15. Jones AC, Hopkinson ND, Pattrick M, Doherty M. Die ''nodale'' Arthrose: Welches Ausmaß an radiographisch gesicherter Arthrose der Hand ist erforderlich, um den Komplex zu definieren? (in German). Rheumatologie in Europa (EULAR) 1995;24:115–7.
16. Kriegel W, Narden M, Offenbächer M, Reckwitz N, Waltz M. ''State of the Art'' in der Arthroseepidemiologie (in German). Z Rheumatol 1995;54:223–40.
17. Samanta A, Jones A, Regan M, Wilson S, Doherty M. Is osteoarthritis in women affected by hormonal changes or smoking? Br J Rheumatol 1993;32:366–70.
18. Loughlin J, Irven C, Fergusson C, Sykes B. Sibling pair analysis shows no linkage of generalized osteoarthritis to the loci encoding type II collagen, cartilage link protein or cartilage matrix protein. Br J Rheumatol 1994; 33:1103–6.
19. Alarcon-Segovia S, Vega-Ortiz JM. Heberden's nodes nails. J Rheumatol 1981;8:509–11.
20. Bach GL. Hyaluronsäurezysten bei der Finger-Polyarthrose (in German). Rheumamed 1982;4:17–9.
21. Eaton RG, Dobranski AI, Littler JW. Marginal osteophyte excision in treatment of mucous cysts. J Bone Joint Surg Am 1973;55:570–4.
22. Jackson DS, Kellgren JH. Hyaluronic acid in Heberden's nodes. Ann Rheum Dis 1957;16:238–40.
23. Irlenbusch U, Enders EM, Traut H. Epidemiologische Untersuchungen zur Generalisierten Osteoarthrose an einer städtischen und ländlichen Population (in German). Akt Rheumatol 1999;24:27–34.
24. Irlenbusch U. Zur Beziehung zwischen den degenerativen Veränderungen an der Halswirbelsäule und den Fingergelenken – eine röntgenologische Untersuchung bei der Generalisierten Osteoarthrose (in German). Z Orthop 1999;137:348–54.
25. Arita M, Li SW, Kopen G, Adachi E, Jimenez SA, Fertala A. Skeletal abnormalities and ultrastructural changes of cartilage in transgenic mice expressing a collagen II gene (COL2A1) with a Cys for Arg-alpha1-519 substitution. Osteoarthritis Cartilage 2002;10:808–15.
26. Bleasel JF, Bisagni-Faure A, Holderbaum D, Vacher-Lavenu MC, Haqqi TM, Moskowitz RW, *et al*. Type II procollagen gene (COL 2A1) mutation in exon 11 associated with spondyloepiphyseal dysplasia, tall stature and precocious osteoarthritis. J Rheumatol 1995; 22:255–61.
27. Priestly L, Fergusson C, Ogilvie D, Wordsworth P, Smith R, Pattrick M, *et al*. A limited association of generalized osteoarthritis with alleles of the type II collagen locus: COL 2A1. Br J Rheumatol 1991;30:272–5.
28. Pun YL, Moskowitz RW, Lie S, Sundstrom WR, Block SR, McEwen C, *et al*. Clinical correlations of osteoarthritis associated with a single-base mutation (arginine 519 to cysteine) in type II procollagen gene. A newly defined pathogenesis. Arthritis Rheum 1994;37:264–9.
29. Vikkula M, Palotie A, Ritvaniemie P, Ott J, Ala-Kokko L, Sievers U, *et al*. Early-onset osteoarthritis linked to the type II procollagen gene. Arthritis Rheum 1993;36:401–9.
30. Naito K, Takahashi M, Kushida K, Suzuki M, Ohishi T, Miura M, *et al*. Measurement of metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases-1 (TIMP-1) in patients with knee osteoarthritis: comparison with generalized osteoarthritis. Rheumatology (Oxford) 1999;38(6):510–5.
31. Stecher RM. Das Problem der Vererbung bei Gelenkerkrankungen. II. Heberdensche Knoten. (in German) Basel: Documenta Rheumatologica Geigy Press 1957;Volume 12:11–22.
32. Stecher RM. Heberden's nodes. Their relation to other degenerative joint diseases. Arch Phys Med Rehabil 1946;27:409–13.
33. Stecher RM. Heberden's nodes: the incidence of hypertrophic arthritis of the fingers. N Engl J Med 1940;222: 300–8.
34. Stecher RM, Hersh A, Hauser H. Heberden's nodes. The family history and radiographic appearance of a large family. Am J Hum Genet 1953;5:46–60.
35. Stecher RM, Hauser H. Traumatic Heberden's nodes. Am J Roentgenol 1954;72:452–61.
36. Irlenbusch U, Schäller Th, Theile H. Genetische Untersuchungen zur Heberden-Arthrose (in German). Akt Rheumatol 1998;23:184–7.
37. Felson DT, Couropmitree NN, Chaisson CE, Hannan MT, Zhang Y, McAlindon TE, *et al*. Evidence for a Mendelian gene in a segregation analysis of generalized radiographic osteoarthritis: the Framingham study. Arthritis Rheum 1999;42:1069–70.
38. Irlenbusch U, Dominick G. Untersuchungen zur Heberden-Arthrose mittels eines histologisch-histochemischen Scores (in German). Z Orthop 1999;137: 355–61.
39. Havelka S, Babicki A, Tesarek B, Musilova J, Rohozkova D. Effect of osteotropic hormones in cartilage metabolism. Wiss Z EM Arndt Univ Greifswald Me Reihe 1980;29:33–4.
40. Rosenberg L. Chemical bases for the histological use of Safranin-O in the study of articular cartilage. J Bone Joint Surg Am 1971;53:69–82.

41. Mankin HJ. Biochemical and metabolic aspects of osteoarthritis. Orthop Clin North Am 1971;2:19–31.
42. Mankin HJ, Dorfman H, Lipiello L, Zarins A. Biomechanical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. J Bone Joint Surg Am 1971;53:523–37.
43. Radin EL, Burr DB, Caterson B, Fyhrie D, Brown TD, Boyd RD. Mechanical determinants of osteoarthrosis. Semin Arthritis Rheum 1991;21(3):12–21.
44. Anderson DD, Brown TD, Radin EL. The influence of basal cartilage calcification on dynamic juxtaarticular stress transmission. Clin Orthop 1992;286:298–307.
45. Meachim G, Bentley G. Horizontal splitting in patellar articular cartilage. Arthritis Rheum 1978;21:669–74.
46. Neusel E, Graf F, Kraft A, Niethard FU. Die Bedeutung des subchondralen Raumes für die Entstehung der Chondromalazia patellae – Morphologische Untersuchung am Gelenkknorpel der Kaninchenpatella nach experimenteller Ischämie (in German). Sportverletz Sportschaden 1992;6:170–8.
47. Wilhelmi G, Maier R. Zur Prüfung potentieller Antiarthrotika an der spontanen Arthrose der Maus (in German). Z Rheumatol 1983;42:203–5.
48. Takahama A. Histological study on spontaneous osteoarthritis of the knee in C 57 black mouse. Nippon Seikeigeka Gakkai Zasshi 1990;64:271–8.
49. Hulth A. Does osteoarthrosis depend on growth of the mineralized layer of cartilage? Clin Orthop 1993;287: 19–24.
50. Sokoloff L. Micro cracks in the calcified layer of articular cartilage. Arch Pathol Lab Med 1993;117:191–5.
51. Aust G, Otte P, Sinterhauf K. Permeabilität der Knochen-/Knorpelgrenze als pathogenetischer Faktor der Osteoarthrose (in German). Presented at the 9th Europ Rheumatologenkongress, Wiesbaden 1979, cit. in Havelka 1980.
52. Green WT, Martin GH, Eanes ED, Sokoloff L. Microradiographic study of the calcified layer of articular cartilage. Arch Pathol 1970;90:151–8.
53. Inoue H, Kodama T. Stereoscopic observations of osteochondral junction in healthy degenerated articular cartilage. Presented at the 14th International Congress of Rheumatology, San Francisco 1977, cit. in Havelka 1980.
54. Norrdin RW, Kawcak C, Capwell BA, McIlwraith CW. Calcified cartilage morphometry and its relation to subchondral bone remodeling in equine arthrosis. Bone 1999;24:109–14.
55. Oegema TR Jr, Carpenter RJ, Hofmeister F, Thompson RC Jr. The interaction of the zone of calcified cartilage and subchondral bone in osteoarthritis. Microsc Res Tech 1997;37:324–32.
56. Oettmeier R, Abendroth K, Oettmeier S. Analyses of the tidemark on humeral femoral heads. Acta Morphol Hung 1989;37:169–80.
57. Hashimoto S, Creighton-Acherman L, Takahashi K, Amiel D, Coutts RD, Lotz M. Development and regulation of osteophyte formation during experimental osteoarthritis. Osteoarthritis Cartilage 2002;10:180–7.
58. Colin JA. Osteoarthritis: a review of old myths and current concepts. Skeletal Radiol 1990;19:327–33.
59. Lane LB, Villacin A, Bullough PG. The vascularity and remodelling of subchondral bone and calcified cartilage in adult human femoral and humeral heads. J Bone Joint Surg Br 1977;59:272–8.
60. Milgram JW. Morphologic alterations of the subchondral bone in advanced degenerative arthritis. Clin Orthop 1983;173:293–312.
61. Mohr W, Doms E, Wessinghage D. Ultrastruktur des Reparationskollagens und seine Beziehungen zum residualen hyalinen Gelenkknorpel bei der entzündlichen Knorpeldestruktion (in German). Z Rheumatol 1989;48:30–8.
62. Mohr W. Gelenkkrankheiten (in German). Stuttgart, New York: Thieme Press 1984;173–202
63. Miyanaga Y. Mechanical behaviour of the subchondral bone in the experimentally induced osteoarthritis. Nippon Seikeigeka Gakkai Zasshi 1979;53:681–95.
64. Gevers G, Dequeker J, Geusens P, Nyssen-Behets C, Dhem A. Physical and histomorphological characteristics of iliac crest differ according to the grade of osteoarthritis at the hand. Bone 1989;10:173–7.
65. Grynpas MD, Alpert B, Katz I, Lieberman I, Pritzker KPH. Subchondral bone in osteoarthritis. Calcif Tissue Int 1991;49:20–6.
66. Lajeunesse D. The role of bone in the treatment of osteoarthritis. Osteoarthritis Cartilage 2004;12:34–8.
67. Naito K, Kushida K, Takahashi M, Ohishi T, Inoue T. Bone mineral density and bone turnover in patients with knee osteoarthritis compared with generalized osteoarthritis. Calcif Tissue Int 2000;66:325–9.
68. Price T, Hesp R, Mitchell R. Bone density in generalized osteoarthritis. J Rheumatol 1987;14:560–2.
69. Puhl W, Günther K. The Importance of Clinical Subsets of Osteoarthritis for Epidemiological Research. In: Puhl W, Brandt KD, Eds. Epidemiology of Osteoarthritis Stuttgart, New York: Thieme Press 1994; 76–86.
70. Burr DB. Anatomy and physiology of the mineralized tissues: role in the pathogenesis of osteoarthritis. Osteoarthritis Cartilage 2004;12:20–30.
71. Dequeker J, Mokassa L, Aerssens J, Boonen. Bone density and local growth factors in generalized osteoarthritis. Microsc Res Tech 1997;37:358–71.
72. Patel N, Buckland-Wright C. Advancement in the zone of calcified cartilage in osteoarthritic hands of patients detected by high definition radiography. Osteoarthritis Cartilage 1999;7:520–5.
73. Gelse K, Söder S, Eger W, Diemtar T, Aigner T. Osteophyte development – molecular characterization of differentiation stages. Osteoarthritis Cartilage 2003; 11:141–8.
74. Shibakawa A, Aoki H, Masuko-Hongo K, Kato T, Tanaka M, Nishioka K, *et al*. Presence of pannus-like tissue on osteoarthritic cartilage and its histological character. Osteoarthritis Cartilage 2003;11:133–40.
75. Böhringer ME, Beyer WF, Weseloh G. Vergleichende Histomorphologie der subchondralen Knochendichte und Gelenkknorpeldicke im Tibiakopf bei humanen Früharthrosen (in German). Z Orthop 1995;133:291–302.
76. Buckland-Wright JC, Macfarlane DG, Lynch J. Osteophytes in the osteoarthritic hand: their incidence, size, distribution and progression. Ann Rheum Dis 1991;50:627–30.
77. Clark JM, Huber JD. The structure of human subchondral plate. J Bone Joint Surg Br 1990;72: 866–73.
78. O'Connor KM. Unweighting accelerates tidemark advancement in articular cartilage at the knee joint of rats. J Bone Miner Res 1997;12:580–9.