pollen analysis, these findings support our conclusion that the murder victims were Soviet soldiers.

R. Szibor*, C. Schubert*, R. Schöning*, D. Krause*, U. Wendt†

**Institut für Rechtsmedizin, Otto-von-Guericke-Universität Magdeburg, Leipziger Strasse 44, D-39120 Magdeburg, Germany*

†Institut für Werkstofftechnik und Werkzeugprüfung, Otto-von-Guericke-Universität Magdeburg, Grosse Steinernetischstrasse, D-39104 Magdeburg, Germany

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Stingray jaws strut their stuff

The cartilaginous skeleton of sharks and rays imposes functional limitations that are not seen in bony fishes. Cartilage is less dense than bone, which helps chondrichthyan (cartilaginous) fishes maintain near neutral buoyancy, but cartilage is also less stiff and strong than bone. Nevertheless, some stingrays routinely use their cartilaginous jaws and pavement-like dentition to crush hard prey, such as snails and mussels[1]. We have studied the cownose ray, *Rhinoptera bonasus*, to investigate how cartilaginous jaws can be used to crush hard-shelled prey. The jaws are composed of 'trabecular cartilage', a material that is structurally and functionally convergent with the trabecular bone found in osteichthyan (bony) fishes and tetrapods.

The cownose ray is pelagic, travelling in large schools that periodically descend to beds of sea-grass to feed on benthic invertebrates. The tooth plates used to crush these hard-shelled prey are similar to the teeth of other vertebrates, but the jaws that bear the teeth are entirely cartilaginous. Superficially, the jaws appear to be made of the calcified cartilage that is common to most chondrichthyan fishes, in which a core of hyaline cartilage is surrounded by a layer of prismatic cartilage arranged in tiny mineralized blocks called tesserae[2,3]. The jaws of the cownose ray exhibit this surface calcification, but cross-sections of the cartilage show that mineralized struts, composed of columns of tesserae, also run through the centre of the tissue (Fig. 1). The struts are concentrated in the region where the tooth plates crush prey, and are well positioned to distribute the bite force load to the layers of tesserae on the opposite side of the jaw.

In most animals, bones are generally stiffened either by thickening the outer layer of compact bone (cortex) or by increasing the internal trabeculation. The struts, or trabeculae, in the marrow cavity of mammalian long bones follow the lines of stress imposed during normal loading[4], for example. The jaws of the cownose ray contain analogues of both of these structural modifications.

The analogue to cortical thickening is the deposition of multiple layers of tesserae on the surface of the cartilage (Fig. 1d, red arrows). This character had been thought to exist only in extinct sharks[5], but the examination of large (>4 m long) carcharhinid and lamnid sharks revealed as many as seven layers of mineralization at the jaw articulation[6]. Two layers of tesserae cover the entire surface of the jaw in the cownose ray, with some areas having up to six layers.

We were surprised to find that cownose rays also stiffen their jaws with internal trabeculation. Because cartilaginous skeletal elements have no blood supply, they usually exhibit only surface mineralization. Examination of neonatal cownose rays, before they begin to feed exogenously, shows that trabeculae are already present. This finding, together with the pattern of trabeculation in large individuals, suggests that the trabeculae grow in length by adding tesserae to the ends of the columns, and increase in complexity by branching at the surface and then increasing in length as the jaws grow.

The biochemistry of trabecular cartilage is distinguishable from hyaline cartilage only by its mineral content (25%), which was higher than normal in the matrix, even with the trabeculae removed. The glycosaminoglycan (4.5% of organic material), collagen (27%) and elastin (0%) contents are all within expected ranges.

Biomaterials testing of trabecular cartilage supports the idea that the trabeculae function to stiffen the cartilage. Small blocks (4-mm cubes) of trabecular cartilage were excised and subjected to mechanical tests of stiffness. To remove the mechanical effects of trabeculae, some blocks were treated with EDTA to decalcify the trabeculae, and others were tested with the struts perpendicular to the applied load rather than parallel to it. Both of these groups were an order of magnitude less stiff (10^6 Pa as opposed to 10^7 Pa) than those with intact struts tested parallel to the load.

To our knowledge, this is the first time that organized, mineralized structures serving a distinct biomechanical role have

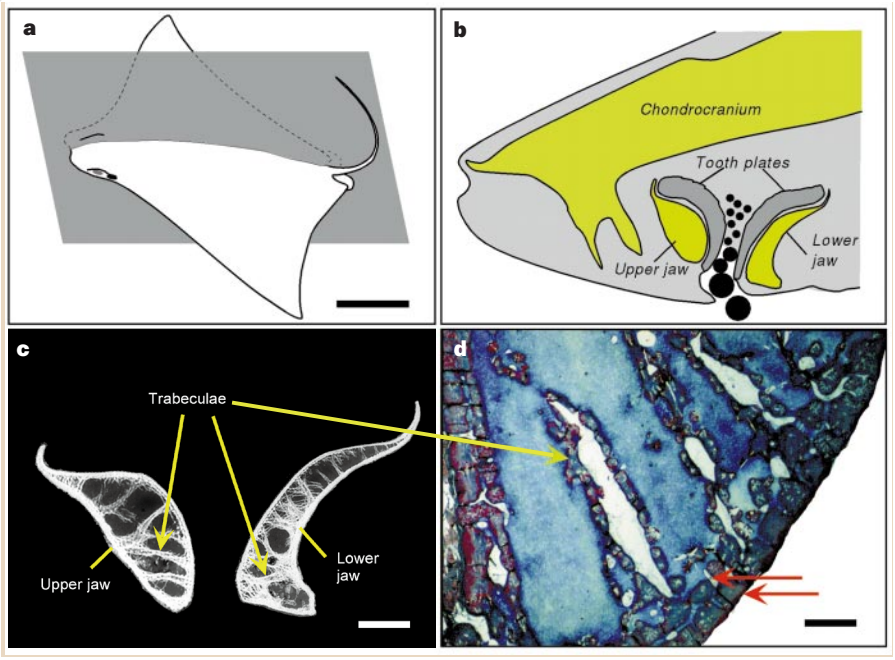

Figure 1 Trabeculae of the cownose stingray, *Rhinoptera bonasus*. **a,** Outline showing the plane through which the head and jaws are sectioned in the other panels. Scale bar, 10 cm. **b,** Sagittal section through the head showing the position of the jaws relative to hard prey items (black circles) that are being crushed. Cartilage is shown in yellow. **c,** Radiograph of a sagittal section through the jaws showing the position of the mineralized struts (trabeculae). The trabeculae are concentrated in the region where the the tooth plates crush prey. Scale bar, 1 cm. **d,** Section through the upper jaw after Masson's staining showing that each trabecula, cut obliquely, is a fluid-filled tube. The walls of each trabecular tube are composed of small blocks of mineralized tissue. There is no epithelial tissue lining the tubes and no cellular material appears in the lumen. Most cartilaginous fishes have a single layer of tesserae in the outer prismatic layer of cartilage, but this species has at least two layers (red arrows), and sometimes as many as six. Scale bar, 1 mm.

been found in the core of a mature cartilaginous tissue. Studies of the palaeohistology of vertebrates have assumed that cartilage can mineralize only on the surface. Any new descriptions of fossilized hard tissues, particularly from fishes, must take this discovery into account, and previous descriptions may need to be re-evaluated.

Adam P. Summers*, Thomas J. Koob†, Elizabeth L. Brainerd*

**Organismic and Evolutionary Biology Program, University of Massachusetts at Amherst, Amherst, Massachusetts 01003, USA*
e-mail: summers@bio.umass.edu
†*Shriner's Hospital for Children, Tampa, Florida 33612, USA*

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The ubiquitin pathway in Parkinson's disease

Mutations of the α-synuclein gene[1,2] have been identified in some familial forms of Parkinson's disease, and α-synuclein protein has been shown to accumulate in the brains of patients with the disease[3]. These findings suggest that Parkinson's disease may be caused by the abnormal aggregation of α-synuclein protein. Here we have identified in a German family with Parkinson's disease a missense mutation in the ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) gene. We show that this mutation, Ile93Met, causes a partial loss of the catalytic activity of this thiol protease, which could lead to aberrations in the proteolytic pathway and aggregation of proteins.

UCH-L1 is one of the most abundant proteins in the brain[4,5], comprising up to 2% of total brain protein. Immunoreactivity for this protein is found in Lewy bodies[6]. It belongs to a family of deubiquitinating enzymes, and is thought to cleave polymeric ubiquitin to monomers and to hydrolyse bonds between ubiquitin molecules and small adducts such as glutathione and cellular amines[7]. The abundance of UCH-L1 in human brain, its presence in Lewy bodies, and its involvement in the ubiquitin-dependent proteolytic pathway implicate it in the pathogenesis of Parkinson's disease.

We have sequenced the coding region of the UCH-L1 gene in probands from 72 families with Parkinson's disease, and identified a missense mutation in the fourth exon of the UCH-L1 gene that changes an isoleucine at position 93 to a methionine in one proband of a German pedigree. This Ile93Met change can be examined by a restriction endonuclease assay, because at the nucleotide level the C277G change introduces a new *Bsm*F1 site (Fig. 1). Mutation analysis of the affected brother of the proband showed that he too carries the Ile93Met mutation (Fig. 2).

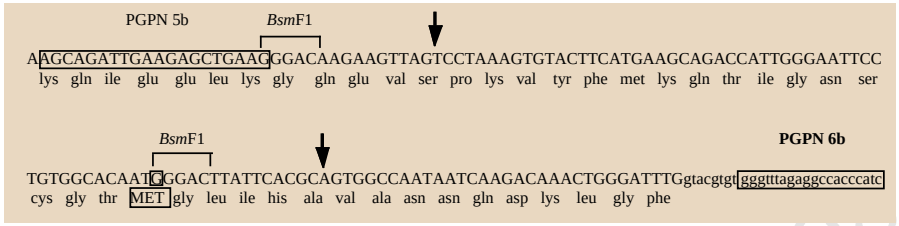

Figure 1 DNA sequence of a portion of exon 4 of the UCH-L1 gene. Boxes indicate polymerase chain reaction (PCR) primer sequences, and arrows indicate restriction sites for the *Bsm*F1 restriction endonuclease.

In both patients, the clinical syndrome is typical for Parkinson's disease. Symptoms began with resting tremor at age 51 for the proband and 49 for her affected brother, and progressed to rigidity, bradykinesia and postural instability. Both individuals showed a beneficial response to L-dopamine replacement therapy. A paternal uncle and the paternal grandmother were also affected, although, with the exception of the two siblings, all other individuals in the pedigree are deceased. The lack of phenotype in the father indicates that the mutation has incomplete penetrance in this family.

We analysed 500 chromosomes from control individuals of different ethnic backgrounds, 204 originating from German backgrounds. None of the 500 control chromosomes examined carried the Ile93Met change. Ile 93 is conserved in UCH-L1, UCH-L3 and the rat and mouse orthologues, as well as in the homologous genes of a yeast and the plant *Arabidopsis thaliana*. Thus, like mutations in α-synuclein, the Ile93Met mutation in the UCH-L1 gene is expected to contribute to the genetic aetiology of only a small number of patients with the familial form of the illness.

The mutant and wild-type proteins were expressed in *Escherichia coli* and assayed using two types of substrate. For the ubiquitin ethyl ester, rate measurements showed that the Ile93Met mutant protein cleaved the substrate (15 μM) at a rate of 2.41 U mg^{-1} compared with 4.08 U mg^{-1} for the wild-type protein. Because the K_m for this substrate is submicromolar, these rates represent V_{max} values and are consistent with previously determined rates[8]. With the substrate ubiquitin-7-amido-4-methycoumarin (Ub-AMC)[9], the V_{max} of the mutant enzyme was 0.20 U mg^{-1}, compared with 0.47 U mg^{-1} for the wild-type enzyme. Mutant and wild-type proteins exhibited similar K_m values, however, indicating that the mutant protein does not show decreased affinity for the substrate. Similarly, ubiquitin was an equally potent inhibitor of both the mutant and normal enzymes. The enzymatic activity values are consistent with Ile93Met UCH-L1 having a lower catalytic activity than the wild-type protein. Molecular modelling of the mutation suggests alteration in the geometry and fluctuation of the active site.

The roughly 50% reduction in catalytic activity of the Ile93Met protein should be interpreted with caution, however, as the natural substrate for the abundant UCH-L1 protein is not known. The reduced catalytic activity may affect the cleavage and turnover of the unknown substrate(s), leading to aggregation over time of the substrate(s), which can in turn act as a seed for other aggregation-prone abundant proteins. Alternatively, the Ile93Met substitution may render UCH-L1 prone to aggregation with the result that the protein accumulates. Finally, both models — reduced enzymatic activity and enhanced aggregation — may be in play at different stages of the illness.

The finding of mutations in the genes encoding α-synuclein and UCH-L1, and the identification of these proteins in Lewy

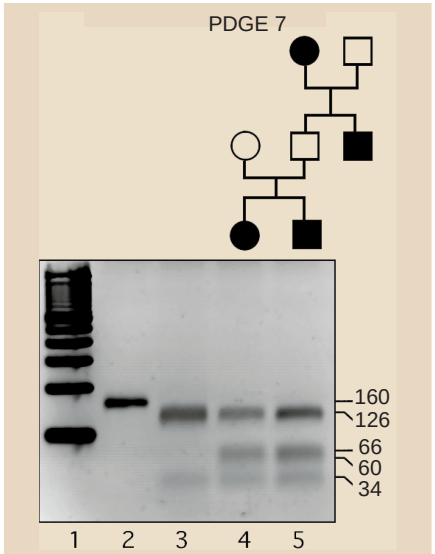

Figure 2 Mutation analysis for the Ile93Met mutation in kindred PDGE7. Lane 1 is a molecular marker; lane 2, undigested PCR product; lane 3, digested PCR product not carrying the mutation; lanes 4 and 5, PCR products from affected individuals carrying the Ile93Met mutation.