**Original** article

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# Presence of L-kynurenine aminotransferase III in retinal ganglion cells and corpora amylacea in the human retina and optic nerve

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## Abstract

***Background:*** *Corpora amylacea (CAm) are a hallmark of aging and neurodegeneration. The presence of kynurenine aminotransferases I and II (KAT I and II) in CAm in the human retina and optic nerve has been already shown. The present study aimed to examine kynurenine aminotransferase III (KAT III) immunoreactivity in CAm in the human retina and optic nerve.*

***Material and methods:*** *Polyclonal antibody against KAT III was used on sections of human eyes enucleated due to malignant uveal melanoma. PAS-stained sections of CAm were compared with KAT III stained ones.*

***Results:*** *KAT III immunoreactivity was observed in CAm in the retina, prelaminar, laminar and retrolaminar region of the optic nerve with similar location to PAS-stained sections. The most intense staining was observed in the retrolaminar part of the optic nerve. KAT III immunoreactivity was also present in the cytoplasm of retinal ganglion cells.*

***Conclusions:*** *Expression of KAT III in CAm in the human retina and optic nerve indicates that this enzyme may be relevant in mechanisms of neurodegeneration leading to CAm formation.*

***Key words:*** *corpora amylacea, degeneration, KAT III, kynurenic acid, retina, immunohistochemistry.*

## Introduction

Kynurenic acid (KYNA) acts as an endogenous modulator of glutamatergic and cholinergic neurotransmission [14,24]. It is the only known endogenous antagonist of the NMDA (N-methyl-D-aspartate) glutamate receptors [24]. KYNA is produced by irreversible transamination of kynurenine (KYN). Four kynurenine aminotransferases have been found in mammalian (human, rat and mouse) brains so far: KAT I/glutamine transaminase/K cysteine conjugate beta-lyase 1, KAT II/aminoadipate aminotransferase,

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KAT III/cysteine conjugate beta-lyase 2 and KAT IV/glutamic-oxaloacetic transaminase 2/mitochondrial aspartate aminotransferase [13,14,16,17]. The role of KAT I and KAT II in the rat brain and the retina has been broadly investigated [10,22,26].

KYNA is important in the development and progression of neurodegenerative disorders of the brain and eye. Abnormal concentrations of KYNA have been found in cerebrospinal fluid or brain tissue in patients suffering from multiple sclerosis, schizophrenia [8], Huntington's disease [2], Alzheimer's disease [2], Parkinson's disease [21], epilepsy [37] and AIDS dementia complex [11]. Presence of L-kynurenine aminotransferases (KAT I and II) in the rat and chicken retina has been already documented [26-31,35,38].

Corpora amylacea (CAm) are homogeneous or laminated oval structures of 10-50 μm diameter. They represent the remnants of degenerated and aggregated neuronal cells [33] and consist of a mass of filamentous tangles within an axonal swelling [1,36] resulting from impaired axonal flow [19]. They are observed in the brain, peripheral nerves and the eye. Intraocular CAm were found in the optic nerve head, nerve fibre layer, ganglion cell layer, as well as in the inner plexiform layer, and inner nuclear layer [17,36]. In the central nervous system (CNS), CAm are regarded as a hallmark of ageing and neurodegeneration [5,7,20]. As CAm are rich in acid polysaccharide content, they are best demonstrable by the PAS (periodic acid-Schiff) stain.

As KAT I and II immunoreactivity in CAm in the human retina and optic nerve has already been reported [32], this study was designed to examine the presence and pattern of KAT III expression in CAm in the human retina and optic nerve.

## Material and methods

Eight human eyes from seven patients (4 females, 4 males, age range: 56-83 years) enucleated due to choroidal malignant melanoma were used for this study. There were no other ocular or systemic diseases. The methods used in this study followed the tenets of the Declaration of Helsinki. The study protocol was approved by the Human Ethics Committee of the University of Erlangen-Nuernberg, Germany.

Following enucleation all globes were fixed immediately in a solution of 4% formaldehyde and 1% glutaraldehyde in 0.1% phosphate buffer (pH 7.2). P-O sections (5 μm) including the centre of the disc and the pupil were stained with PAS, HE (haematoxylin-eosin) or subjected to immunohistochemistry.

We used anti-KAT III polyclonal antibody (1 : 50) [22,23] at least twice for staining, using the streptavidin-biotin method, as described previously [6]. Subsequently – after deparaffinization and rehydration – sections were digested with proteinase K (Dako) before incubation with peroxidase for 10 minutes. Afterwards sections were incubated with primary antibody (30 minutes) and horseradish peroxidase (HRP)-conjugated secondary antibody before development with 3-amino-9-ethylcarbazole (AEC)+ substrate (red reaction product). In the final stage, the sections were counterstained with Mayer haemalaun (Chroma, Münster, Germany) and mounted in an aqueous-based medium (Faramount; Dako). As the negative control preimmune serum was included and there was no staining of CAm observed. Sections were photographed with a microscope (Axiophot; Carl Zeiss, Oberkochen, Germany) using colour film (Ektachrome 64 T; Eastman Kodak, Rochester, NY).

## Results

CAm were observed in all cases in the retina (Fig. 1) and prelaminar, laminar and retrolaminar regions of the optic nerve (Figs. 2-4). Moreover, KAT III immunoreactivity was present in the cytoplasm of retinal ganglion cells (Fig. 5).

KAT III expression in CAm was observed in the retina and optic nerve (Fig. 1 R2, Fig. 2 PL2, Fig. 3 L2 and Fig. 4 RL2) with similar location to PAS-stained sections (Fig. 1 R1, Fig. 2 PL1, Fig. 3 L1 and Fig. 4 RL1). CAm appeared as round, oval, smooth, or laminated bodies with dense centres in PAS-stained sections. These results are in agreement with the previous study of Kubota and co-workers [17].

There was more pronounced staining of KAT III in the retrolaminar part of the optic nerve (Fig. 4 RL2). Some of the CAm showed only faint KAT III immunoreactivity and occasionally there was no staining (data not shown). No correlation was found between the size of CAm and immunoreactivity of KAT III.

## Discussion

This study is the first investigation demonstrating the immunoreactivity of KAT III in CAm in the human retina and optic nerve. CAm expressing KAT III

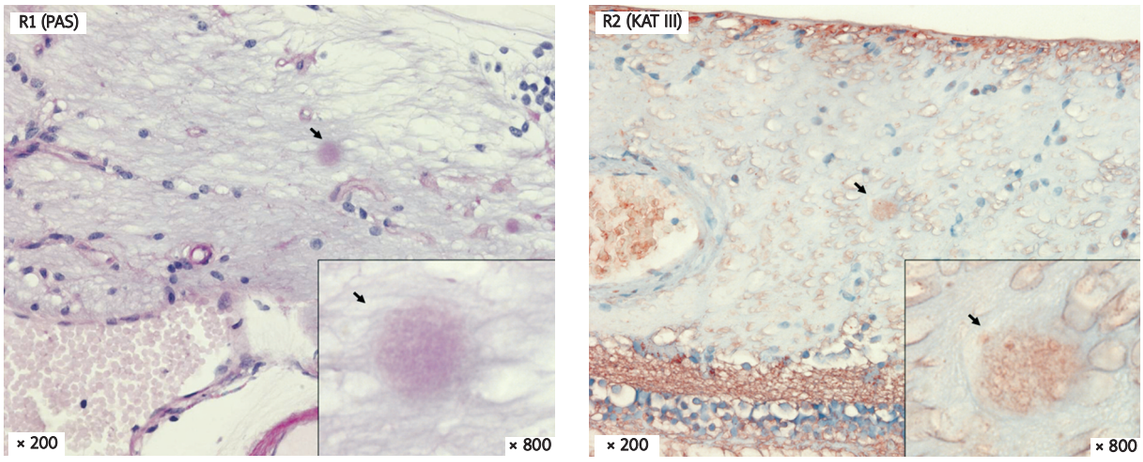


**Fig. 1.** PAS and immunohistochemical staining of KAT III in CAm in the human retina.
In PAS-stained sections CAm were observed in all cases in the retina (R1). Immunoreactivity of KAT III was detected in CAm in the retina (R2) showing intense staining. Some CAm showed only faint KAT immunoreactivity. CAm are indicated by black arrows. Magnifications are indicated on the pictures.

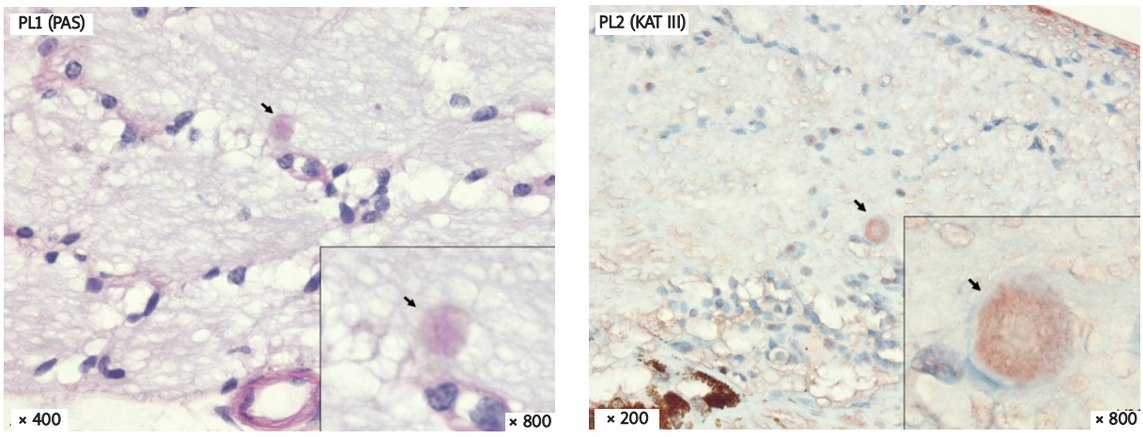


**Fig. 2.** PAS and immunohistochemical staining of KAT III in CAm in prelaminar region of the human optic nerve.
In PAS-stained sections CAm were observed in all cases in the prelaminar (PL1) region of the optic nerve. KAT III immunoreactivity was observed in CAm in the prelaminar (PL2) region of the optic nerve revealing a pattern of intensive staining. CAm are indicated by black arrows. Magnifications are indicated on the pictures.

enzyme were found in all cases in the retina and in the optic nerve – in the prelaminar, laminar and retrolaminar regions. The most pronounced staining was found in the retrolaminar part of the optic nerve. Presence of KAT III was observed not only extracellularly but also in the cytoplasm of retinal ganglion cells.

The results of this study are similar to our previous study with KAT I and II showing that both enzymes are present in the human retina and optic nerve [32]. KAT I was localised on Muller cell endfeet while KAT II was expressed in cells within the ganglion cell layer. In the optic nerve KAT I staining was more intense than KAT II and was observed in the prelaminar, laminar and retrolaminar regions of the optic nerve. Cellular expression of KATs in neurons and glial cells of CNS has already been described [16,26,29,31].

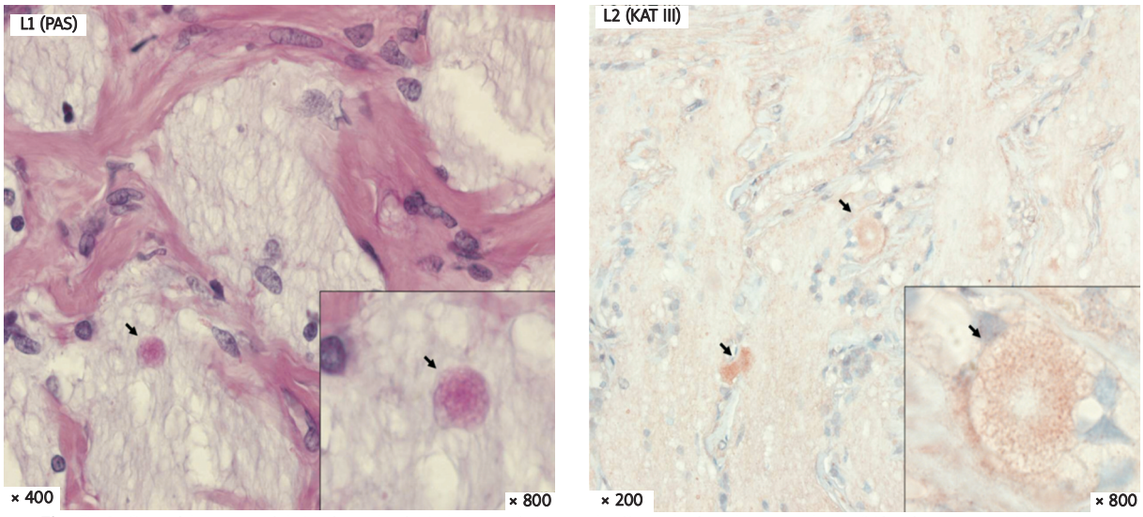


**Fig. 3.** PAS and immunohistochemical staining of KAT III in CAm in laminar region of the human optic nerve. In PAS-stained sections CAm were observed in all cases in the laminar (L1) region of the optic nerve. KAT III immunoreactivity was observed in CAm in the laminar region of the optic nerve (L2) and the pattern of its staining in most cases was intense. CAm are indicated by black arrows. Magnifications are indicated on the pictures.

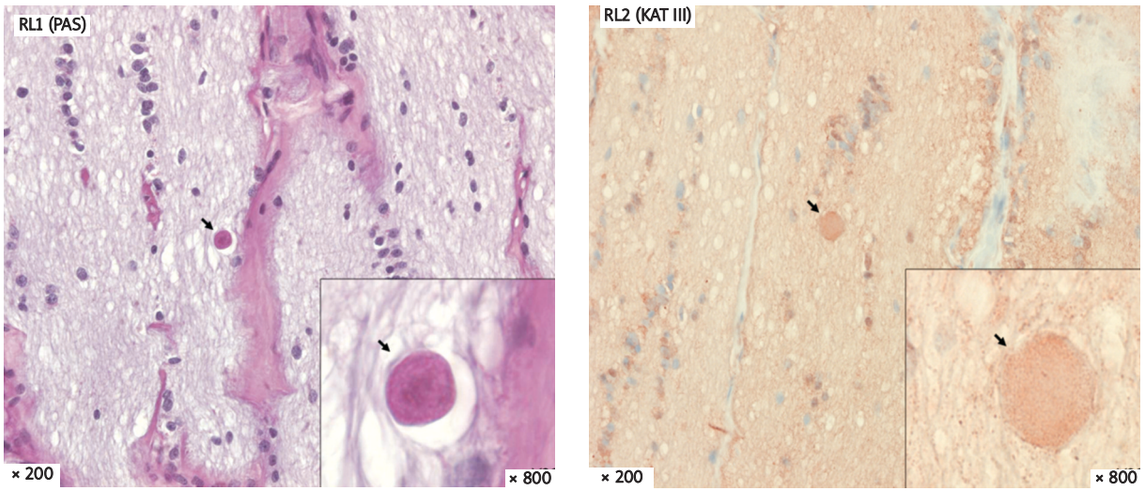


**Fig. 4.** PAS and immunohistochemical staining of KAT III in CAm in retrolaminar region of the human optic nerve. In PAS-stained sections CAm were observed in all cases in the retrolaminar (RL1) region of the optic nerve. KAT III immunoreactivity was observed in CAm in the retrolaminar region of the optic nerve (RL2) and the pattern of its staining in most cases was more pronounced as compared to the retina and other regions of the optic nerve. CAm are indicated by black arrows. Magnifications are indicated on the pictures.

There are only limited data in the literature concerning CAm and their formation. CAm were originally described by Purkinje in 1837 [25] and their pathological relevance has not been considered for a long time. They were found in subpial regions in brains of normal elderly subjects. The presence of CAm was also found in post-mortem brains of patients suffering from various neurological conditions such as Parkinson disease [4], Alzheimer's disease, Pick's disease [34] and sclerosis multiplex [33]. Mechanisms leading to extracellular occurrence of KAT III are not very well known.

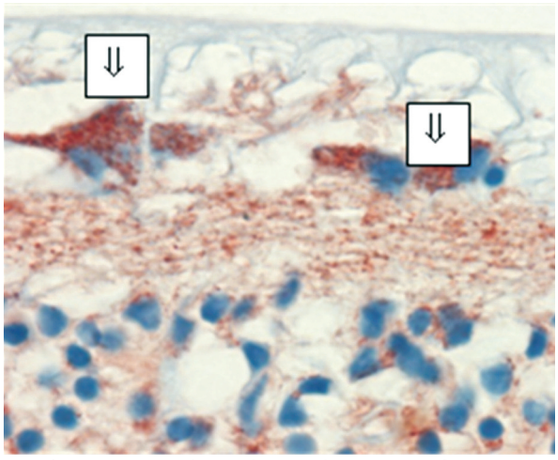

**Fig. 5.** Immunohistochemical staining of KAT III in the cytoplasm of the retinal ganglion cell.

Many substances were found to contribute to the formation of CAm, as components of the degraded cells, metabolites originating from the cerebrospinal fluid, blood and the mesenchyme of pia mater and adventitia of the vessel wall [15]. It has been shown that CAm consist of an inert mucopolysaccharide matrix encasing ubiquitinated proteins, resulting from death of and damage to neurons, myelin and oligodendrocytes [34]. Positive staining of CAm was reported for ubiquitin [5], tau [19], heat shock protein [9], a neuronal GABA releasing enzyme [15], ferritin [34], nestin [4] and other proteins. Some of these substances are "protective" to nerve cells and can rescue them from the devastating effects of ischaemia or ageing [3,5,9]. A function of CAm, therefore, could be to prevent the recognition of these immunogenic proteins by lymphocytes and microglia and thus protect the CNS from further injury [34].

We have already shown [30] the age-dependant decrease of cellular expression of both KAT I and II in the retina of DBA/2J mice (model for ocular hypertension). Moreover, it was proven that KYNA deficiency is related to the pathology of excitotoxic retinal diseases and that NMDA-induced retinal ganglion cell loss may cause alterations of KYNA content in the rat retina [28]. Cellular expression of KAT in neurons and glial cells of CNS is well described [16,26,29,31]. The present study, similarly to results of our studies in rodents [26,30,31] and a previous study with KAT I and II in the human retina [32], showed that KAT III was present in the human retina. The mechanisms leading to extracellular occurrence of KAT III in CAm are still not known. It may be a primary event in the CAm formation or a secondary mechanism induced by some products of a degenerative process (ageing, neurodegeneration) or by recurrent functional disturbances of the cellular barriers. KAT III as well as KAT I and KAT II may be released from cells dying due to degeneration and accumulated in CAm.

The presence of KAT III in CAm in the human retina and optic nerve indicates that KYNA synthesis might be involved in the mechanisms of retinal ageing and neurodegeneration. However, mechanisms leading to the occurrence of KAT enzymes in CAm need further investigations.

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