

DISSERTATIONS IN  
**HEALTH  
SCIENCES**

**ALLAN SEPPÄNEN**

*Collagen XVII in the Human Brain*

PUBLICATIONS OF THE UNIVERSITY OF EASTERN FINLAND  
*Dissertations in Health Sciences*



UNIVERSITY OF  
EASTERN FINLAND

ALLAN SEPPÄNEN

# *Collagen XVII in the Human Brain*

To be presented by permission of the Faculty of Health Sciences, University of Eastern Finland for public examination in Auditorium 8, Oulu University Hospital, on Friday, November 25<sup>th</sup> 2011, at 12 noon

Publications of the University of Eastern Finland  
Dissertations in Health Sciences  
Number 83

Department of Neurology, Institute of Clinical Medicine, School of Medicine,  
Faculty of Health Sciences, University of Eastern Finland  
Kuopio  
and  
Department of Neurology, Institute of Clinical Medicine,  
Faculty of Medicine, University of Oulu and Clinical Research Center, Oulu University Hospital  
Oulu  
2011

Kopijyvä Oy  
Kuopio, 2011

Series Editors:

Professor Veli-Matti Kosma, M.D., Ph.D.  
Institute of Clinical Medicine, Pathology  
Faculty of Health Sciences

Professor Hannele Turunen, Ph.D.  
Department of Nursing Science  
Faculty of Health Sciences

Professor Olli Gröhn, Ph.D.  
A.I. Virtanen Institute for Molecular Sciences  
Faculty of Health Sciences

Distributor:

University of Eastern Finland  
Kuopio Campus Library  
P.O.Box 1627  
FI-70211 Kuopio, Finland  
<http://www.uef.fi/kirjasto>

ISBN (print): 978-952-61-0583-3

ISBN (pdf): 978-952-61-0584-0

ISSN (print): 1798-5706

ISSN (pdf): 1798-5714

ISSN-L: 1798-5706

- Author's address: Department of Neurology, Institute of Clinical Medicine  
School of Medicine, Faculty of Health Sciences  
University of Eastern Finland  
KUOPIO  
FINLAND
- Supervisors: Professor Irina Alafuzoff, M.D., Ph.D.  
Department of Neurology, Institute of Clinical Medicine  
School of Medicine, Faculty of Health Sciences  
University of Eastern Finland  
and Department of Genetics and Pathology, Uppsala University, Sweden  
KUOPIO  
FINLAND  
and  
UPPSALA  
SWEDEN
- Professor Kari Majamaa, M.D., Ph.D.  
Department of Neurology, Institute of Clinical Medicine,  
Faculty of Medicine  
University of Oulu  
and Clinical Research Center, Oulu University Hospital  
OULU  
FINLAND
- Reviewers: Professor Veli-Matti Kähäri, M.D., Ph.D.  
Department of Dermatology  
University of Turku  
TURKU  
FINLAND
- Head of Section Safa Al-Sarraj, M.D., Ph.D.  
Section of Neuropathology, Institute of Psychiatry  
King's College London  
LONDON  
UK
- Opponent: Professor Hannu Kalimo, M.D., Ph.D.  
Department of Pathology  
Haartman Institute  
University of Helsinki  
HELSINKI  
FINLAND



Seppänen, Allan

Collagen XVII in the Human Brain, 46 p.

University of Eastern Finland, Faculty of Health Sciences, 2011

Publications of the University of Eastern Finland. Dissertations in Health Sciences 83. 2011. 46 p.

ISBN (print): 978-952-61-0583-3

ISBN (pdf): 978-952-61-0584-0

ISSN (print): 1798-5706

ISSN (pdf): 1798-5714

ISSN-L: 1798-5706

## **ABSTRACT**

Collagens have previously been overlooked for roles in the brain since fibrillar collagens, the best known and most widely studied example of collagens, are not present in the mature central nervous system (CNS). However, over the last decade it has become increasingly apparent that collagens are not merely structural proteins giving strength to tissue, but bio-active molecules with a dynamic role within the CNS. In fact, a role in the CNS, albeit often transient, has been identified for nearly every type of collagen during some phase of CNS development. Thus, collagens are now thought to have a decisive role in various aspects of neural maturation and are currently being studied in relation to various neurological disorders.

Collagen XVII is one of the four non-fibril-forming transmembrane collagens, which function as both matrix proteins and cell-surface receptors. It is known to be a structural component of hemidesmosomes, which mediate adhesion of epidermal keratinocytes and certain other epithelial cells to the underlying basement membrane. Based on numerous case, animal and epidemiological studies, collagen XVII could be one of the most interesting putative antigens common to both dermatological and neurological disease.

In this thesis, collagen XVII was studied in the mature human brain, using brain samples obtained at autopsy and an array of standard histological and molecular research methods. The aim was to establish whether collagen XVII is present in the human central nervous system and if so, to define the anatomical regions, cells and intracellular locations in which it is expressed. Also, possible changes in expression due to motor neuron disease-related neuropathology, as visualized with p62, were studied.

This study found that collagen XVII is expressed in human CNS neurons and that it is widely distributed in different anatomical regions of the human brain. Intraneuronally, the immunoreactivity is localised to lipofuscin granules. The study also established that the expression of collagen XVII is not altered in motor neuron disease and that the presence of p62- positive inclusions outside the motor system in motor neuron disease could be a marker for psychiatric morbidity.

National Library of Medical Classification: QW 504.5, QZ 35, WE 550, WL 300

Medical Subject Headings: Collagen Type XVII; Non-Fibrillar Collagens; Central Nervous System; Immunohistochemistry; Lipofuscin; Motor Neuron Disease; Autopsy; Brain; Humans



Seppänen, Allan

Collagen XVII in the Human Brain, 46 p.

Itä-Suomen yliopisto, Terveystieteiden tiedekunta, 2011

Publications of the University of Eastern Finland. Dissertations in Health Sciences 83. 2011. 46 p.

ISBN (print): 978-952-61-0583-3

ISBN (pdf): 978-952-61-0584-0

ISSN (print): 1798-5706

ISSN (pdf): 1798-5714

ISSN-L: 1798-5706

## TIIVISTELMÄ

Kollageenit ovat aiemmin jääneet huomiotta aivotutkimuksen saralla, sillä kollageenisäikeitä, kollageenien tunnetuinta esiintymismuotoa, ei kypsässä keskushermostossa esiinny. Viime vuosikymmenen aikana on kuitenkin käynyt ilmeiseksi, että kollageenit eivät ainoastaan strukturoi ja vahvista kudoksia, vaan ovat bioaktiivisia molekyylejä joilla on dynaaminen rooli keskushermostossa. Itse asiassa, lähes jokaisella kollageenityypillä on jonkinlainen rooli keskushermoston kehityksessä, joskin useimmiten vain väliaikaisesti ja vain tietyssä kehitysvaiheessa. Näin ollen nykyisin kollageeneilla ajatellaan olevan merkittäviä tehtäviä hermoston kypsymisessä ja kollageeneja on alettu tutkia erilaisten hermostollisten sairauksien yhteydessä.

Kollageeni XVII on yksi neljästä transmembraani kollageenista. Nämä kollageenit eivät muodosta säikeitä ja toimivat sekä soluvälitilaproteiineina, että solukalvoreseptoreina. Kollageeni XVII:n tiedetään olevan osa hemidesmosomia, joka toimii sitomalla keratinosyyttejä ja tiettyjä muita epiteelisoluja niiden alaiseen tyvikalvoon. Useat epidemiologiset tutkimukset, tapausselostukset ja eläinkokeet viittaavat siihen, että kollageeni XVII voisi toimia antigeeninä sekä hermostollisissa sairauksissa, että ihotaudeissa.

Tässä väitöskirjassa tyyppin XVII kollageenia tutkittiin kypsissä ihmisen aivoissa, käyttäen ruumiinavauksissa otettuja aivonäytteitä sekä valikoimaa vakiintuneita histologisia ja molekylaarisia tutkimusmenetelmiä. Tarkoituksena oli tutkia esiintykö kollageeni XVII ihmisen keskushermostossa ja jos esiintyy, niin millä anatomisilla alueilla, missä soluissa ja missä solunosassa. Myös mahdollisia muutoksia kollageeni XVII:n ekspressiotasossa suhteessa p62-visualisoiuihin motoneuronitautimuutoksiin tutkittiin. Tutkimus toi esiin, että kollageeni XVII esiintyy ihmisen aivojen neuroneissa useilla eri aivoalueilla. Solunsisäisesti kollageeni XVII sijaitsee lipofuskiinirakkuloissa. Tutkimus osoitti myös, ettei kollageeni XVII:n ekspressiotaso muutu motoneuronitaudissa ja että p62-positiivisten inklusioiden läsnäolo motoristen aivoalueiden ulkopuolella motoneuronitaudissa voi liittyä psykiatriseen oireiluun.

Luokitus: QW 504.5, QZ 35, WE 550, WL 300

Yleinen Suomalainen asiasanasto: amyotrofinen lateraaliskleroosi; aivot; ihminen; kollageenit; ruumiinavaus; aivotutkimus; keskushermosto



# Acknowledgements

This thesis was carried out in the Department of Neurology, University of Oulu, the Clinical Research Center, Oulu University Hospital and the Department of Neurology, Institute of Clinical Medicine, University of Eastern Finland during the years 2004–2011. It has been financially supported by the Sigrid Juselius Foundation and grants from the Research Council for Health within the Academy of Finland and the Finnish Medical Society Duodecim.

First and foremost my gratitude goes to my two supervisors, professors Kari Majamaa and Irina Alafuzoff. Of particular note is their enthusiastic and unprejudiced attitude towards the subject of this thesis, which is a substantial diversion from the core-work of their groups. It is due to the vast knowledge-base of these two scientists that it was possible to seize the significance of the results of what seemed at the time like a failed lab-experiment, but what then grew into this thesis. It has been a privilege to work with these two, rather contrasting, personalities.

Similarly, professors Matti Hillbom and Hilikka Soininen are gratefully acknowledged as the heads of the departments in which I worked for creating an environment in which individual research ideas can be hypothesized and tested according to the best scientific practises. Professor Veli-Matti Kähäri and head of neuropathology Safa Al-Sarraj are sincerely appreciated for their valuable commenting as pre-examiners.

The confidence Dr Terttu Särkioja and Dr Helena Autio-Harmanen showed in the, frankly, dubious early stages of my research work and the willingness to provide time, thought, tissue and facilities has been absolutely indispensable. It seems that particularly time in the competitive and driven academic environment has become a rare commodity indeed. I am also indebted to Drs Johanna Veijola and Maria Pikkarainen for the hands-on supervising of the day-to-day work involved in this thesis. Similarly, the expert technical guidance I have received from Anja Heikkinen, Pirjo Keränen, Irma Moilanen, Heli Auno and Tarja Kauppinen has been fundamental in achieving the skills necessary to produce results in any past or future research project. Significant secretarial help has been provided by Tiia Knuutila at various stages of this work, for which I am extremely grateful.

Within the scope of this thesis I have had the privilege to collaborate with a wide variety of scholars, representing various disciplines and medical specialities. I wish to thank professor Leena-Bruckner-Tuderman, docents Kaisa Tasanen-Määttä and Riitta Miettinen, and Drs Silke C. Hofmann, Tiina Hurskainen, Riitta Miettinen, Tiina Suuronen and Päivi Hartikainen for the expertise they were willing to share with me as co-authors.

My clinical training has coincided with completing this thesis. This challenging administrative equation has been solved by my boss, docent Markku Eronen, for which I am extremely thankful. His pro-research attitude promises a lot in the continual development of research activity at Vanha Vaasa Hospital and psychiatric science in general. Thanks are also due to all my other colleagues at Vaasa, particularly my immediate superior Dr Erkki Kivilinna, who have been kind enough to take on my clinical responsibilities when I was caught up in research dilemmas (and no, research-leave is not really a holiday). I also want to single out Dr Kyösti Väisänen and thank him for the far-ranging intellectual, and geographical, excursions that comprise his particular brand of clinical supervision. Other scholars and teachers that have affected me with their intellectual attitudes are professors Howy Jacobs and Markku Ryytänen, and lecturer Richard W. Goymer: thank you.

Finally, I thank my father Reijo Seppänen and brother Mikael Seppänen for helping me revise when I decided that natural science was not a complete waste of time after all and my mother Ann Seppänen for all that mothers are usually thanked for plus excellent language consultancy. Anna: thank you for Onni and Hilla and my apologies for all the forgotten birthdays.

Vaasa, October 2011

Allan Seppänen

## List of the original publications

This dissertation is based on the following original publications, referred to in the text by the Roman numerals I-IV:

- I Seppänen A, Autio-Harminen H, Alafuzoff I, Särkioja T, Veijola J, Hurskainen T, Bruckner-Tuderman L, Tasanen K, Majamaa K. Collagen XVII is expressed in human CNS neurons. *Matrix Biol* 25(3): 185-188, 2006.
- II Seppänen A, Suuronen T, Hofmann SC, Majamaa K, Alafuzoff I. Distribution of collagen XVII in the human brain. *Brain Res* 1158: 50-56, 2007.
- III Seppänen A, Miettinen R, Alafuzoff I. Neuronal collagen XVII is localized to lipofuscin granules. *Neuroreport* 21(17): 1090-1094, 2010.
- IV Seppänen A, Pikkarainen M, Hartikainen P, Hofmann SC, Majamaa K, Alafuzoff I. Expression of collagen XVII and ubiquitin-binding protein p62 in motor neuron disease. *Brain Res* 1247:171-177, 2009.

The publications were adapted with the permission of the copyright owners.



# Contents

<b>1 INTRODUCTION</b> .....	<b>1</b>
<b>2 REVIEW OF THE LITERATURE</b> .....	<b>2</b>
2.1 Collagens .....	2
2.2 Collagens in the nervous system .....	2
2.3 Collagen involvement in neurological disorders .....	6
2.4 Collagen XVII .....	6
2.4.1 Collagen XVII in skin disease .....	8
2.4.2 Bullous pemphigoid and neurological disorders .....	8
2.5 Visualization of neuronal lesions in neurodegenerative diseases .....	10
2.5.1 p62.....	11
<b>3 AIMS OF THE STUDY</b> .....	<b>12</b>
<b>4 MATERIALS AND METHODS</b> .....	<b>13</b>
4.1 Summary .....	13
4.2 Human brain samples .....	13
4.2.1 Samples of normal brain obtained at forensic autopsy (I).....	13
4.2.2 Samples of normal brain obtained at clinical autopsy (II, III).....	15
4.2.3 Motor neuron disease- related samples obtained at clinical autopsy (IV).....	15
4.3 In situ hybridisation .....	16
4.4 RT-PCR.....	16
4.5 Immunohistochemistry .....	17
4.5.1 Antibodies .....	17
4.6 Western blot.....	19
4.7 Electron microscopy .....	19
4.7.1 Pre-embedding method.....	19
4.7.2 Post-embedding method .....	19
4.7.3 Analysis.....	20
4.8 Ethical aspects .....	20
<b>5 RESULTS</b> .....	<b>21</b>
5.1 Collagen XVII RNA is expressed in neurons of the human brain (I).....	21
5.2 Collagen XVII is widely expressed in the human brain (I, II) .....	22
5.3 The intraneuronal localisation of collagen XVII is to lipofuscin granules (III) .....	24
5.4 Motor neuron disease does not alter the expression of neuronal collagen XVII (IV).....	26
<b>6 DISCUSSION</b> .....	<b>27</b>
6.1 The strengths and limitations of the study .....	27
6.2 The possible role of collagen XVII as a common antigen in neurological and immunobullous skin disorders .....	28
6.3 Lipofuscin.....	29

6.4 p62 and clinical presentation in MND.....	30
<b>7 CONCLUSIONS.....</b>	<b>31</b>
<b>8 REFERENCES.....</b>	<b>32</b>
<b>APPENDIX: ORIGINAL PUBLICATIONS I-IV</b>	

# Abbreviations

ADAM	a disintegrin and a metalloprotease
ALS	amyotrophic lateral sclerosis
BP	bullous pemphigoid
BPAG1	bullous pemphigoid antigen 1, dystonin, BP230
BPAG2	bullous pemphigoid antigen 2, collagen XVII, BP180
CA	cornu ammonis- region in hippocampus
CLAC	collageneous Alzheimer amyloid plaque component
CNS	central nervous system
ELISA	enzyme-linked immunosorbent assay
FTLD-u	frontotemporal lobar degeneration with ubiquitinated inclusions
HE	hematoxylin-eosin
IHC	immunohistochemistry
IR	immunoreactive, immunoreactivity
MND	motor neuron disease
mRNA	messenger ribonucleic acid
NCI	neuronal cytoplasmic inclusions
p62	ubiquitin binding protein p62, sequestome 1
RNA	ribonucleic acid
RT-PCR	reverse transcriptase-polymerase chain reaction
TDP-43	TAR DNA-binding protein 43



# 1 Introduction

Collagens have generally been overlooked for roles in the brain since fibrillar collagens, the best known and most widely studied example of collagens, are not present in the mature central nervous system (CNS). However, over the last decade it has become increasingly apparent that collagens are not merely structural proteins giving strength to tissue but bio-active molecules with a dynamic role within the CNS (Fox 2008, Hubert et al. 2009).

Previously, collagen XVII has primarily been studied in relation to blistering skin diseases. It is abundantly expressed in the skin, which is ectodermal in origin, as is the nervous system. This common ontogenetic background could be one of the reasons behind the often noted connection between psychiatric and dermatological symptomology (Locala 2009) and explain neuro-dermatological disease associations via immunological pathways to common antigens (Sperner-Unterweger 2005). Based on numerous case-, animal and epidemiological studies, collagen XVII could be one of the most interesting putative antigens common to both dermatological and neurological disease (see for example Claudepierre et al. 2005, Langan et al. 2011, Li et al. 2009, Stinco et al. 2005).

In this thesis, collagen XVII was studied in the mature human brain, using brain tissue samples obtained at autopsy and an array of standard molecular and histological research methods, such as RT-PCR, immunohistochemistry and electron microscopy. The associated literature review provides an overview of the current evidence pointing toward immunologically mediated neuro-dermatological interactions in various pathological states.

## 2 *Review of the literature*

### 2.1 COLLAGENS

Collagens, the most abundant protein in the human body (Myllyharju and Kivirikko 2004), and, in fact, on earth (Buehler 2006), are a family of extracellular or transmembrane proteins made up of three polypeptide strands called  $\alpha$ -chains. These three chains, which in most collagen types are identical (Kadler et al. 2007), twist together with the aid of hydrogen bonds in order to create a triple helix. The final superstructural folding of the triple helix in each type of collagen is determined by the  $\alpha$ -chains' distinguishing type of Gly-X-Y repetition, where X and Y can represent any amino acid but Y is often proline or 4-hydroxyproline. Some collagens have an uninterrupted stretch of Gly-X-Y triplets whereas some, such as collagen XVII, present with several stretches, interrupted by non-collagenous sequences (Hubert et al. 2009). All collagens have non-collagenous domains at their N- and C-termini (Kadler et al. 2007).

That having been said, there is no universally accepted definition for a collagen. The distinction between proteins accepted into the collagen family and other proteins with collagenous, triple-helical domains is blurred (Kadler et al. 2007). The current literature accepts that there are 29 collagen types, although it has been argued that type XXIX is not, in fact, a distinct collagen subtype but a variant of collagen VI (Fitzgerald et al. 2008). In any case, the collagens differ from each other significantly in size, structure and function (Hubert et al. 2009). Because of their ability to form superstructures, the collagens can be further divided into seven subfamilies, based on their varying functional roles (Table 1). The role collagens are best known for is forming fibrils and assembling into elongated fibres in the extracellular matrix in order to add structural strength to connective tissues (Fox 2008).

### 2.2 COLLAGENS IN THE NERVOUS SYSTEM

In the nervous system, collagens have traditionally been thought to occupy a marginal position, such as the basement membrane in the blood-brain barrier between vascular and neural tissues (Cardoso et al. 2010), the meninges (Sajanti et al. 1999) and around sensory end organs such as inner ear hair cells, skin receptors and muscle spindles (Cueva et al. 2007, Russell et al. 2007). In mature brain tissue itself, fibrillar collagen is absent (Hubert et al. 2009).

However, collagens are not merely structural molecules, but bio-active adhesion molecules. Indeed, the non-structural activities of collagens within the nervous system have become more of a focus of interest in the last decade, as many recently discovered collagens are not assembled into fibers (Kadler et al. 2007, Myllyharju and Kivirikko 2004) and some of them are expressed by neurons themselves (Claudepierre et al. 2005, Hashimoto et al. 2002, Sund et al. 2001). Functions such as establishment of brain architecture (Sertie et al. 2000), neuronal differentiation (Ali et al. 1998), regulation of axonal outgrowth (Schneider and Granato 2006) and targeting (Xiao and Baier 2007) and synaptic differentiation (Fox et al. 2007) have been attributed to various types of collagen. In fact, a role in the CNS, albeit often transient, has been identified for nearly every type of collagen during some phase of CNS development (Table 1). Thus, collagens are now thought to have a decisive role in various aspects of neural maturation (Fox 2008, Heffron et al. 2009).

*Table 1.* The 29 collagen types and their division into seven subfamilies. Adapted from the following reviews: Hubert et al. (2009), Fox (2008) and Kadler (2007).

<b>Type</b>	<b>Major expression site(s)</b>	<b>Expression and/or role in the nervous system</b>	<b>References</b>
1. Fibril-forming collagens			
Collagen I	Bone, skin, tendon, arteries, intestine, tendon, ligament	Expressed in the meninges. Neurologic defects in osteogenesis imperfecta. Intracranial aneurysms associated with certain COL1A2 polymorphisms.	(Charnas and Marini 1995, Ruigrok and Rinkel 2008, Sajanti et al. 1999)
Collagen II	Cartilage	Expressed in the brain during embryogenesis.	(Leung et al. 1998)
Collagen III	Co-distributes with type I except absent in bone and tendon	Expressed in the meninges.	(Myllyharju and Kivirikko 2001, Sajanti et al. 1999)
Collagen V	Cornea, co-distributes with type I	Expressed in Schwann cells, regulates axonal outgrowth and Schwann cell migration.	(Chernousov et al. 2001)
Collagen XI	Co-distributes with type II	Expressed in the brain during embryogenesis.	(Lui et al. 1995)
Collagen XXIV	Bone, cornea	None known.	(Matsuo et al. 2008)
Collagen XXVII	Cartilage in adult, varying tissues during embryogenesis	mRNA expressed in brain during embryogenesis.	(Boot-Handford et al. 2003, Plumb et al. 2007)
2. Beaded-filament forming collagens			
Collagen VI	Most connective tissues and muscle	Regulates Schwann cell differentiation.	(Vitale et al. 2001)
Collagen XXVI	Testis, ovaries	None known.	(Sato et al. 2002)
Collagen XXVIII	Peripheral nervous system	Basement membrane around Schwann cells.	(Veit et al. 2006)
Collagen XXIX	Skin, lung, the gastrointestinal tract	None known.	(Soderhall et al. 2007)

3. Network-forming collagens			
Collagen IV	Ubiquitous tissue distribution in basement membranes	Col4a1 mutations cause porencephaly and cerebral vasculopathy. Present in pia mater and subependymal basement membrane. Role in neuronal differentiation.	(Ali et al. 1998, Gordon and Hahn 2010, Gould et al. 2005, Lanfranconi and Markus 2010, Urabe et al. 2002)
Collagen VIII	Eye, skin , glomeruli	Expressed in meninges and spinal cord. Expressed in blood vessels of various kinds of brain tumors.	(Gordon and Hahn 2010, Kapoor et al. 1988, Paulus et al. 1991)
Collagen X	Hypertrophic cartilage	None known.	(Gordon and Hahn 2010)
4. Fibril-associated collagen with interrupted triple helices and related collagens			
Collagen IX	Co-distributes with type II	Possibly contributes to segmentation of peripheral nervous system, expressed by meninges.	(Ring et al. 1996, Ring et al. 1995)
Collagen XII	Co-distributes with type I	Present in meninges during development.	(Berthod et al. 1997, Oh et al. 1993, Walchli et al. 1994)
Collagen XIV	Co-distributes with type I	Present in nervous tissue during development.	(Berthod et al. 1997, Walchli et al. 1994)
Collagen XVI	Skin, heart, kidney, intestine, ovary, testis, eye, arterial walls and smooth muscles	Low expression in normal brain, strongly upregulated in gliomas.	(Bauer et al. 2011, Grassel et al. 1999, Lai and Chu 1996)
Collagen XIX	Muscle, basement membrane zone	Expressed by interneurons and contributes to formation of hippocampal synapses.	(Su et al. 2010)
Collagen XX	Widespread, especially corneal epithelium	None known.	(Koch et al. 2001)
Collagen XXI	Widespread	Low expression in brain.	(Fitzgerald and Bateman 2001)
Collagen XXII	Tissue junctions	None known.	(Koch et al. 2004)

5. Transmembrane collagens			
Collagen XIII	Neuromuscular junctions, skin	Expressed in nervous system.	(Sund et al. 2001)
Collagen XVII	Epithelium, see text	Expressed in brain and retina, see text.	(Claudepierre et al. 2005)
Collagen XXIII	Lung, cornea, brain, skin, tendon, kidney	Expressed in brain.	(Koch et al. 2006)
Collagen XXV	Amyloid plaques in brain	Associated with Alzheimer's disease pathology.	(Forsell et al. 2010, Hashimoto et al. 2002, Tong et al. 2010)
6. Multiplexin collagens			
Collagen XV	Eye, heart, skeletal muscle, microvessels	Expressed in brain vasculature.	(Muona et al. 2002, T. Sasaki et al. 2000)
Collagen XVIII	Ubiquitous tissue distribution in basement membranes	Mutations responsible for neural tube defects (Knobloch syndrome). Present in amyloid plaques in Alzheimer's disease and brains affected with cerebral malaria and traumatic injury.	(T. Sasaki et al. 2000, Seppinen and Pihlajaniemi 2011, Sertie et al. 2000, van Horsen et al. 2002)
7. Anchoring fibril-forming collagen			
Collagen VII	Skin, cornea and several other epithelial tissues	Expressed in choroid plexus epithelial cells, around the pineal gland and pituitary gland cell nests.	(Paulus et al. 1995, Uitto and Pulkkinen 1996)

The human genes coding for the ca. 40 distinct  $\alpha$ -chains appearing in the different collagens are expressed as, for example, COL1A2, where the first arabic numeral stands for the collagen type and the second for the number of the  $\alpha$ -chain (Myllyharju and Kivirikko 2004).

### 2.3 COLLAGEN INVOLVEMENT IN NEUROLOGICAL DISORDERS

To date, most CNS pathology associated with collagens is developmental in origin, in line with the role that collagens have in neurodevelopment (Table 1, collagens I, IV and XVIII). A notable exception is Alzheimer's disease. In the extracellular plaques present in brains with Alzheimer's disease, a collagenous component called CLAC (collagenous Alzheimer amyloid plaque component) is present (Hashimoto et al. 2002). CLAC is the furin-cleaved extracellular part of the transmembrane collagen XXV, which is expressed by neurons. Interestingly, COL25A1 alleles have been associated with increased risk for Alzheimer's disease (Forsell et al. 2010) and over-expression of the Col25a1- gene in mice leads to Alzheimer's disease-like brain pathology (Tong et al. 2010).

Although these non-structural functions of collagens in the CNS have gained attention recently, fibrillar collagens have also been studied in relation to neurological disease. In fact, cutaneous involvement in the motor neuron disease (MND) amyotrophic lateral sclerosis (ALS) was first suggested as early as 1880, prompted by the lack of bedsores in ALS patients (Charcot 1880). Contemporary clinicians have also noted that the skin of ALS patients feels supple and loses elasticity. When the skin is stretched, it returns only sluggishly to its original position (Ono 2007). Accordingly, several studies have pointed towards cutaneous and collagen involvement in MND. Using light and electron microscopy Ono and colleagues (1998, 1990, 1986) found abnormalities in the amount and diameter of fibril-forming collagens in both the skin and perivascular spaces of the spinal cord in ALS patients, whereas Ono and Yamauchi (1992) showed that there was an increased amount of immature soluble collagen in relation to the duration of illness in the skin of ALS patients. Also, irregularity in dermal collagen fibrils has been reported (Kolde et al. 1996, Provinciali et al. 1994, Watanabe et al. 1987). Furthermore, the amount of collagen-associated amino acids is markedly decreased in the lateral corticospinal tract and the anterior horn of ALS patients (Ono et al. 1999) and the amount of glucosylgalactosyl hydroxylysine, a collagen metabolite, is decreased in the urine of ALS patients (Ono et al. 2001). These findings have suggested that an alteration of collagen metabolism takes place in ALS.

Subarachnoid haemorrhage is followed by a transient increase in the rate of fibrillar collagen synthesis in both the arachnoid and the dura (Sajanti et al. 1999, Sajanti et al. 2000). The leptomeningeal cells and dural fibroblasts have thus a considerable potential for collagen synthesis and can function similarly to fibroblasts in other tissues during wound healing. Highly elevated levels of propeptides of procollagens have also been measured in chronic subdural haematoma (Sajanti and Majamaa 2003).

Collagen aberrations have also been widely studied in relation to intracranial aneurysms and indeed intracranial aneurysms are a typical feature of the heritable connective tissue disorder Ehlers-Danlos syndrome type IV, which involves collagen mutations (Borck et al. 2010). However, although connective tissue alterations have been found in skin biopsies from a minority of patients with intracranial aneurysms without Ehlers-Danlos syndrome (Grond-Ginsbach et al. 2002), no definite linkage between collagen- gene mutations and intracranial aneurysms has been established in the general population (Kuivaniemi et al. 1993, Ruigrok and Rinkel 2008).

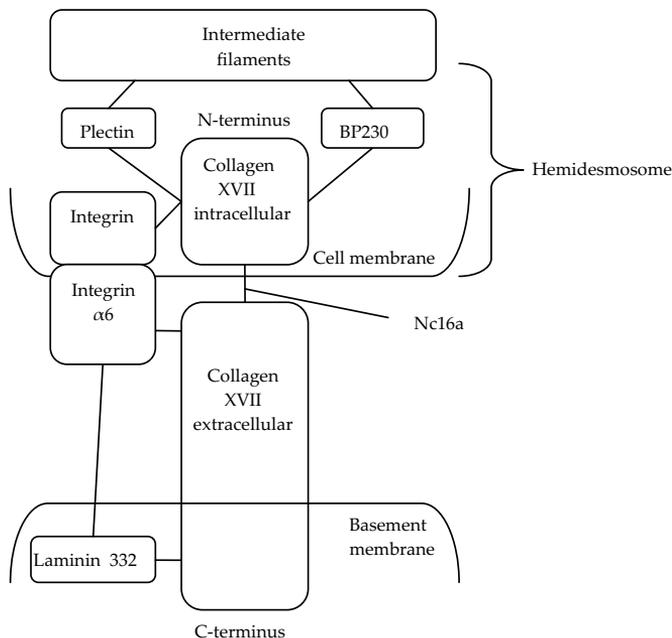
### 2.4 COLLAGEN XVII

Collagen XVII, also known as bullous pemphigoid antigen 2 (BPAG2) or BP180, is one of the four non-fibril-forming transmembrane collagens (Table 1), which function as both

matrix proteins and cell-surface receptors. In addition, there are also other transmembrane proteins, such as ectodysplasins-A and gliomedin that have not been accepted into the family of collagens, despite harbouring collagenous domains. All of these proteins exist in two different forms, one being a type II- oriented transmembrane protein and the other being a shorter soluble molecule derived by post-translational proteolysis (Franzke et al. 2003, Hooper et al. 1997). Interestingly, all the transmembrane collagens have been shown to be present in the nervous system and gliomedin has been implicated in the genesis of the nodes of Ranvier (Maertens et al. 2007).

The structure of collagen XVII, its binding ligands and pathological alterations in various genetic and acquired skin disorders have been described in detail (Franzke et al. 2005, Franzke et al. 2003). It is a homotrimer of three 180 kDa  $\alpha 1$ (XVII) chains, each with a long intracellular N-terminal domain of 466 amino acids, a short transmembrane stretch of 23 amino acids and an extracellular C-terminus of 1008 amino acids (Giudice et al. 1992). Collagen XVII is known to be a structural component of hemidesmosomes, which mediate adhesion of epidermal keratinocytes and certain other epithelial cells to the underlying basement membrane.

The intracellular component of collagen XVII interacts with the  $\beta 4$ -integrin subunit, plectin, and BP230 (Hopkinson et al. 1998, Hopkinson and Jones 2000) to form a stable attachment of hemidesmosomes to keratin intermediate filaments within the cell (Figure 1). The 120-kDa ectodomain of collagen XVII binds to both the  $\alpha 6$  integrin subunit (Hopkinson et al. 1995) and laminin 332, previously known as laminin 5 (Marinkovich 2007, Tasanen et al. 2004), and is constitutively shed from the cell surface by the metalloproteases ADAM 9 and ADAM 10 (Franzke et al. 2009), yielding a soluble form of the molecule into the extracellular matrix (Franzke et al. 2002, Schacke et al. 1998). Although the physiological implications of the shedding are not certain, it has been proposed that this allows the anchored cell to detach, migrate and differentiate during morphogenesis and during regeneration in wound healing (Franzke et al. 2005, Tasanen et al. 2004).



*Figure 1.* Schematic representation of collagen XVII and its main ligands attaching intracellular intermediate filaments via hemidesmosomes to the basement membrane.

Interestingly, ADAM 10 is expressed by both oligodendrocytes and developing neurons (Lin et al. 2008), and has several important functional roles in the CNS. These include processing of amyloid precursor protein (Marcello et al. 2007, Rosenberg 2009), involvement in neurogenesis and axon extension (Y. Y. Chen et al. 2007), the establishment of the brain cortex (Jorissen et al. 2010), spine maturation and control of the structure and function of glutamatergic synapses (Malinverno et al. 2010).

Collagen XVII is abundantly expressed in the cell membranes of epithelia. It is particularly copious in the skin (Nishizawa et al. 1993), but is also present in the human ocular cornea and conjunctiva, buccal mucosa, upper oesophagus, placenta, umbilical cord, urine bladder, (Fairley et al. 1995), bronchial epithelia (Michelson et al. 2000), amniotic fluid and fetal membranes (Huilaja et al. 2008) and the ring fibers of the spleen (Määttä et al. 2004).

In the nervous system, collagen XVII has been previously studied using bovine and rat tissue (Claudepierre et al. 2005). Collagen XVII was detected, often co-localizing with its epithelial ligand BPAG1 and complexing with various laminins, in Muller glial cells, photoreceptors and synaptic regions of the retina, and the cerebellum.

#### **2.4.1 Collagen XVII in skin disease**

Lack of collagen XVII or the loss of its function results in diminished epidermal adhesion and skin blistering in response to minimal shearing forces. In non-Herlitz-type junctional epidermolysis bullosa this can be caused by mutations in the collagen XVII gene, COL17A1, leading to rudimentary hemidesmosomes and separation of the basal keratinocytes from the underlying basement membrane (Powell et al. 2005). In the pemphigoids, i.e. bullous pemphigoid, pemphigoid gestationis, linear IgA disease and mucous membrane pemphigoid, the cause can be autoimmunity against collagen XVII. The autoantibodies are primarily directed against two antigenic extracellular regions, namely the NC16a domain (Nishie et al. 2010) and the carboxyterminal domain (Figure 1), although reactivity to other parts has been reported (Patricio et al. 2009, Powell et al. 2005).

Among pemphigoids, bullous pemphigoid is the most frequent, with a reported annual incidence of 43 per million population in the UK (Taghipour et al. 2010) and six to seven cases per million population in France and Germany. It usually affects the elderly, and both sexes are similarly affected. Clinically, BP is characterized by tense blisters, variably associated with severe itching. In most BP- cases serological diagnostics reveal circulating autoantibodies (Leuci et al. 2010), and the examination of the skin by direct immunofluorescence shows linear complement C3 deposition along the basement membrane and in most cases IgG as well (Kirtschig et al. 2010).

In addition to collagen XVII, autoantibodies in bullous pemphigoid can be directed against BP230, also called BPAG1 or dystonin. Interestingly, BP230 has an isoform expressed by neurons and is implicated in dystonia musculorum in mice (Brown et al. 1995). BP230 belongs to the plakin family of cytolinkers and interacts with collagen XVII in hemidesmosomes (Figure 1.), functioning as a cytoskeletal organizer (Brown et al. 1995, Thoma-Uszynski et al. 2004). It is noteworthy that there is no significant nucleotide or amino acid sequence homology between collagen XVII and BP230 (Yamada et al. 1996).

#### **2.4.2 Bullous pemphigoid and neurological disorders**

In line with the findings associating ALS and collagen abnormalities, Chosidow et al. (2000) suggested an association between pemphigoid and ALS by reporting 3 cases of BP and 2 cases of dyshidrosiform pemphigoid, an unusual localised variant of BP, in a population of 168 French ALS patients.

More robust statistical associations between BP and other neurological disorders have also been repeatedly implied (Table 2). Foureur et al. (2001) found neurological disorders in 30 out of 46 consecutive day-unit patients with BP, chief amongst which were senile dementia (17/46) and cerebral stroke (6/46). Likewise, Cordel (2007) found that 123 (36%) of 341 BP patients from French dermatology departments had a neurological disorder: 68 (55%) of these were dementia, primarily Alzheimer's disease followed by vascular dementia, and (52) 42% were cerebral stroke. These findings were repeated by Jedlickova et al. (2010). Their analysis showed that psycho-neurological disease, again primarily cerebral stroke and dementia, was found in 42.7% of 89 BP patients and in 19.1% of controls. Similar figures were reported by Taghipour et al (2010): at least one neurological disease was present in 46% of 90 consecutive BP patients from an immunobullous referral centre, as compared to 11% in controls. Identically, a statistically significant association with BP was found for cerebrovascular disease and dementia. Dementia, or severe cognitive impairment, has since been consistently reported in association with BP in three more studies (Bastuji-Garin et al. 2011, Y. J. Chen, Wu et al. 2011, Langan et al. 2011) and a case-report (Kanda et al. 2010). What is more, a study of 138 elderly subjects with no dermatological symptoms discovered that the presence of anti-collagen XVII antibodies in the serum was significantly correlated with a mini-mental test score of under 24/30, i.e. the cut-off score for dementia (Foureur et al. 2006).

Table 2. Epidemiological studies linking bullous pemphigoid and neurological morbidity.

Reference	Country of study	Type of study	Number of BP cases in study	Neurological disorders associated to BP
Foureur et al., 2001	France	Retrospective case-control	46	Senile dementia/Alzheimer Cerebral stroke
Stinco et al., 2005	Italy	Retrospective	238	Multiple sclerosis Parkinson's disease
Cordel et al., 2007	France	Retrospective	341	Dementia Cerebral stroke Parkinson's disease or parkinsonism
Jedlickova et al., 2010	Czech Republic	Retrospective case-control	89	Dementia Cerebral stroke
Taghipour et al., 2010	UK	Retrospective case-control	90	Cerebrovascular disease Dementia
Langan et al., 2011	UK	Retrospective population based case-control	868	Dementia Parkinson's disease Stroke Epilepsy
Bastuji-Garin et al., 2011	France	Prospective case-control	201	Severe cognitive impairment (MMSE <17) Parkinsons' disease Uni- or bipolar disorder Chronic neuroleptic drug use
Chen Y.J. et al., 2011	Taiwan	Retrospective population based case-control	3485	Dementia Stroke Schizophrenia Epilepsy Parkinson's disease

A retrospective study on the discharge records of all hospitalised patients in a region in northern Italy of ca. 1 200 000 inhabitants during a 5-year period supported an association between bullous pemphigoid, multiple sclerosis and Parkinson's disease (Stinco et al. 2005). In line with these findings, the literature reports several cases of bullous pemphigoid developing in patients with multiple sclerosis (Kirtschig et al. 1995, Simjee et al. 1985, Stinco et al. 2002) and at least one in a patient with Parkinson's disease (Forschner et al. 2002). The association of Parkinson's disease with bullous pemphigoid has been subsequently reported in epidemiological studies by others as well (Bastuji-Garin et al. 2011, Y. J. Chen, Wu et al. 2011, Cordel et al. 2007, Langan et al. 2011). There are also case reports of unilateral BP on the paralysed side of hemiplegic patients (Bunker and Brown 1993, Foureur et al. 2001, Long et al. 1992).

Psychiatric morbidity may also be associated with BP (Wijeratne and Webster 1996). Bastuji-Garin found that unipolar or bipolar mood disorders and the use of psycholeptics, particularly neuroleptics, were a risk factor for BP in the elderly (2011, 1996). One large study has also associated schizophrenia with BP in females (Y. J. Chen, Wu et al. 2011).

Although BP usually affects people over 65 years of age, cases among younger people are not unheard of. Interestingly, a retrospective study of 74 patients with BP before 60 years of age found neurological disorders in 12 and use of psychiatric drugs in 33 cases (Bourdon-Lanoy et al. 2005) (not included in Table 2 due to age-difference of cases as compared to the other studies).

## **2.5 VISUALIZATION OF NEURONAL LESIONS IN NEURODEGENERATIVE DISEASES**

Neurodegenerative disorders, clinically characterized by dementia and/or motor syndromes, each present with characteristic gross and microscopic lesions in distinct cell populations and anatomical regions of the brain. This enables diagnostic distinction between various diseases and what can be considered as normal variability and changes due to aging (Fjell and Walhovd 2010). For instance, in Alzheimer's disease cortical atrophy is most pronounced in the frontal, parietal and temporal lobes, whereas in corticobasal degeneration depigmentation of the substantia nigra and asymmetric frontoparietal atrophy is typically seen (Tolnay and Probst 2003). In addition to neurodegeneration and resulting gross atrophy, neurodegenerative diseases are characterized by various types of cytoplasmic or intranuclear inclusions. Inclusions are abnormal accumulations of intracellular constituents, primarily ubiquitinated proteins, appearing as discrete bodies within the neuron when visualized in tissue sections using IHC (Alves-Rodrigues et al. 1998, Lowe 1998).

Ubiquitin is a small protein with which other proteins are marked for degradation in the ubiquitin-proteasome degradation pathway, which is one of the two main pathways of degradation of intracellular components. This pathway plays a crucial role in the selective degradation of short-lived regulatory proteins and abnormal proteins that need to be eliminated from the cells (S. Sasaki 2011). The ubiquitinated protein accumulations mentioned above are thought to result from dysfunction of the ubiquitin-proteasome degradation pathway or from structural changes in the protein substrates that prevent their degradation. Impairment in autophagy, the other main degradation pathway, has also been implicated in the formation of the ubiquitinated protein aggregates (Myeku and Figueiredo-Pereira 2011). In contrast to the ubiquitin-proteasome system, autophagy is generally thought to be a less selective degradation system, as it can deal with entire portions of cytoplasmic organelles (S. Sasaki 2011).

In MND, by definition, upper or lower motor neuron lesions with corticospinal tract degeneration are seen. In addition, distinctive neuronal cytoplasmic inclusions (NCI) are

visualized in both sporadic and familial cases of MND by applying ubiquitin or TAR DNA-binding protein 43 (TDP-43) IHC. TDP-43 is a nuclear factor that functions in regulating transcription and alternative splicing, but in sites affected by MND it is mislocalized to the cytoplasm where it aggregates into inclusions (Ito and Suzuki 2011). However, it has become evident that TDP-43-positive inclusions are not present only in MND but also in a high proportion of cases with frontotemporal lobar degeneration with ubiquitinated, tau-negative inclusions (FTLD-U) (Arai et al. 2006). Based on these findings, an appreciation of a common neuropathological spectrum encompassing FTLD-U, FTLD-MND/ALS and MND (King et al. 2010) has developed, dubbed TDP-43 proteinopathies (Arai et al. 2006), which not only affect the motor system, but rather are multisystem proteinopathies (Geser et al. 2008). The common core of the neurodegenerative cascade associated with this disease-entity is thought to be an impairment of the RNA quality control system (Ito and Suzuki 2011).

### 2.5.1 p62

Likewise, the ubiquitin binding protein p62/sequestosome 1 (p62) has been shown to be a component of NCIs in neurodegenerative disorders, including MND (Arai et al. 2003, Furukawa et al. 2004, Mizuno et al. 2006, Parkinson et al. 2006, Seelaar et al. 2007). In fact, there are reports that p62-IHC can visualize TDP-43-negative inclusions within the cerebellum in a proportion of cases across the range of the TDP-43 proteinopathy spectrum (King et al. 2010, Pikkarainen, Hartikainen et al. 2010). In addition, it has been reported that p62-immunoreactive (IR) pathology is seen in MND not only in the pyramidal motor system but more widely in the CNS (Hiji et al. 2008). Because of its wide ability to readily visualize neuropathological inclusions, p62 has been promoted as a “general inclusion stain” (Kuusisto et al. 2008).

Physiologically, p62 is a multidomain signaling adaptor that functions as an organizer of receptor-mediated signalling in cells (Kuusisto et al. 2008). It is best-known for its function in the regulation of atypical protein kinase C in response to cell-surface receptor stimulation. By thus interacting and mediating in many different cellular pathways, such as autophagy and the ubiquitin-proteasome pathway, it has a critical role in the control of cell survival and death (Moscat and Diaz-Meco 2009). p62 mRNA is ubiquitously expressed in human tissues (Joung et al. 1996).

### *3 Aims of the study*

The molecular features and ligands of collagen XVII are well characterized, as is its necessity for epidermal stability. Previous studies have indeed mainly focused on epithelia, while very little is known about collagen XVII in neural tissue. However, earlier studies have implied that collagen XVII could have a functional and neuropathological role in the human central nervous system.

The aims of the study were:

1. To study whether or not collagen XVII is expressed in the human central nervous system (study I).
2. To define in which cells and anatomical regions in the human brain collagen XVII is expressed (study II).
3. To define the intraneuronal location of collagen XVII (study III).
4. To detect any change in neuronal collagen XVII- expression in motor neuron disease (study IV).

## 4 *Materials and methods*

### 4.1 SUMMARY

A summary of the main materials, methods and purpose of each step in this study is given in Table 3, and the details are described in the following chapters. In short, the materials in this study consisted of both pathological and neuropathologically unimpaired human brain samples, in which the presence of collagen XVII and its RNA were detected with several methods.

### 4.2 HUMAN BRAIN SAMPLES

#### 4.2.1 Samples of normal brain obtained at forensic autopsy (I)

As the first step in the study, ten human brain samples were obtained at autopsy in the Department of Forensic Medicine, University of Oulu. The autopsies were performed as part of the Finnish death investigation procedure and as such the cases represented a wide age group (21-75 years at death) of neuropathologically healthy individuals with a very short agonal period and a low probability of suffering from diseases that could compromise tissue quality.

The brain samples were dissected by a forensic pathologist and obtained within 6-51 hours (median 29h) post-mortem. Deceased that posed a risk of infection or had any neurological diagnoses indicating pre-mortem brain pathology were excluded from the study. Demographics of each subject including gender, age at death, cause of death, mode and rapidity of death and post-mortem delay are given in the original publication (I). IHC detecting astrocytes and microglia detected a small infarct in one case, but all samples fulfilled the quality requirements for molecular and histological studies (Hynd et al. 2003).

At this point of the study the focus was simply whether or not collagen XVII could be detected in the human brain. Therefore two anatomical regions sufficed: the cortex and the basal forebrain. In each case cortical brain samples were taken from both the frontal and temporal lobe. Samples from the amygdaloid complex were obtained in three random cases. The samples were fixed in formalin and embedded in paraffin and consecutive sections of 3-4  $\mu\text{m}$  thickness were cut to be used for routine histochemical stainings, *in situ* hybridisation and immunohistochemistry (IHC). Furthermore, two additional hippocampal and amygdaloid samples were snap frozen to be used for reverse transcriptase-polymerase chain reaction (RT-PCR). Hematoxylin-eosin (HE) and cresyl fast violet stains were used to evaluate the basic histology of the paraffin embedded samples and to identify the neuroanatomical structures.

Table 3. A summary of the main materials and methods of the present study.

Human brain samples from (number of cases)	Presence of neuropathology	Methods	Purpose	Level of analysis	Publication	
Forensic autopsy (n=10)	no	In situ hybridisation	Detection of collagen XVII mRNA	Histological	I	
		RT-PCR	Detection of collagen XVII mRNA	Molecular		
		Sequencing	Detection of collagen XVII mRNA	Nucleotide		
		IHC NC16a polyclonal	Detection of collagen XVII protein	Histological		
Clinical autopsy (n=12)	no	IHC NC16a-3 monoclonal	Detection of collagen XVII protein	Histological	II	
		Western blotting	Detection of collagen XVII protein	Molecular		
		Electron microscopy NC16a polyclonal	Detection of collagen XVII protein	Intracellular		III
		Electron microscopy NC16a-3 monoclonal	Detection of collagen XVII protein	Intracellular		
Clinical autopsy (n=9)	motor neuron disease	Electron microscopy NC16a-1 monoclonal	Detection of collagen XVII protein	Intracellular	IV	
		IHC NC16a -3 monoclonal	Detection of collagen XVII protein	Histological		
		IHC p62	Detection of motor neuron disease related NCI's	Histological		

#### 4.2.2 Samples of normal brain obtained at clinical autopsy (II, III)

In order to study the distribution of collagen XVII more widely in the human brain, 12 cases were chosen from the Kuopio Brain Bank. All subjects, aged from 17 to 78 years at death from cardiovascular causes, lacked any signs of neurological dysfunction. Routine gross- and microscopic neuropathological assessment, using  $\beta$ -amyloid,  $\alpha$ -synuclein and hyperphosphorylated tau- IHC, did not reveal any disease- or age-related neuropathological changes. The deaths had been virtually instantaneous in seven subjects; three subjects had died within 24 h with no evidence of cerebral hypoxia and one subject displayed evidence of final cerebral hypoxia. The mode of death was categorized as previously described (Hynd et al. 2003).

The brains (n=11) were fixed at autopsy with 10% buffered formalin by in situ perfusion via the carotid artery for 1 h in order to achieve consistent fixation throughout the entire brain. The brains were then removed, weighed and fixed in 10% buffered formalin for 23 to 65 days. After fixation, the brains were grossly evaluated, cut into coronal slices and examined for macroscopically detectable lesions. The same neuropathologist dissected all the brains, using a standardized sampling protocol. Brain specimens were taken from cortical, subcortical and subtentorial gray matter structures as follows. Cortical: frontal (Brodmann 9), temporal (Brodmann 22), parietal (Brodmann 39), occipital and motor, or precentral, and insular cerebral cortices and gyrus cinguli and hippocampus, including the subiculum and entorhinal cortex. Subcortical: basal forebrain, including amygdala, basal ganglia and thalamus. Subtentorial: midbrain, including substantia nigra, pons, medulla, cerebellar cortex, vermis and dentatus. All specimens were embedded in paraffin.

For electron microscopy, autopsied tissue from the human brainstem was obtained from a neurologically unimpaired male subject, age at death 68 years. The tissue was cut into 0.5cm thick slices and fixed in 4% formaldehyde (in 0.1M phosphate buffer, pH 7.4) overnight. The slices were then cut at 50 $\mu$ m with a vibratome (Leica VT 100S, Leica Instruments GmbH, Wetzlar, Germany).

#### 4.2.3 Motor neuron disease- related samples obtained at clinical autopsy (IV)

Brain specimens were obtained from nine subjects (age 35-82 years at death) that had shown clinical symptoms of MND. In the neuropathological examination, most of the cases fulfilled the criteria for ALS, i.e. both lower and upper motor neuron involvement was noted. However, a case merited the histopathological diagnosis of progressive muscular atrophy when upper motor neurons were intact. The diagnosis of frontotemporal lobar degeneration with ubiquitinated inclusions and ALS (FTLD-u/ALS) was applied when, in addition to widespread ubiquitin immunoreactive lesions, clinical signs of dementia had been registered. Thus, 7 cases fulfilled the criteria for ALS, whereas one was diagnosed as progressive muscular atrophy and one as FTLD-U/ALS.

All cases were negative for  $\alpha$ -synuclein in substantia nigra. All cases were Braak-staged and the clinical records were re-examined. The one case that had been diagnosed with frontotemporal dementia also had the diagnosis of paranoid schizophrenia. In addition, three other subjects had been diagnosed with a non-psychotic psychiatric disorder. None of the examined cases had any history of skin disorders.

At autopsy, all the brains were fixed and dissected in the same way as the non-neuropathological samples described above. Brain specimens were taken from cortical, subcortical and subtentorial gray matter structures as follows: frontal (Brodmann 9), temporal (Brodmann 22), parietal (Brodmann 39), occipital cortex including calcarine sulcus, up to five specimens from the motor cortex, hippocampus, midbrain including substantia nigra, medulla, cerebellar cortex and spinal cord.

7- $\mu$ m-thick brain tissue sections from all blocks were cut for hematoxylin-eosin and IHC stains. For IHC, the sections were deparaffinized in xylene, rehydrated in graded alcohol series and subjected to epitope unmasking treatments.

### 4.3 IN SITU HYBRIDISATION

Radioactive *in situ* hybridisation was performed as previously described (Hurskainen et al. 1996). In short, the paraffin sections were deparaffinized with xylene and dehydrated. For proteolysis the sections were incubated with proteinase K (100 $\mu$ g/ml). Acetylation was carried out in 0.25% to 0.5% acetic anhydride in 0.1M triethanolamine for 10min. Prehybridisation was carried out for 2 hrs at +50°C. In order to produce the RNA probes, a cDNA fragment covering amino acids 1365-1497 of human collagen XVII ectodomain (Ecto-4) was cloned into pGEM 4Z vector (Promega, Madison, WI, USA) and linearized with suitable restriction enzymes. RNA probes labelled with <sup>35</sup>S-UTP (800Ci/mmol) were transcribed using a riboprobe transcription kit (Promega) (Parikka et al., 2003). The <sup>35</sup>S-UTP-labelled antisense or sense probe (3x 10<sup>6</sup> c.p.m) in 40-50 $\mu$ l hybridisation buffer was applied on each section and the hybridisation was carried out in a humid chamber at +50°C overnight. After posthybridisation washes, the sections were dehydrated in ethanol containing 0.3M ammonium acetate. For autoradiography the slides were dipped into NTB-2 film emulsion (Kodak, New York, NY, USA) and placed in light-tight boxes for 12-14 days. The slides were developed in D-19 (Kodak) developer, fixed in Agefix (Kodak) and counterstained with HE. Corresponding sense probes were always used as negative controls.

### 4.4 RT-PCR

RT-PCR followed by sequencing was performed in order to further confirm the presence of collagen XVII mRNA in the brain. After RNA isolation from the snap-frozen brain samples using TRIzol (Gibco, Invitrogen Co., Carlsbad, CA, USA) 1.5 $\mu$ g of total RNA was reverse transcribed to complementary DNA (cDNA) using M-MLV RT (Finnzymes, Helsinki, Finland) with random primers (Promega) and RNase inhibitor (Amersham Biosciences, Little Chalfont, UK). PCR assays were performed in 50 $\mu$ l volumes using 3 $\mu$ l of cDNA, 160  $\mu$ M of each nucleotide, 1 x reaction buffer, 1 U of Dynazyme polymerase (Finnzymes) and 200nM of each specific primer. Collagen XVII and  $\beta$ -actin (control) reactions were done in separate tubes using the following primers: human collagen XVII (GenBank accession number M911669) 22-F (5'-GGAAGCCCTGGCCCTAAAGGTG AC-3') and 22-R (5'-AACCTTCATGCCAGGCTCGCCTGT-3'), and human  $\beta$ -actin BA-F (5'-TGCAGAAGGGAGTCACTGCC-3') and BA-R (5'GTGAACTTTGGGGGATGCTC-3'). The PCR products were analyzed on 1% agarose gels, stained with ethidium bromide (EtBr), and visualized under ultraviolet light. As a control, human HaCaT keratinocyte total RNA was used.

After cloning to pCRII-TOPO vector (Invitrogen, Paisley, UK) the identity of the collagen XVII positive bands were confirmed by sequencing using the collagen XVII primers 22-F and 22-R.

## **4.5 IMMUNOHISTOCHEMISTRY**

### **4.5.1 Antibodies**

The antibodies used in this study are given in Table 4. All three collagen XVII antibodies used in this thesis were a gift from the Department of Dermatology, University of Freiburg, Germany, where they were developed and produced (Hofmann et al. 2009, Schönau 2009). The monoclonal NC16a-1 antibody specifically binds to the N-terminal part of the NC16a domain, whereas NC16a-3 recognizes a more C-terminal region. The NC16a- domain of collagen XVII is the part of the non-collagenous ectodomain that is situated adjacent to the cell membrane (Figure 1). The antibodies used are able to detect both the full-length and shed form of collagen XVII (Hofmann et al. 2009). The epitope of the polyclonal antibody used encompasses that of the monoclonal antibodies (Table 4) and is, as is typical for polyclonal antibodies, more sensitive but less specific than the monoclonal antibodies (Schönau 2009).

Either the PowerVision+ Poly-HRP IHC Detection Kit (ImmunoVision Technologies, Brisbane, CA, USA) (II, IV) or DAKO EnVision Detection Kit, Peroxidase/DAB, Rabbit/Mouse (DakoCytomation, Glostrup, Denmark) (I) was used for detection of coll XVII and for all other stainings, Histostain-Plus Bulk Kit (Zymed Laboratories, San Francisco, CA, USA) was employed. All stainings were visualized with Romulin AEC Chromogen (Biocare Medical, Concord, CA, USA). As a negative control, sections not incubated with the primary antibody were prepared.

Table 4. Antibodies, pretreatments and dilutions.

Detection of	Source/Code	Epitope	Dilution	Pre-treatment
astrocytes	Z0334 (Dako, Glostrup, Denmark)	GFAP	1:4000	MW in CB
$\alpha$ -synuclein	NCL-ASYN (Novocastra, Newcastle upon Tyne, UK)	AA 1-140	1:1000	AC in CB + FA 5 minutes
$\beta$ -amyloid	M0872 (Dako)	AA 9-14	1:100	FA 6 hours
CD68 macrophages	M0814 (Dako)	not given	1:1000	AC in CB
collagen XVII	NC16a-1 <sup>1</sup>	AA 513-523	none in electron microscopy	see text
collagen XVII	NC16a-3 <sup>2</sup>	AA 545-557	none in immunohistochemistry none in Western blot 1:100 in electron microscopy	AC in CB see text
collagen XVII	NC16a polyclonal <sup>3</sup>	AA 490-566	1:1500 in immunohistochemistry 1:100 in electron microscopy	MW in CB see text
hyperphosphorylated tau	90206 (Innogenetics, Gent, Belgium)	Ser202	1:500	none
microglia	M0755 (Dako)	MHC Class II ag	1:500	MW in CB
p62 sequestosome	610832 (BD Biosciences Pharmingen, San Jose, CA, USA)	p62 Ick ligand	1:1000	AC in CB
TDP-43	H00023435-M01 (Abnova, Taipei City, Taiwan)	AA 1-261	1:1000	AC in distilled H <sub>2</sub> O + FA 5 min

<sup>1,2</sup>(Hofmann et al. 2009), <sup>3</sup>(Schumann et al. 2000). AA= amino acid, AC= autoclave 120C 10min, CB= citrate buffer pH 6.0, FA= formic acid 80%, MW= microwave

## 4.6 WESTERN BLOT

For Western blotting, samples of non-neuropathological human temporal cortex grey matter were used. Protein was extracted from the tissue using T-PER™ Tissue Protein Extraction Reagent (Thermo Fisher Scientific, Rockford, IL, USA). As a control, 100µg of protein extract was subjected to collagenase, as described previously (Claudepierre et al. 2005), in order to verify the protein as a collagen. The samples were solubilized in Laemmli-PAGE sample buffer, boiled for four minutes, resolved at 200V on 7.5% SDS-PAGE gel and electrophoretically transferred to ECL-nitrocellulose membrane (Amersham). Blots were blocked with 3% nonfat dry milk in Tris-buffered saline and 0.05% Tween-20. Membranes were probed for 3 hours at room temperature with the undiluted monoclonal collagen XVII antibody NC16a-3, washed and incubated for 2 hours with horse radish peroxidase-conjugated sheep anti-mouse secondary antibody (1:4000, Amersham). Results were detected by Immobilon Western Chemiluminiscent HRP Substrate (Millipore, Billerica, MA, USA) and exposed against SuperRX-film (Fujifilm, Tokyo, Japan).

## 4.7 ELECTRON MICROSCOPY

### 4.7.1 Pre-embedding method

Free-floating sections were washed in 0.1M phosphate buffer and then immersed in a solution containing 25% sucrose and 10% glycerol in 0.05M phosphate buffer. In order to increase the penetration of the antibodies, sections were freeze-thawed three times in liquid nitrogen. To block unspecific binding, sections were incubated in 10% normal goat serum in Tris-buffered saline for 40min. Sections were then incubated with the primary monoclonal antibody for collagen XVII (NC16a-1 undiluted and NC16a-3 1:100 solutions) for two days at 4°C. Negative control sections were incubated without the primary antibody. After extensive washings, sections were incubated with biotinylated horse anti-mouse (1:300, Vector #BA-2000) for 16h at 4°C and finally in avidin-biotinylated horseradish peroxidase complex (ABC, 1:500, Vector #PK-4000) for 3h at room temperature. The immunoperoxidase reaction was developed using 3,3-diaminobenzidine as a chromogen. During immunostaining, sections were washed with 1% normal goat serum in Tris-buffered saline three times for 30min after each antibody solution. After immunostaining, sections were processed for correlated light and electron microscopy. They were treated with 1% osmium tetroxide (OsO<sub>4</sub>) for 1 hour, dehydrated in graded ethanol and propylene oxide, and embedded in Durcupan (AMC) (Fluka, Sigma-Aldrich, St. Louis, MO, USA) between a glass microscope slide and coverslip. After polymerization at 60°C, sections were first studied and photographed under the light microscope. Then the coverslip was removed and the areas of interest were cut out from the section and re-embedded for further ultrathin sectioning. Ultrathin sections were mounted on copper grids.

### 4.7.2 Post-embedding method

Sections were first washed in 0.1M phosphate buffer and then dehydrated in graded ethanol and embedded in LR White (London Resin Company Ltd, London, UK) containing accelerator followed by mounting between a glass microscope slide and coverslip. After polymerization at 60°C, the coverslip was removed and the areas of interest were cut out from the section and re-embedded for ultrathin sectioning. A neighbouring section stained with thionin was used to provide landmarks for the suitable area. Ultrathin sections were cut on nickel grids and stained using the immunogold technique. The staining was carried out on droplets in a humid Petri dish. Sections were first treated with 0.05M glycine in

phosphate buffered saline for 15min and then for 15min in a solution containing 5% bovine serum albumin and 0.1% cold water fish skin gelatine in phosphate buffered saline. After washing in 0.1% BSA-c in phosphate buffered saline, sections were incubated in the primary antibody (monoclonal NC16a-3 1:100 or polyclonal NC16a 1:100 solutions) for 2h at room temperature. Then they were incubated in goat anti-mouse IgG (GAM) conjugated to 15nm gold particles (1:30) (Aurion, Wageningen, The Netherlands) or in protein A (1:30) conjugated to 14nm gold particles, respectively. Antibody dilutions were made in 0.1% BSA-c in phosphate buffered saline containing 0.5% Tween 20. Sections were washed with 1% normal goat serum in Tris-buffered saline six times for 5min after each antibody solution. All ultrathin sections were treated for 30min in uranyl acetate and for two minutes in lead citrate in order to improve the contrast.

#### **4.7.3 Analysis**

The sections were examined in a Jeol (Tokyo, Japan) 1200 EX electron microscope at 80kV. The light microscopy digital micrographs were taken with an Olympus (Tokyo, Japan) DP50 digital camera mounted on an Olympus BX40 microscope. For verifications of autofluorescence, the samples were viewed under fluorescence light. For excitation, bandpass filter 460-490nm was used and emission was detected using BA520IF highpass filter.

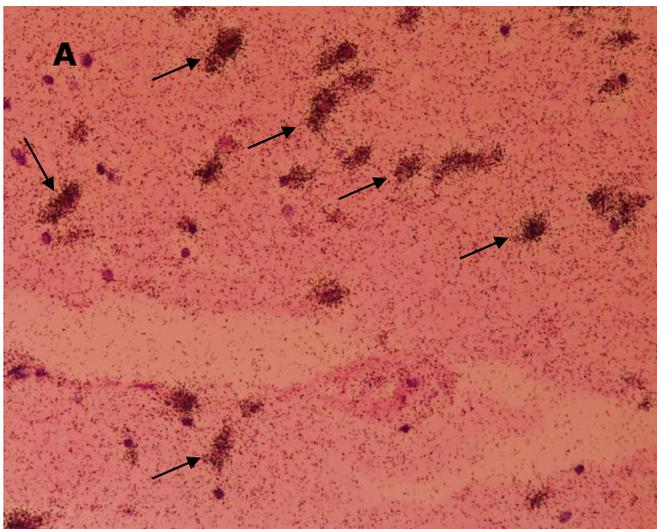
## **4.8 ETHICAL ASPECTS**

This study has been reviewed and approved by the ethics committees of The Northern Ostrobothnian Hospital District and Kuopio University Hospital. It adheres to the Finnish law concerning the use of human tissues for research purposes (*Laki ihmisen elimien, kudoksien ja solujen lääketieteellisestä käytöstä* 2.2.2001/101). This study in no way affected the standard diagnostic procedures for which the autopsies were performed.

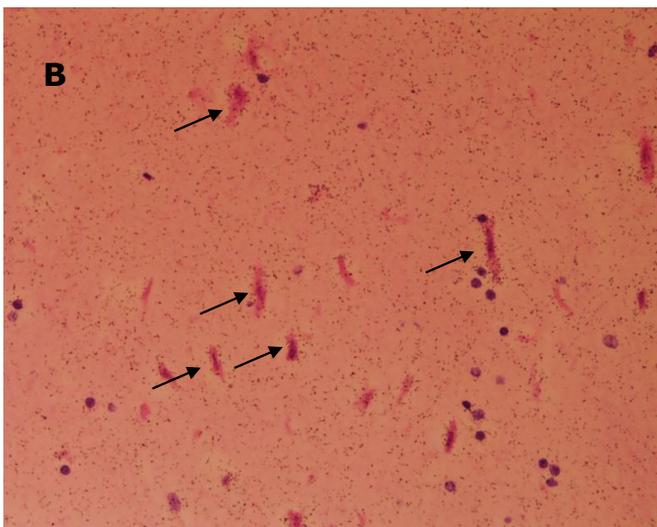
## 5 Results

### 5.1 COLLAGEN XVII RNA IS EXPRESSED IN NEURONS OF THE HUMAN BRAIN (I)

Neuropathologically unimpaired human brain tissue was used for in situ hybridisation, RT-PCR and sequencing. In situ hybridisation results demonstrated that collagen XVII mRNA was present in neurons, predominantly in the pyramidal cells, in all sections of all of the ten forensic cases. The localization was clearly concentrated to soma and proximal axon. The sense probe used as a control detected no signals in any of the brain samples (Figure 2). Using tissue from the hippocampus and amygdala, RT-PCR and sequencing further confirmed the presence of collagen XVII mRNA in the human brain.



*Figure 2. A)* In situ hybridization with a collagen XVII RNA antisense probe in the human hippocampus. The probe has a strong affinity to neurons (some marked by arrows). **B)** The same sample using a sense probe as a control. Note the lack of binding in neurons (some marked with arrows) or elsewhere. Magnification x200.



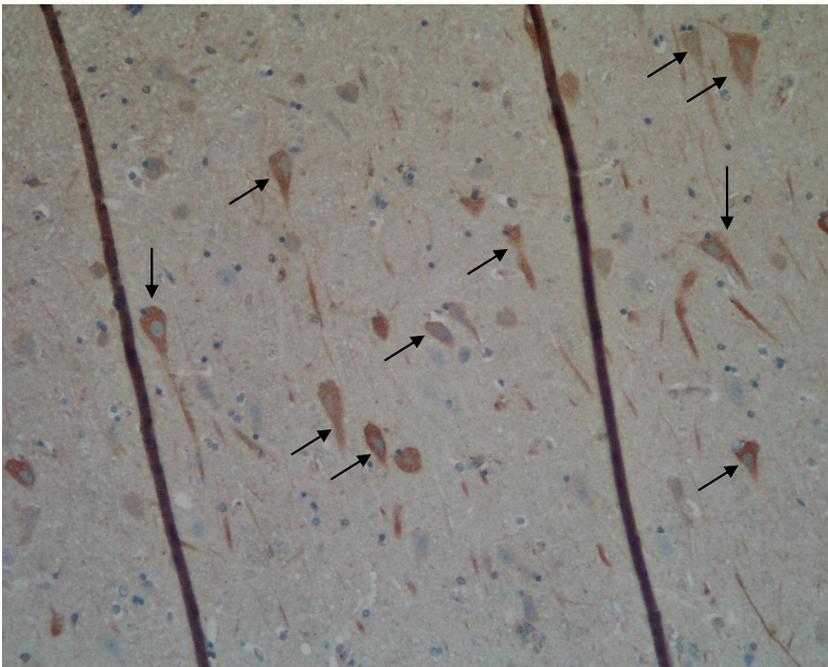
## 5.2 COLLAGEN XVII IS WIDELY EXPRESSED IN THE HUMAN BRAIN (I, II)

Using IHC, collagen XVII was detected in nearly all of the studied brain regions, although the immunoreactivity (IR) did vary markedly from one area to another (see Table 5 for exact details). Areas of consistently prominent IR were the hypoglossal nucleus (nucleus XII), oculomotor nucleus (nucleus III), nucleus basalis of Meynert, supraoptic nucleus, subthalamic nuclei and pyramidal cells of the hippocampal regions of cornu ammonis (CA) 4-2. In the cerebral cortex, staining was strongest in the deeper layers, i.e. layer V (ganglionic layer) but expression varied somewhat from one cortical area to another. IR was most prominent in the motor cortex, which is mainly accounted for by strong staining of Betz cells. In contrast, either none at all or only very faint IR was seen in the granular cells of the hippocampal CA and cerebellar cortex, and in the neurons of the mamillary body and caudatus.

In some brain regions, particularly in the cerebral cortex, basal ganglia, and the cerebellum, collagen XVII-IR varied from one case to another. The differences in IR were not associated with age or gender, and only one clear association with pre-mortem events could be deduced: there was an almost total lack of collagen XVII-IR in the one case that suffered pre-mortem cerebral hypoxia and that displayed numerous ischemic neurons in the brain samples (Table 5, case 11).

On a cellular level, the staining primarily localised to the cytoplasm of the cell body in either a diffuse or a slightly granular pattern (Figure 3), though there was also a light staining of the neuropil. No staining of white matter was seen and neither glial cells nor endothelial cells were labelled. In contrast, the ependymal lining was clearly stained.

Western blotting using human brain tissue failed to recognize the expected 180kDa  $\alpha$ -chain band of collagen XVII. Instead, a single band of ca. 150kDa was detected (original publication II, figure 2). It was subjected to collagenase digestion, which obliterated the band, supporting the authenticity of the antigen as a collagen. Therefore, the detected 150kDa size could be caused by partial degradation of collagen XVII by endogenous proteases during the post-mortem delay.



*Figure 3.* A section of human hippocampus labeled using a polyclonal collagen XVII Nc16a antibody. Note the labeling of the pyramidal neurons (some marked by arrows) throughout the pictured area. Magnification x200.



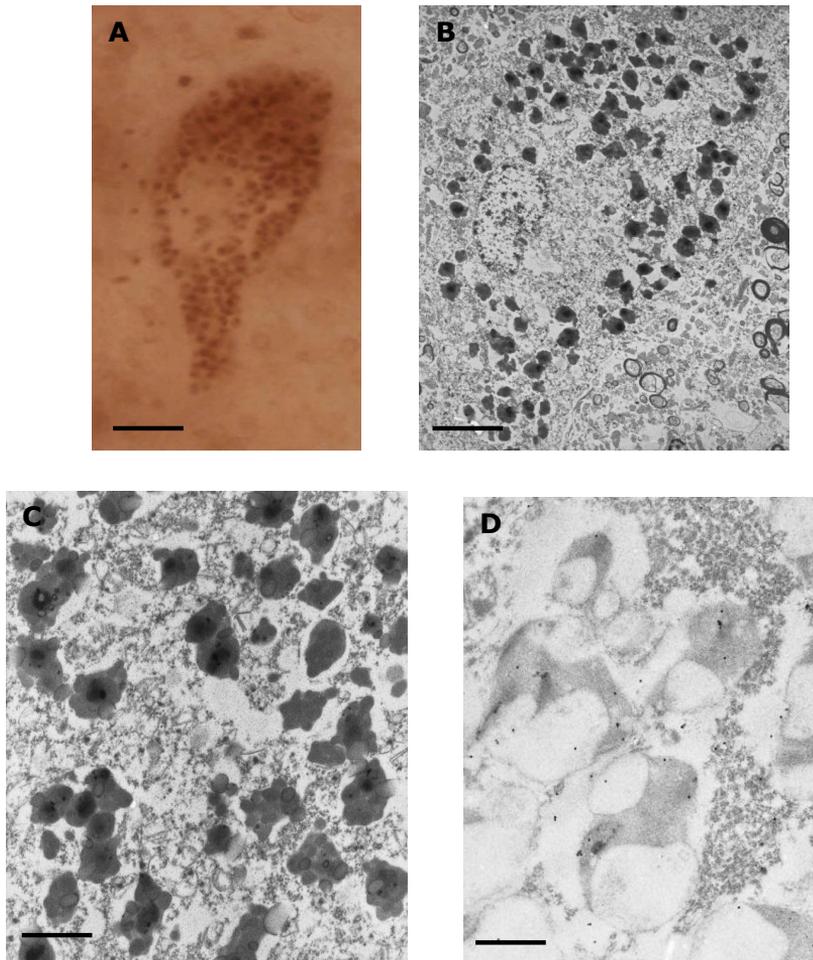
### **5.3 THE INTRANEURONAL LOCALISATION OF COLLAGEN XVII IS TO LIPOFUSCIN GRANULES (III)**

In order to define the intraneuronal localisation of collagen XVII, electron microscopy on human brain samples was utilized. In line with the *in situ* hybridisation and IHC results, collagen XVII- IR was seen in the cell soma and proximal dendrites of large to medium-sized neurons, although usually only in less than 10% of the neuronal population. The IR was concentrated in irregularly-shaped granules around the nucleus. (Figure 4A). Correlated light and electron microscopy revealed that these granules had the appearance of lipofuscin (Figure 4B and C) and they were confirmed to be lipofuscin due to their autofluorescence under fluorescent light.

As is typical for lipofuscin, these granules were membrane-bound particles having two different compartments: one containing more electron-dense, dark substance and one containing more translucent material. However, in contrast with the usual appearance of lipofuscin granules, those cells that were immunopositive for collagen XVII had even darker granules within the darker lipofuscin compartment (Figure 4C). Furthermore, the lighter compartment had lost its translucency and was darker than what is usually seen in lipofuscin.

In order to confirm the presence of collagen XVII within these lipofuscin granules, the immunogold technique was used (Figure 4D). Indeed, gold particles conjugated to the secondary antibody were found to locate to lipofuscin granules in the same way as described above, using both the monoclonal and polyclonal antibodies.

Omission of the primary antibody was used as a control, and resulted in negligible staining and presence of gold particles.



*Figure 4. A)* With the light microscope, collagen XVII-IR is seen as cytoplasmic clusters in the neuron soma. Scale bar 10 $\mu$ m. *B)* Correlated electron microscopy of the neuron shown in A reveals that the immunoreactivity is located to granules having a lipofuscin-like appearance. Scale bar 5 $\mu$ m. *C)* High power electron microscopy shows that within the darker compartment of the lipofuscin particles, there are unusual electron-dense granules. Furthermore, the lighter compartment of lipofuscin is darker than in normal lipofuscin. Scale bar 2 $\mu$ m. *D)* In immunogold- stained sections, gold particles (small black particles) are found both in the dark and light lipofuscin compartments. Scale bar 0,5 $\mu$ m.

#### **5.4 MOTOR NEURON DISEASE DOES NOT ALTER THE EXPRESSION OF NEURONAL COLLAGEN XVII (IV)**

Based on previous work pointing towards a possible role of CNS collagen in the pathogenesis of ALS (see for example (Chosidow et al. 2000, Ono et al. 1998), we decided to study collagen XVII- expression in brain samples affected with MND. However, we found the expression of collagen XVII in MND cases to be the same as in unaffected subjects. It should be mentioned, though, that collagen XVII- IR was seen also in frontal horn  $\alpha$ -motor neurons in the MND- samples, but we have no data of collagen XVII expression in the spinal cord of neurologically unimpaired cases for comparison.

The collagen XVII- IR was widely dispersed in the neuronal cytoplasm as usual, whereas the staining of p62, a robust marker of neuronal inclusion bodies (Kuusisto et al. 2008), was sharp and demarcated to the NCIs and neurites in all 9 subjects. There was no correlation between the intensity of the collagen XVII-IR and the quantity of p62-positive neurites or NCIs (nonparametric Spearman's correlation tests).

Both TDP-43- and p62-IR was seen in the motor cortex, at the level of the medulla in the nucleus hypoglossus, and in the spinal cord in all cases, as expected in MND. However, in addition, p62-IHC visualized NCIs in the frontal cortex, in the hippocampus (both the granular cell layer of the dentate gyrus and CA4 region), and in the cerebellum in subjects with a clinical history of psychiatric morbidity.

## 6 Discussion

### 6.1 THE STRENGTHS AND LIMITATIONS OF THE STUDY

The present study has made use of current standard research methods and therefore relies heavily on their validity.

All the tissue studied has been from humans and obtained at autopsy. In many ways, human brain samples are the most appropriate tissue for studies on brain disorders, but high-quality samples are also the most difficult to obtain. Samples are collected post-mortem, and lack the kind of pre-mortem control that animal studies and cell lines derived from lymphocytes or fibroblasts can be subjected to. For instance, heterogeneity in cause of death, pre-mortem medical interventions, lifestyle habits, such as alcohol and drug consumption, smoking and dietary preferences, can cause difficulties in interpreting results. Also, agonal and post-mortem events, such as rapidity of death and the interval between death and appropriate tissue storage, may influence the results obtained (Konradi et al. 2011).

However, in this study care has been taken to define the quality of the tissues and to ensure that they fulfil the requirements for the methods used (Hynd et al. 2003). For instance, a long fixation time can compromise antigen retrieval and result in false negative findings, but in the clinical tissue samples, where the fixation time was exactly recorded, the maximum time was 65 days, which can be considered short-term fixation (Pikkarainen, Martikainen et al. 2010). In addition, the epitopes probed with the collagen XVII antibodies seemed to be relatively insensitive to agonal and post-mortem changes: no difference in collagen XVII-IR was seen in relation to post-mortem delay, which ranged from 48 to 124 h, or the fixation time. Likewise, no differences in collagen XVII-IR were noted between subjects dying instantaneously or within 24 h after the onset of symptoms but lacking signs of cerebral ischemia. The only noted influential variable was a prolonged mode of death, causing irreversible ischemic damage and depletion of collagen XVII-IR (study II). A notable exception is the Western blot. Here, although recognized by collagen XVII antibodies, the size of the detected protein differed from that expected in keratinocytes (c.a. 150kDa vs. 180kDa). This could be due to post-mortem degradation and, indeed, earlier work has also reported a band differing from the expected size (less than 110kDa) due to apparent degradation (Claudepierre et al. 2005) but no absolute answer can be given within the scope of this thesis. It is worth noting, though, that subsequent work (J. Chen, Li et al. 2011) has been able to demonstrate the full length 180kDa collagen XVII product in the human brain.

In order to ensure specific antibody binding to collagen XVII, three antibodies, targeting different epitopes, have been used. The results were identical, as was the localisation of collagen XVII mRNA using *in situ* hybridisation: all methods localized collagen XVII to the soma and proximal cell extensions.

The autopsied brain material in study III consisted of a single 68-year old male. Because lipofuscin accumulates in the aging brain, and is largely originated from autophagocytosed mitochondria (Terman and Brunk 2006), one is tempted to speculate that in a younger brain the intracellular location could be elsewhere.

Finally, it must be mentioned that the decision to study MND in relation to collagen XVII-expression (IV) was made based on research available at the time (2007-2008). However, subsequent work (Bastuji-Garin et al. 2011, Y. J. Chen, Wu et al. 2011, Jedlickova et al. 2010, Langan et al. 2011, Taghipour et al. 2010) points towards dementia, primarily

Alzheimer's disease, and cerebral stroke having a more probable pathogenetic connection with collagen XVII. Therefore, the fact that these diseases were not studied within this thesis must be considered a limitation.

## **6.2 THE POSSIBLE ROLE OF COLLAGEN XVII AS A COMMON ANTIGEN IN NEUROLOGICAL AND IMMUNOBULLOUS SKIN DISORDERS**

By demonstrating that collagen XVII is present in the human brain, our study raises the possibility of collagen XVII having a role in neurological disorders, particularly in association with subsequent BP. Some authors have discussed the possibility that BPAG1, or BP230, the other antigen targeted in BP, could be the common autoantigen in neurological and dermatological disorders, as variants of BPAG1 are expressed in the nervous system (Garcia-Estevez et al. 2008, Laffitte et al. 2005, Li et al. 2009). Indeed, antibodies in the serum of patients with both BP and neurological disease, primarily cerebrovascular disease, have been shown to recognize proteins corresponding to BPAG1 in mouse brain extract (Li et al. 2009). It is interesting, however, that inspection of the immunoblots in Li and colleagues' study reveals that in one case the serum reacts with a 180 kDa protein, which is compatible with coll XVII, and subsequent work (J. Chen, Li et al. 2011) found that sera of BP-patients with neurological disease reacted with both 230 and 180 kDa antigens in human brain extract, corresponding to BPAG1 and collagen XVII, respectively. Also, immunoblotting of the cerebrospinal fluid of MS patients against BPAG1 has revealed only a low frequency of reactivity (Laffitte et al. 2005). What is more, in reply to Foureur and colleagues (2010), Wieland et al. specify that in their study of collagen XVII and BPAG1 autoantibodies in the serum of 337 individuals with no signs of BP (Wieland et al. 2010), of the 25 found to be positive, 5 had neurologic diseases listed in their medical records. In all but one case, the amount of BP180, i.e. collagen XVII, antibodies, as measured with enzyme-linked immunosorbent assay (ELISA), is greater than that of BP230. These findings underline the possibility that collagen XVII, the primary autoantigen in BP (Thoma-Uszynski et al. 2004), could be the common etiopathogenetic denominator in dermatological and neurological disorders, perhaps in addition to BPAG1. On the other hand, Soni (2009) found evidence of neither collagen XVII nor BPAG1 antibodies in a case of autoimmune encephalopathy with BP, raising the possibility of an antigenic target common to both the skin and CNS which is neither of the above.

In any case, when both BP and neurological disorder are present, neurological disease usually precedes BP (J. Chen, Li et al. 2011, Langan et al. 2011) by months to years (Cordel et al. 2007, Stinco et al. 2005). This supports the idea that neuronal antigen exposure, conceivably via a compromised blood-brain barrier, is causatively involved in subsequent development of BP. In light of this, the hypothesis of immunoglobulin-mediated neurodegeneration in Alzheimer's disease (Bouras et al. 2005) is particularly intriguing, as dementia is the disorder most consistently associated with BP. This hypothesis involves an age-related compromise of the blood-brain barrier (Zeevi et al. 2010) and loss of the "immunological privilege" of the brain. In a murine model of senescence, vascular permeability to the brain has been shown to be particularly prevalent in the hippocampal region (Pelegri et al. 2007), where we have shown strong collagen XVII expression and which is a well-recognized predilection area for Alzheimer's disease-related lesions (Barnes et al. 2009). Notably, mRNA of ADAM 10, the metalloprotease responsible for the cleavage of the extracellular domain of collagen XVII and which is also implicated in processing the amyloid precursor protein (Jorissen et al. 2010), is also detectable in the same anatomical areas as collagen XVII, including the hippocampus (Kärkkäinen et al. 2000).

The other condition most frequently reported in association with BP is cerebrovascular disease, or stroke (Table 2). Of course, etiopathogenetically, this is not a neuronal disease,

but vascular in origin. Therefore, it is interesting that in contrast with neurological disease in general, stroke has been shown to often occur also after the onset of BP (Yang et al. 2011). This could be explained by the fact that the inflammatory state present in BP is not confined to the skin but also involves vascular endothelium (Ameglio et al. 1997), as does the pathogenesis of atherosclerosis, atheroma plaque rupture and thrombosis (Ross 1999). Also, there is some evidence that antiphospholipid antibodies are present in the serum of BP-patients (Echigo et al. 2007). Thus, the BP- associated inflammation, perhaps together with the hypercoagulable state associated with antiphospholipid antibodies, could exacerbate pre-existing atherosclerosis and promote thrombosis and stroke (Yang et al. 2011).

In terms of inflammation, the recent findings linking affective disorder and schizophrenia with BP (Bastuji-Garin et al. 2011, Y. J. Chen, Wu et al. 2011) are interesting, as there is evidence of upregulation of immune response genes in these disorders (Konradi et al. 2011). In fact for schizophrenia, a neuroimmune hypothesis has been debated for decades (Arion et al. 2007). Although the current consensus concerning the inflammatory etiology of schizophrenia involves the idea of a long-lasting consequence of an infective-immune challenge during early brain-development, numerous other explanations have been offered, including autoimmunity towards brain structures (Pandey et al. 1981), particularly in the hippocampus (Ganguli et al. 1993). However, as inflammation is also closely linked with behavioral parameters such as exercise, alcohol abuse, and smoking, as well as with medical conditions including coronary artery disease, obesity and insulin resistance (Goldstein et al. 2009), interpreting the inflammatory findings in psychiatric disease is exceedingly complex (Sperner-Unterweger 2005).

Although there is a case report in which mental retardation has been present with junctional epidermolysis bullosa (Nakar et al. 1992), it is not a common feature (Pfundner and Lucky 2008). Genetic studies concerning collagen XVII involvement in CNS- pathology are beyond the scope of this thesis.

### **6.3 LIPOFUSCIN**

Lipofuscin is an undegradable autofluorescent pigment that accumulates in aging, postmitotic cells, including neurons, throughout the animal kingdom (Double et al. 2008, Sulzer et al. 2008). It is usually considered waste material, the accumulation of which has a detrimental effect on various cellular functions, particularly by interfering with lysosomal degradation (Brunk and Terman 2002). Although lipofuscin is produced by nearly all brain cells, it is, similarly to collagen XVII, most abundant in larger neurons and in brain areas involved in movement (Double et al. 2008).

Physiologically, lipofuscin can be thought of as the end-product of incomplete degradation of various cytoplasmic substances through autophagy. Autophagy is an evolutionarily conserved catabolic process, the function of which is to maintain cellular homeostasis by, for instance, clearing out damaged intracellular organelles and macromolecules, eliminating pathogens and suppressing tumour growth (Kang et al. 2011). The final breakdown of these intracellular substances to simple, recyclable molecules takes place within lysosomes by various degrading enzymes, such as hydrolases and proteases. However, due to intra- and intermolecular cross-links being formed by oxidation products of various cellular components, insoluble and non-degradable material accumulates and aggregates over time: this material is referred to as lipofuscin (Jung et al. 2007). The primary constituents of neuronal lipofuscin are oxidatively modified protein (30-58%) and lipid clusters (19-51%) (Sulzer et al. 2008, Terman and Brunk 2004), which largely originate from oxidatively damaged mitochondria (Terman and Brunk 2006).

That having been said, it has been argued that there is not just one type of lipofuscin, but that this term encompasses a variety of differing lipopigments (Seehafer and Pearce 2006).

Indeed, the presence of collagen XVII in a specific, ultrastructurally definable type of lipofuscin granule supports this idea and may be helpful in the process of further exploring and defining the variety of neuronal lipopigments.

Although our results show that collagen XVII expression was confined to lipofuscin, it is tempting to speculate that in a younger brain the intracellular location of collagen XVII could be different. It is worth noting, though, that both BP and the neurological morbidity most strongly associated with BP, particularly dementia, are associated with aging, as is the accumulation of neuronal lipofuscin. However, what the function of neuronal collagen XVII is and whether or not it has a role in any neuropathological or –physiological processes, we have not been able to discover within the scope of this thesis. That notwithstanding, the suggestion that lipofuscin actually benefits the neuron by incorporating potentially damaging metabolites (Stojanovic et al. 1994) is of great interest. Indeed, neuronal populations that regularly contain abundant lipofuscin are among the most resistant to age-related degenerative changes (Gray and Woulfe 2005) and, more specifically, there is evidence that lipofuscin plays a neuroprotective role in Parkinson’s disease (Kanaan et al. 2007).

#### **6.4 P62 AND CLINICAL PRESENTATION IN MND**

During the last decade, there has been increasing awareness of the overlap between frontotemporal dementia and ALS (Lillo et al. 2010). Previous research has suggested that an alteration in collagen metabolism may take place in ALS (Ono et al. 1998, Ono et al. 1999, Ono et al. 2001), that BP may be associated with ALS (Chosidow et al. 2000) and several studies have associated dementia with BP (Table 2). However, we found no evidence of a change in collagen XVII- expression in either brains from ALS- or ALS with frontotemporal dementia- patients.

On the other hand, we found that in MND p62-IR inclusions were present not only in the pyramidal motor system but more widely in the CNS. Interestingly, the distribution of p62-IR varied according to clinical presentation: psychiatric disorders had been diagnosed only in the four subjects with p62-IR NCIs in the frontal cortex, hippocampus and cerebellum. Also, the only case in study IV with the diagnosis of frontotemporal dementia and ALS also had a diagnosis of paranoid schizophrenia, which supports the findings by Lillo et al. (2010) that psychotic symptoms are relatively common in frontotemporal dementia-patients who go on to develop ALS. However, the other psychiatric diagnoses in our cases were non-psychotic and all differed from each other (please see article IV, Table 3 for details), so no absolute conclusions should be made based on these findings. It can merely be speculated whether some of the diagnosed psychiatric disorders, such as personality disorder, in fact represent the prodromal phase of a psychotic or dementing disease. In any case, our findings support the idea to think of ALS not only as a disease affecting the pyramidal motor system, but as a multisystem proteinopathy with a wide clinicopathological spectrum (Geser et al. 2008), including psychiatric morbidity.

## 7 *Conclusions*

A number of earlier animal, case, and epidemiological studies have pointed towards the possibility of collagen XVII having a physiological and pathological role in the human central nervous system. In this thesis our knowledge of collagen XVII in humans was expanded by the following conclusions:

1. Collagen XVII is expressed in human CNS neurons and is widely distributed in the human brain.
2. Neuronal collagen XVII is localised to lipofuscin granules.
3. A change in the expression of collagen XVII in the human brain is not an immunohistochemically detectable feature of motor neuron disease.
4. The presence of p62- positive inclusions outside the motor system in motor neuron disease could be a marker for psychiatric morbidity.

## 8 References

Ali SA, Pappas IS, Parnavelas JG. 1998. Collagen type IV promotes the differentiation of neuronal progenitors and inhibits astroglial differentiation in cortical cell cultures. *Brain Res Dev Brain Res* 110(1): 31-38.

Alves-Rodrigues A, Gregori L, Figueiredo-Pereira ME. 1998. Ubiquitin, cellular inclusions and their role in neurodegeneration. *Trends Neurosci* 21(12): 516-520.

Ameglio F, D'Auria L, Cordiali-Fei P, Mussi A, Valenzano L, D'Agosto G, Ferraro C, Bonifati C, Giacalone B. 1997. Bullous pemphigoid and pemphigus vulgaris: correlated behaviour of serum VEGF, sE-selectin and TNF-alpha levels. *J Biol Regul Homeost Agents* 11(4): 148-153.

Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, Mann D, Tsuchiya K, Yoshida M, Hashizume Y, Oda T. 2006. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* 351(3): 602-611.

Arai T, Nonaka T, Hasegawa M, Akiyama H, Yoshida M, Hashizume Y, Tsuchiya K, Oda T, Ikeda K. 2003. Neuronal and glial inclusions in frontotemporal dementia with or without motor neuron disease are immunopositive for p62. *Neurosci Lett* 342(1-2): 41-44.

Arion D, Unger T, Lewis DA, Levitt P, Mirnics K. 2007. Molecular evidence for increased expression of genes related to immune and chaperone function in the prefrontal cortex in schizophrenia. *Biol Psychiatry* 62(7): 711-721.

Barnes J, Bartlett JW, van de Pol LA, Loy CT, Scahill RI, Frost C, Thompson P, Fox NC. 2009. A meta-analysis of hippocampal atrophy rates in Alzheimer's disease. *Neurobiol Aging* 30(11): 1711-1723.

Bastuji-Garin S, Joly P, Lemordant P, Sparsa A, Bedane C, Delaporte E, Roujeau JC, Bernard P, Guillaume JC, Ingen-Housz-Oro S, Maillard H, Pauwels C, Picard-Dahan C, Dutronc Y, Richard MA. 2011. Risk Factors for Bullous Pemphigoid in the Elderly: A Prospective Case-Control Study. *J Invest Dermatol* 131(3): 637-643.

Bastuji-Garin S, Joly P, Picard-Dahan C, Bernard P, Vaillant L, Pauwels C, Salagnac V, Lok C, Roujeau JC. 1996. Drugs associated with bullous pemphigoid. A case-control study. *Arch Dermatol* 132(3): 272-276.

Bauer R, Ratzinger S, Wales L, Bosserhoff A, Senner V, Grifka J, Grassel S. 2011. Inhibition of Collagen XVI Expression Reduces Glioma Cell Invasiveness. *Cell Physiol Biochem* 27(3-4): 217-226.

- Berthod F, Germain L, Guignard R, Lethias C, Garrone R, Damour O, van der Rest M, Auger FA. 1997. Differential expression of collagens XII and XIV in human skin and in reconstructed skin. *J Invest Dermatol* 108(5): 737-742.
- Boot-Handford RP, Tuckwell DS, Plumb DA, Rock CF, Poulsom R. 2003. A novel and highly conserved collagen (pro(alpha)1(XXVII)) with a unique expression pattern and unusual molecular characteristics establishes a new clade within the vertebrate fibrillar collagen family. *J Biol Chem* 278(33): 31067-31077.
- Borck G, Beighton P, Wilhelm C, Kohlhase J, Kubisch C. 2010. Arterial rupture in classic Ehlers-Danlos syndrome with COL5A1 mutation. *Am J Med Genet A* 152A(8): 2090-2093.
- Bouras C, Riederer BM, Kovari E, Hof PR, Giannakopoulos P. 2005. Humoral immunity in brain aging and Alzheimer's disease. *Brain Res Brain Res Rev* 48(3): 477-487.
- Bourdon-Lanoy E, Roujeau JC, Joly P, Guillaume JC, Bernard P, Prost C, Tancrede-Bohin E, Delaporte E, Picard-Dahan C, Albes B, Bedane C, Doutre MS, Chosidow O, Lok C, Pauwels C, Chevrand-Breton J, Sassolas B, Richard MA. 2005. [Bullous pemphigoid in young patients: a retrospective study of 74 cases]. *Ann Dermatol Venereol* 132(2): 115-122.
- Brown A, Bernier G, Mathieu M, Rossant J, Kothary R. 1995. The mouse dystonia musculorum gene is a neural isoform of bullous pemphigoid antigen 1. *Nat Genet* 10(3): 301-306.
- Brunk UT, Terman A. 2002. Lipofuscin: mechanisms of age-related accumulation and influence on cell function. *Free Radic Biol Med* 33(5): 611-619.
- Buehler MJ. 2006. Nature designs tough collagen: explaining the nanostructure of collagen fibrils. *Proc Natl Acad Sci U S A* 103(33): 12285-12290.
- Bunker CB, Brown E. 1993. Unilateral bullous pemphigoid in a hemiplegic patient. *Br J Dermatol* 129(4): 502.
- Cardoso FL, Brites D, Brito MA. 2010. Looking at the blood-brain barrier: molecular anatomy and possible investigation approaches. *Brain Res Rev* 64(2): 328-363.
- Charcot JM. *Leçon sur le maladies du système nerveux faites à la Salpêtrière* (Vol. II). Paris, France 1880.
- Charnas LR, Marini JC. 1995. Neurologic profile in osteogenesis imperfecta. *Connect Tissue Res* 31(4): S23-26.
- Chen J, Li L, Zeng Y, Xu H, Song Y, Wang B. 2011. Sera of Elderly Bullous Pemphigoid Patients with Associated Neurological Diseases Recognize Bullous Pemphigoid Antigens in the Human Brain. *Gerontology* 57(3): 211-216.

Chen YJ, Wu CY, Lin MW, Chen TJ, Liao KK, Chen YC, Hwang CY, Chu SY, Chen CC, Lee DD, Chang YT, Wang WJ, Liu HN. 2011. Comorbidity profiles among patients with bullous pemphigoid: a nationwide population-based study. *Br J Dermatol*.

Chen YY, Hehr CL, Atkinson-Leadbetter K, Hocking JC, McFarlane S. 2007. Targeting of retinal axons requires the metalloproteinase ADAM10. *J Neurosci* 27(31): 8448-8456.

Chernousov MA, Stahl RC, Carey DJ. 2001. Schwann cell type V collagen inhibits axonal outgrowth and promotes Schwann cell migration via distinct adhesive activities of the collagen and noncollagen domains. *J Neurosci* 21(16): 6125-6135.

Chosidow O, Doppler V, Bensimon G, Joly P, Salachas F, Lacomblez L, Prost C, Camu W, Frances C, Herson S, Meininger V. 2000. Bullous pemphigoid and amyotrophic lateral sclerosis: a new clue for understanding the bullous disease? *Arch Dermatol* 136(4): 521-524.

Claudepierre T, Manglapus MK, Marengi N, Radner S, Champliaud MF, Tasanen K, Bruckner-Tuderman L, Hunter DD, Brunken WJ. 2005. Collagen XVII and BPAG1 expression in the retina: evidence for an anchoring complex in the central nervous system. *J Comp Neurol* 487(2): 190-203.

Cordel N, Chosidow O, Hellot MF, Delaporte E, Lok C, Vaillant L, Bernard P, D'Incan M, Roujeau JC, Joly P. 2007. Neurological disorders in patients with bullous pemphigoid. *Dermatology* 215(3): 187-191.

Cueva JG, Mulholland A, Goodman MB. 2007. Nanoscale organization of the MEC-4 DEG/ENaC sensory mechanotransduction channel in *Caenorhabditis elegans* touch receptor neurons. *J Neurosci* 27(51): 14089-14098.

Double KL, Dedov VN, Fedorow H, Kettle E, Halliday GM, Garner B, Brunk UT. 2008. The comparative biology of neuromelanin and lipofuscin in the human brain. *Cell Mol Life Sci* 65(11): 1669-1682.

Echigo T, Hasegawa M, Inaoki M, Yamazaki M, Sato S, Takehara K. 2007. Antiphospholipid antibodies in patients with autoimmune blistering disease. *J Am Acad Dermatol* 57(3): 397-400.

Fairley JA, Heintz PW, Neuburg M, Diaz LA, Giudice GJ. 1995. Expression pattern of the bullous pemphigoid-180 antigen in normal and neoplastic epithelia. *Br J Dermatol* 133(3): 385-391.

Fitzgerald J, Bateman JF. 2001. A new FACIT of the collagen family: COL21A1. *FEBS Lett* 505(2): 275-280.

Fitzgerald J, Rich C, Zhou FH, Hansen U. 2008. Three novel collagen VI chains, alpha4(VI), alpha5(VI), and alpha6(VI). *J Biol Chem* 283(29): 20170-20180.

Fjell AM, Walhovd KB. 2010. Structural brain changes in aging: courses, causes and cognitive consequences. *Rev Neurosci* 21(3): 187-221.

- Forschner A, Ulmer A, Rassner G, Fierlbeck G. 2002. Bullous pemphigoid in a patient with Parkinson's disease. *Eur J Dermatol* 12(6): 615.
- Forsell C, Bjork BF, Lilius L, Axelman K, Fabre SF, Fratiglioni L, Winblad B, Graff C. 2010. Genetic association to the amyloid plaque associated protein gene COL25A1 in Alzheimer's disease. *Neurobiol Aging* 31(3): 409-415.
- Foureur N, Descamps V, Lebrun-Vignes B, Picard-Dahan C, Grossin M, Belaich S, Crickx B. 2001. Bullous pemphigoid in a leg affected with hemiparesia: a possible relation of neurological diseases with bullous pemphigoid? *Eur J Dermatol* 11(3): 230-233.
- Foureur N, Grootenboer-Mignot S, Descamps V. 2010. Value of the detection of circulating antibodies against BP antigens in unaffected subjects. *Arch Dermatol* 146(7): 801; author reply 802.
- Foureur N, Mignot S, Senet P, Verpillat P, Picard-Dahan C, Crickx B, Labarre C, Nicaise-Roland P, Descamps V. 2006. [Correlation between the presence of type-2 anti-pemphigoid antibodies and dementia in elderly subjects with no clinical signs of pemphigoid]. *Ann Dermatol Venereol* 133(5 Pt 1): 439-443.
- Fox MA. 2008. Novel roles for collagens in wiring the vertebrate nervous system. *Curr Opin Cell Biol* 20(5): 508-513.
- Fox MA, Sanes JR, Borza DB, Eswarakumar VP, Fassler R, Hudson BG, John SW, Ninomiya Y, Pedchenko V, Pfaff SL, Rheault MN, Sado Y, Segal Y, Werle MJ, Umemori H. 2007. Distinct target-derived signals organize formation, maturation, and maintenance of motor nerve terminals. *Cell* 129(1): 179-193.
- Franzke CW, Bruckner-Tuderman L, Blobel CP. 2009. Shedding of collagen XVII/BP180 in skin depends on both ADAM10 and ADAM9. *J Biol Chem* 284(35): 23386-23396.
- Franzke CW, Bruckner P, Bruckner-Tuderman L. 2005. Collagenous transmembrane proteins: recent insights into biology and pathology. *J Biol Chem* 280(6): 4005-4008.
- Franzke CW, Tasanen K, Schacke H, Zhou Z, Tryggvason K, Mauch C, Zigrino P, Sunnarborg S, Lee DC, Fahrenholz F, Bruckner-Tuderman L. 2002. Transmembrane collagen XVII, an epithelial adhesion protein, is shed from the cell surface by ADAMs. *EMBO J* 21(19): 5026-5035.
- Franzke CW, Tasanen K, Schumann H, Bruckner-Tuderman L. 2003. Collagenous transmembrane proteins: collagen XVII as a prototype. *Matrix Biol* 22(4): 299-309.
- Furukawa Y, Iseki E, Hino H, Odawara T, Ikeda K, Tsuchiya K, Kosaka K. 2004. Ubiquitin and ubiquitin-related proteins in the brains of patients with atypical Pick's disease without Pick bodies and dementia with motor neuron disease. *Neuropathology* 24(4): 306-314.
- Ganguli R, Brar JS, Chengappa KN, Yang ZW, Nimgaonkar VL, Rabin BS. 1993. Autoimmunity in schizophrenia: a review of recent findings. *Ann Med* 25(5): 489-496.

- Garcia-Estevez DA, Peon-Curras G, Bal-Nieves F. 2008. [Bullous pemphigoid and amyotrophic lateral sclerosis]. *Rev Neurol* 47(10): 525-526.
- Geser F, Brandmeir NJ, Kwong LK, Martinez-Lage M, Elman L, McCluskey L, Xie SX, Lee VM, Trojanowski JQ. 2008. Evidence of multisystem disorder in whole-brain map of pathological TDP-43 in amyotrophic lateral sclerosis. *Arch Neurol* 65(5): 636-641.
- Giudice GJ, Emery DJ, Diaz LA. 1992. Cloning and primary structural analysis of the bullous pemphigoid autoantigen BP180. *J Invest Dermatol* 99(3): 243-250.
- Goldstein BI, Kemp DE, Soczynska JK, McIntyre RS. 2009. Inflammation and the phenomenology, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature. *J Clin Psychiatry* 70(8): 1078-1090.
- Gordon MK, Hahn RA. 2010. Collagens. *Cell Tissue Res* 339(1): 247-257.
- Gould DB, Phalan FC, Breedveld GJ, van Mil SE, Smith RS, Schimenti JC, Aguglia U, van der Knaap MS, Heutink P, John SW. 2005. Mutations in Col4a1 cause perinatal cerebral hemorrhage and porencephaly. *Science* 308(5725): 1167-1171.
- Grassel S, Unsold C, Schacke H, Bruckner-Tuderman L, Bruckner P. 1999. Collagen XVI is expressed by human dermal fibroblasts and keratinocytes and is associated with the microfibrillar apparatus in the upper papillary dermis. *Matrix Biol* 18(3): 309-317.
- Gray DA, Woulfe J. 2005. Lipofuscin and aging: a matter of toxic waste. *Sci Aging Knowledge Environ* 2005(5): re1.
- Grond-Ginsbach C, Schnippering H, Hausser I, Weber R, Werner I, Steiner HH, Luttgen N, Busse O, Grau A, Brandt T. 2002. Ultrastructural connective tissue aberrations in patients with intracranial aneurysms. *Stroke* 33(9): 2192-2196.
- Hashimoto T, Wakabayashi T, Watanabe A, Kowa H, Hosoda R, Nakamura A, Kanazawa I, Arai T, Takio K, Mann DM, Iwatsubo T. 2002. CLAC: a novel Alzheimer amyloid plaque component derived from a transmembrane precursor, CLAC-P/collagen type XXV. *EMBO J* 21(7): 1524-1534.
- Heffron DS, Landreth GE, Samuels IS, Mandell JW. 2009. Brain-specific deletion of extracellular signal-regulated kinase 2 mitogen-activated protein kinase leads to aberrant cortical collagen deposition. *Am J Pathol* 175(6): 2586-2599.
- Hiji M, Takahashi T, Fukuba H, Yamashita H, Kohriyama T, Matsumoto M. 2008. White matter lesions in the brain with frontotemporal lobar degeneration with motor neuron disease: TDP-43-immunopositive inclusions co-localize with p62, but not ubiquitin. *Acta Neuropathol* 116(2): 183-191.
- Hofmann SC, Voith U, Schonau V, Sorokin L, Bruckner-Tuderman L, Franzke CW. 2009. Plasmin plays a role in the in vitro generation of the linear IgA dermatosis antigen LAD97. *J Invest Dermatol* 129(7): 1730-1739.

Hooper NM, Karran EH, Turner AJ. 1997. Membrane protein secretases. *Biochem J* 321 ( Pt 2): 265-279.

Hopkinson SB, Baker SE, Jones JC. 1995. Molecular genetic studies of a human epidermal autoantigen (the 180-kD bullous pemphigoid antigen/BP180): identification of functionally important sequences within the BP180 molecule and evidence for an interaction between BP180 and alpha 6 integrin. *J Cell Biol* 130(1): 117-125.

Hopkinson SB, Findlay K, deHart GW, Jones JC. 1998. Interaction of BP180 (type XVII collagen) and alpha6 integrin is necessary for stabilization of hemidesmosome structure. *J Invest Dermatol* 111(6): 1015-1022.

Hopkinson SB, Jones JC. 2000. The N terminus of the transmembrane protein BP180 interacts with the N-terminal domain of BP230, thereby mediating keratin cytoskeleton anchorage to the cell surface at the site of the hemidesmosome. *Mol Biol Cell* 11(1): 277-286.

Hubert T, Grimal S, Carroll P, Fichard-Carroll A. 2009. Collagens in the developing and diseased nervous system. *Cell Mol Life Sci* 66(7): 1223-1238.

Huilaja L, Hurskainen T, Autio-Harmainen H, Hofmann SC, Sormunen R, Rasanen J, Ilves M, Franzke CW, Bruckner-Tuderman L, Tasanen K. 2008. Pemphigoid gestationis autoantigen, transmembrane collagen XVII, promotes the migration of cytotrophoblastic cells of placenta and is a structural component of fetal membranes. *Matrix Biol* 27(3): 190-200.

Hurskainen T, Höyhty M, Tuuttila A, Oikarinen A, Autio-Harmainen H. 1996. mRNA expressions of TIMP-1, -2, and -3 and 92-KD type IV collagenase in early human placenta and decidual membrane as studied by in situ hybridization. *J Histochem Cytochem* 44(12): 1379-1388.

Hynd MR, Lewohl JM, Scott HL, Dodd PR. 2003. Biochemical and molecular studies using human autopsy brain tissue. *J Neurochem* 85(3): 543-562.

Ito D, Suzuki N. 2011. Conjoint pathologic cascades mediated by ALS/FTLD-U linked RNA-binding proteins TDP-43 and FUS. *Neurology*.

Jedlickova H, Hlubinka M, Pavlik T, Semradova V, Budinska E, Vlasin Z. 2010. Bullous pemphigoid and internal diseases - A case-control study. *Eur J Dermatol* 20(1): 96-101.

Jorissen E, Prox J, Bernreuther C, Weber S, Schwanbeck R, Serneels L, Snellinx A, Craessaerts K, Thathiah A, Tesseur I, Bartsch U, Weskamp G, Blobel CP, Glatzel M, De Strooper B, Saftig P. 2010. The disintegrin/metalloproteinase ADAM10 is essential for the establishment of the brain cortex. *J Neurosci* 30(14): 4833-4844.

Joung I, Strominger JL, Shin J. 1996. Molecular cloning of a phosphotyrosine-independent ligand of the p56lck SH2 domain. *Proc Natl Acad Sci U S A* 93(12): 5991-5995.

- Jung T, Bader N, Grune T. 2007. Lipofuscin: formation, distribution, and metabolic consequences. *Ann N Y Acad Sci* 1119: 97-111.
- Kadler KE, Baldock C, Bella J, Boot-Handford RP. 2007. Collagens at a glance. *J Cell Sci* 120(Pt 12): 1955-1958.
- Kanaan NM, Kordower JH, Collier TJ. 2007. Age-related accumulation of Marinesco bodies and lipofuscin in rhesus monkey midbrain dopamine neurons: relevance to selective neuronal vulnerability. *J Comp Neurol* 502(5): 683-700.
- Kanda N, Soga Y, Meguro M, Tanabe A, Yagi Y, Himuro Y, Fujiwara Y, Takashiba S, Kobayashi N. 2010. Discovery of a patient with strongly suspected bullous pemphigoid in a ward by oral health care providers. *Int J Dent Hyg* 9(2): 159-162.
- Kang HT, Lee KB, Kim SY, Choi HR, Park SC. 2011. Autophagy impairment induces premature senescence in primary human fibroblasts. *PLoS One* 6(8): e23367.
- Kapoor R, Sakai LY, Funk S, Roux E, Bornstein P, Sage EH. 1988. Type VIII collagen has a restricted distribution in specialized extracellular matrices. *J Cell Biol* 107(2): 721-730.
- King A, Maekawa S, Bodi I, Troakes C, Al-Sarraj S. 2010. Ubiquitinated, p62 immunopositive cerebellar cortical neuronal inclusions are evident across the spectrum of TDP-43 proteinopathies but are only rarely additionally immunopositive for phosphorylation-dependent TDP-43. *Neuropathology*.
- Kirtschig G, Middleton P, Bennett C, Murrell DF, Wojnarowska F, Khumalo NP. 2010. Interventions for bullous pemphigoid. *Cochrane Database Syst Rev* 10: CD002292.
- Kirtschig G, Walkden VM, Venning VA, Wojnarowska F. 1995. Bullous pemphigoid and multiple sclerosis: a report of three cases and review of the literature. *Clin Exp Dermatol* 20(6): 449-453.
- Koch M, Foley JE, Hahn R, Zhou P, Burgeson RE, Gerecke DR, Gordon MK. 2001. alpha 1(Xx) collagen, a new member of the collagen subfamily, fibril-associated collagens with interrupted triple helices. *J Biol Chem* 276(25): 23120-23126.
- Koch M, Schulze J, Hansen U, Ashwodt T, Keene DR, Brunken WJ, Burgeson RE, Bruckner P, Bruckner-Tuderman L. 2004. A novel marker of tissue junctions, collagen XXII. *J Biol Chem* 279(21): 22514-22521.
- Koch M, Veit G, Stricker S, Bhatt P, Kutsch S, Zhou P, Reinders E, Hahn RA, Song R, Burgeson RE, Gerecke DR, Mundlos S, Gordon MK. 2006. Expression of type XXIII collagen mRNA and protein. *J Biol Chem* 281(30): 21546-21557.
- Kolde G, Bachus R, Ludolph AC. 1996. Skin involvement in amyotrophic lateral sclerosis. *Lancet* 347(9010): 1226-1227.

Konradi C, Sullivan SE, Clay HB. 2011. Mitochondria, oligodendrocytes and inflammation in bipolar disorder: Evidence from transcriptome studies points to intriguing parallels with multiple sclerosis. *Neurobiol Dis*.

Kuivaniemi H, Prockop DJ, Wu Y, Madhatheri SL, Kleinert C, Earley JJ, Jokinen A, Stolle C, Majamaa K, Myllyla VV, et al. 1993. Exclusion of mutations in the gene for type III collagen (COL3A1) as a common cause of intracranial aneurysms or cervical artery dissections: results from sequence analysis of the coding sequences of type III collagen from 55 unrelated patients. *Neurology* 43(12): 2652-2658.

Kuusisto E, Kauppinen T, Alafuzoff I. 2008. Use of p62/SQSTM1 antibodies for neuropathological diagnosis. *Neuropathol Appl Neurobiol* 34(2): 169-180.

Kärkkäinen I, Rybnikova E, Pelto-Huikko M, Huovila AP. 2000. Metalloprotease-disintegrin (ADAM) genes are widely and differentially expressed in the adult CNS. *Mol Cell Neurosci* 15(6): 547-560.

Laffitte E, Burkhard PR, Fontao L, Jaunin F, Saurat JH, Chofflon M, Borradori L. 2005. Bullous pemphigoid antigen 1 isoforms: potential new target autoantigens in multiple sclerosis? *Br J Dermatol* 152(3): 537-540.

Lai CH, Chu ML. 1996. Tissue distribution and developmental expression of type XVI collagen in the mouse. *Tissue Cell* 28(2): 155-164.

Lanfranconi S, Markus HS. 2010. COL4A1 mutations as a monogenic cause of cerebral small vessel disease: a systematic review. *Stroke* 41(8): e513-518.

Langan SM, Groves RW, West J. 2011. The Relationship between Neurological Disease and Bullous Pemphigoid: A Population-Based Case-Control Study. *J Invest Dermatol* 131(3): 631-636.

Leuci S, Gurcan HM, Ahmed AR. 2010. Serological studies in bullous pemphigoid: a literature review of antibody titers at presentation and in clinical remission. *Acta Derm Venereol* 90(2): 115-121.

Leung KK, Ng LJ, Ho KK, Tam PP, Cheah KS. 1998. Different cis-regulatory DNA elements mediate developmental stage- and tissue-specific expression of the human COL2A1 gene in transgenic mice. *J Cell Biol* 141(6): 1291-1300.

Li L, Chen J, Wang B, Yao Y, Zuo Y. 2009. Sera from patients with bullous pemphigoid (BP) associated with neurological diseases recognized BP antigen 1 in the skin and brain. *Br J Dermatol* 160(6): 1343-1345.

Lillo P, Garcin B, Hornberger M, Bak TH, Hodges JR. 2010. Neurobehavioral features in frontotemporal dementia with amyotrophic lateral sclerosis. *Arch Neurol* 67(7): 826-830.

Lin J, Luo J, Redies C. 2008. Differential expression of five members of the ADAM family in the developing chicken brain. *Neuroscience* 157(2): 360-375.

- Locala JA. 2009. Current concepts in psychodermatology. *Curr Psychiatry Rep* 11(3): 211-218.
- Long CC, Lever LR, Marks R. 1992. Unilateral bullous pemphigoid in a hemiplegic patient. *Br J Dermatol* 126(6): 614-616.
- Lowe J. 1998. Establishing a pathological diagnosis in degenerative dementias. *Brain Pathol* 8(2): 403-406.
- Lui VC, Kong RY, Nicholls J, Cheung AN, Cheah KS. 1995. The mRNAs for the three chains of human collagen type XI are widely distributed but not necessarily co-expressed: implications for homotrimeric, heterotrimeric and heterotypic collagen molecules. *Biochem J* 311 ( Pt 2): 511-516.
- Maertens B, Hopkins D, Franzke CW, Keene DR, Bruckner-Tuderman L, Greenspan DS, Koch M. 2007. Cleavage and oligomerization of gliomedin, a transmembrane collagen required for node of ranvier formation. *J Biol Chem* 282(14): 10647-10659.
- Malinverno M, Carta M, Epis R, Marcello E, Verpelli C, Cattabeni F, Sala C, Mulle C, Di Luca M, Gardoni F. 2010. Synaptic localization and activity of ADAM10 regulate excitatory synapses through N-cadherin cleavage. *J Neurosci* 30(48): 16343-16355.
- Marcello E, Gardoni F, Mauceri D, Romorini S, Jeromin A, Epis R, Borroni B, Cattabeni F, Sala C, Padovani A, Di Luca M. 2007. Synapse-associated protein-97 mediates alpha-secretase ADAM10 trafficking and promotes its activity. *J Neurosci* 27(7): 1682-1691.
- Marinkovich MP. 2007. Tumour microenvironment: laminin 332 in squamous-cell carcinoma. *Nat Rev Cancer* 7(5): 370-380.
- Matsuo N, Tanaka S, Yoshioka H, Koch M, Gordon MK, Ramirez F. 2008. Collagen XXIV (Col24a1) gene expression is a specific marker of osteoblast differentiation and bone formation. *Connect Tissue Res* 49(2): 68-75.
- Michelson PH, Tigue M, Jones JC. 2000. Human bronchial epithelial cells secrete laminin 5, express hemidesmosomal proteins, and assemble hemidesmosomes. *J Histochem Cytochem* 48(4): 535-544.
- Mizuno Y, Amari M, Takatama M, Aizawa H, Mihara B, Okamoto K. 2006. Immunoreactivities of p62, an ubiquitin-binding protein, in the spinal anterior horn cells of patients with amyotrophic lateral sclerosis. *J Neurol Sci* 249(1): 13-18.
- Moscat J, Diaz-Meco MT. 2009. p62 at the crossroads of autophagy, apoptosis, and cancer. *Cell* 137(6): 1001-1004.
- Muona A, Eklund L, Vaisanen T, Pihlajaniemi T. 2002. Developmentally regulated expression of type XV collagen correlates with abnormalities in Col15a1(-/-) mice. *Matrix Biol* 21(1): 89-102.

- Myeku N, Figueiredo-Pereira ME. 2011. Dynamics of the degradation of ubiquitinated proteins by proteasomes and autophagy: association with sequestosome 1/p62. *J Biol Chem* 286(25): 22426-22440.
- Myllyharju J, Kivirikko KI. 2001. Collagens and collagen-related diseases. *Ann Med* 33(1): 7-21.
- Myllyharju J, Kivirikko KI. 2004. Collagens, modifying enzymes and their mutations in humans, flies and worms. *Trends Genet* 20(1): 33-43.
- Määttä M, Liakka A, Salo S, Tasanen K, Bruckner-Tuderman L, Autio-Harmanen H. 2004. Differential expression of basement membrane components in lymphatic tissues. *J Histochem Cytochem* 52(8): 1073-1081.
- Nakar S, Ingber A, Kremer I, Hodak E, Garty BZ, Ben-David E, David M, Shohat M. 1992. Late-onset localized junctional epidermolysis bullosa and mental retardation: a distinct autosomal recessive syndrome. *Am J Med Genet* 43(5): 776-779.
- Nishie W, Lamer S, Schlosser A, Licarete E, Franzke CW, Hofmann SC, Jackow J, Sitaru C, Bruckner-Tuderman L. 2010. Ectodomain shedding generates Neoepitopes on collagen XVII, the major autoantigen for bullous pemphigoid. *J Immunol* 185(8): 4938-4947.
- Nishizawa Y, Uematsu J, Owaribe K. 1993. HD4, a 180 kDa bullous pemphigoid antigen, is a major transmembrane glycoprotein of the hemidesmosome. *J Biochem* 113(4): 493-501.
- Oh SP, Griffith CM, Hay ED, Olsen BR. 1993. Tissue-specific expression of type XII collagen during mouse embryonic development. *Dev Dyn* 196(1): 37-46.
- Ono S. 2007. [Skin changes in amyotrophic lateral sclerosis]. *Brain Nerve* 59(10): 1099-1107.
- Ono S, Imai T, Munakata S, Takahashi K, Kanda F, Hashimoto K, Yamano T, Shimizu N, Nagao K, Yamauchi M. 1998. Collagen abnormalities in the spinal cord from patients with amyotrophic lateral sclerosis. *J Neurol Sci* 160(2): 140-147.
- Ono S, Imai T, Takahashi K, Jinnai K, Kanda F, Fukuoka Y, Hashimoto K, Shimizu N, Nagao K. 1999. Alteration in amino acids in motor neurons of the spinal cord in amyotrophic lateral sclerosis. *J Neurol Sci* 167(2): 121-126.
- Ono S, Mechanic GL, Yamauchi M. 1990. Amyotrophic lateral sclerosis: unusually low content of collagen in skin. *J Neurol Sci* 100(1-2): 234-237.
- Ono S, Shimizu N, Imai T, Rodriguez GP. 2001. Urinary collagen metabolite excretion in amyotrophic lateral sclerosis. *Muscle Nerve* 24(6): 821-825.
- Ono S, Toyokura Y, Mannen T, Ishibashi Y. 1986. Amyotrophic lateral sclerosis: histologic, histochemical, and ultrastructural abnormalities of skin. *Neurology* 36(7): 948-956.

- Ono S, Yamauchi M. 1992. Amyotrophic lateral sclerosis: increased solubility of skin collagen. *Neurology* 42(8): 1535-1539.
- Pandey RS, Gupta AK, Chaturvedi UC. 1981. Autoimmune model of schizophrenia with special reference to antibrain antibodies. *Biol Psychiatry* 16(12): 1123-1136.
- Parkinson N, Ince PG, Smith MO, Highley R, Skibinski G, Andersen PM, Morrison KE, Pall HS, Hardiman O, Collinge J, Shaw PJ, Fisher EM. 2006. ALS phenotypes with mutations in CHMP2B (charged multivesicular body protein 2B). *Neurology* 67(6): 1074-1077.
- Patricio P, Ferreira C, Gomes MM, Filipe P. 2009. Autoimmune bullous dermatoses: a review. *Ann N Y Acad Sci* 1173: 203-210.
- Paulus W, Baur I, Liszka U, Drlicek M, Leigh I, Bruckner-Tuderman L. 1995. Expression of type VII collagen, the major anchoring fibril component, in normal and neoplastic human nervous system. *Virchows Arch* 426(2): 199-202.
- Paulus W, Sage EH, Liszka U, Iruela-Arispe ML, Jellinger K. 1991. Increased levels of type VIII collagen in human brain tumours compared to normal brain tissue and non-neoplastic cerebral disorders. *Br J Cancer* 63(3): 367-371.
- Pelegri C, Canudas AM, del Valle J, Casadesus G, Smith MA, Camins A, Pallas M, Vilaplana J. 2007. Increased permeability of blood-brain barrier on the hippocampus of a murine model of senescence. *Mech Ageing Dev* 128(9): 522-528.
- Pfendner G, Lucky A. (2008). Junctional Epidermolysis Bullosa. Retrieved 3.2.2011, from University of Washington <http://www.ncbi.nlm.nih.gov/books/NBK1125/>
- Pikkarainen M, Hartikainen P, Alafuzoff I. 2010. Ubiquitinated p62-positive, TDP-43-negative inclusions in cerebellum in frontotemporal lobar degeneration with TAR DNA binding protein 43. *Neuropathology* 30(2): 197-199.
- Pikkarainen M, Martikainen P, Alafuzoff I. 2010. The effect of prolonged fixation time on immunohistochemical staining of common neurodegenerative disease markers. *J Neuropathol Exp Neurol* 69(1): 40-52.
- Plumb DA, Dhir V, Mironov A, Ferrara L, Poulsom R, Kadler KE, Thornton DJ, Briggs MD, Boot-Handford RP. 2007. Collagen XXVII is developmentally regulated and forms thin fibrillar structures distinct from those of classical vertebrate fibrillar collagens. *J Biol Chem* 282(17): 12791-12795.
- Powell AM, Sakuma-Oyama Y, Oyama N, Black MM. 2005. Collagen XVII/BP180: a collagenous transmembrane protein and component of the dermoepidermal anchoring complex. *Clin Exp Dermatol* 30(6): 682-687.
- Provinciali L, Cangiotti A, Tulli D, Carboni V, Cinti S. 1994. Skin abnormalities and autonomic involvement in the early stage of amyotrophic lateral sclerosis. *J Neurol Sci* 126(1): 54-61.

- Ring C, Hassell J, Halfter W. 1996. Expression pattern of collagen IX and potential role in the segmentation of the peripheral nervous system. *Dev Biol* 180(1): 41-53.
- Ring C, Lemmon V, Halfter W. 1995. Two chondroitin sulfate proteoglycans differentially expressed in the developing chick visual system. *Dev Biol* 168(1): 11-27.
- Rosenberg GA. 2009. Matrix metalloproteinases and their multiple roles in neurodegenerative diseases. *Lancet Neurol* 8(2): 205-216.
- Ross R. 1999. Atherosclerosis is an inflammatory disease. *Am Heart J* 138(5 Pt 2): S419-420.
- Ruigrok YM, Rinkel GJ. 2008. Genetics of intracranial aneurysms. *Stroke* 39(3): 1049-1055.
- Russell IJ, Legan PK, Lukashkina VA, Lukashkin AN, Goodyear RJ, Richardson GP. 2007. Sharpened cochlear tuning in a mouse with a genetically modified tectorial membrane. *Nat Neurosci* 10(2): 215-223.
- Sajanti J, Bjorkstrand AS, Finnila S, Heikkinen E, Peltonen J, Majamaa K. 1999. Increase of collagen synthesis and deposition in the arachnoid and the dura following subarachnoid hemorrhage in the rat. *Biochim Biophys Acta* 1454(3): 209-216.
- Sajanti J, Heikkinen E, Majamaa K. 2000. Transient increase in procollagen propeptides in the CSF after subarachnoid hemorrhage. *Neurology* 55(3): 359-363.
- Sajanti J, Majamaa K. 2003. High concentrations of procollagen propeptides in chronic subdural haematoma and effusion. *J Neurol Neurosurg Psychiatry* 74(4): 522-524.
- Sasaki S. 2011. Autophagy in spinal cord motor neurons in sporadic amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol* 70(5): 349-359.
- Sasaki T, Larsson H, Tisi D, Claesson-Welsh L, Hohenester E, Timpl R. 2000. Endostatins derived from collagens XV and XVIII differ in structural and binding properties, tissue distribution and anti-angiogenic activity. *J Mol Biol* 301(5): 1179-1190.
- Sato K, Yomogida K, Wada T, Yorihuri T, Nishimune Y, Hosokawa N, Nagata K. 2002. Type XXVI collagen, a new member of the collagen family, is specifically expressed in the testis and ovary. *J Biol Chem* 277(40): 37678-37684.
- Schacke H, Schumann H, Hammami-Hauasli N, Raghunath M, Bruckner-Tuderman L. 1998. Two forms of collagen XVII in keratinocytes. A full-length transmembrane protein and a soluble ectodomain. *J Biol Chem* 273(40): 25937-25943.
- Schneider VA, Granato M. 2006. The myotomal diwanka (Ih3) glycosyltransferase and type XVIII collagen are critical for motor growth cone migration. *Neuron* 50(5): 683-695.
- Schumann H, Baetge J, Tasanen K, Wojnarowska F, Schacke H, Zillikens D, Bruckner-Tuderman L. 2000. The shed ectodomain of collagen XVII/BP180 is targeted by autoantibodies in different blistering skin diseases. *Am J Pathol* 156(2): 685-695.

Schönau VM. Characterization of monoclonal antibodies to collagen XVII and detection of the ectodomain in blister fluids. Thesis. Albert-Ludwigs-Universität Freiburg 2009. [http://www.freidok.uni-freiburg.de/volltexte/6814/pdf/DruckversionDISS\\_neu.pdf](http://www.freidok.uni-freiburg.de/volltexte/6814/pdf/DruckversionDISS_neu.pdf)

Seehafer SS, Pearce DA. 2006. You say lipofuscin, we say ceroid: defining autofluorescent storage material. *Neurobiol Aging* 27(4): 576-588.

Seelaar H, Schelhaas HJ, Azmani A, Kusters B, Rosso S, Majoor-Krakauer D, de Rijk MC, Rizzu P, ten Brummelhuis M, van Doorn PA, Kamphorst W, Willemsen R, van Swieten JC. 2007. TDP-43 pathology in familial frontotemporal dementia and motor neuron disease without Progranulin mutations. *Brain* 130(Pt 5): 1375-1385.

Seppinen L, Pihlajaniemi T. 2011. The multiple functions of collagen XVIII in development and disease. *Matrix Biol* 30(2): 83-92.

Sertie AL, Sossi V, Camargo AA, Zatz M, Brahe C, Passos-Bueno MR. 2000. Collagen XVIII, containing an endogenous inhibitor of angiogenesis and tumor growth, plays a critical role in the maintenance of retinal structure and in neural tube closure (Knobloch syndrome). *Hum Mol Genet* 9(13): 2051-2058.

Simjee S, Konqui A, Razzaque Ahmed A. 1985. Multiple sclerosis and bullous pemphigoid. *Dermatologica* 170(2): 86-89.

Soderhall C, Marenholz I, Kerscher T, Ruschendorf F, Esparza-Gordillo J, Worm M, Gruber C, Mayr G, Albrecht M, Rohde K, Schulz H, Wahn U, Hubner N, Lee YA. 2007. Variants in a novel epidermal collagen gene (COL29A1) are associated with atopic dermatitis. *PLoS Biol* 5(9): e242.

Soni A, Irani SR, Lang B, Taghipour K, Mann R, Vincent A, Collins D. 2009. Immunotherapy: responsive autoimmune encephalopathy associated with bullous pemphigoid. *J Neurol Neurosurg Psychiatry* 80(12): 1412-1413.

Sperner-Unterweger B. 2005. Immunological aetiology of major psychiatric disorders: evidence and therapeutic implications. *Drugs* 65(11): 1493-1520.

Stinco G, Codutti R, Scarbolo M, Valent F, Patrone P. 2005. A retrospective epidemiological study on the association of bullous pemphigoid and neurological diseases. *Acta Derm Venereol* 85(2): 136-139.

Stinco G, Mattighello P, Zanchi M, Patrone P. 2002. Multiple sclerosis and bullous pemphigoid: a casual association or a pathogenetic correlation? *Eur J Dermatol* 12(2): 186-188.

Stojanovic A, Roher AE, Ball MJ. 1994. Quantitative analysis of lipofuscin and neurofibrillary tangles in the hippocampal neurons of Alzheimer disease brains. *Dementia* 5(5): 229-233.

- Su J, Gorse K, Ramirez F, Fox MA. 2010. Collagen XIX is expressed by interneurons and contributes to the formation of hippocampal synapses. *J Comp Neurol* 518(2): 229-253.
- Sulzer D, Mosharov E, Tallozy Z, Zucca FA, Simon JD, Zecca L. 2008. Neuronal pigmented autophagic vacuoles: lipofuscin, neuromelanin, and ceroid as macroautophagic responses during aging and disease. *J Neurochem* 106(1): 24-36.
- Sund M, Vaisanen T, Kaukinen S, Ilves M, Tu H, Autio-Harmanen H, Rauvala H, Pihlajaniemi T. 2001. Distinct expression of type XIII collagen in neuronal structures and other tissues during mouse development. *Matrix Biol* 20(4): 215-231.
- Taghipour K, Chi CC, Vincent A, Groves RW, Venning V, Wojnarowska F. 2010. The association of bullous pemphigoid with cerebrovascular disease and dementia: a case-control study. *Arch Dermatol* 146(11): 1251-1254.
- Tasanen K, Tunggal L, Chometon G, Bruckner-Tuderman L, Aumailley M. 2004. Keratinocytes from patients lacking collagen XVII display a migratory phenotype. *Am J Pathol* 164(6): 2027-2038.
- Terman A, Brunk UT. 2004. Lipofuscin. *Int J Biochem Cell Biol* 36(8): 1400-1404.
- Terman A, Brunk UT. 2006. Oxidative stress, accumulation of biological 'garbage', and aging. *Antioxid Redox Signal* 8(1-2): 197-204.
- Thoma-Uszynski S, Uter W, Schwietzke S, Hofmann SC, Hunziker T, Bernard P, Treudler R, Zouboulis CC, Schuler G, Borradori L, Hertl M. 2004. BP230- and BP180-specific auto-antibodies in bullous pemphigoid. *J Invest Dermatol* 122(6): 1413-1422.
- Tolnay M, Probst A. 2003. The neuropathological spectrum of neurodegenerative tauopathies. *IUBMB Life* 55(6): 299-305.
- Tong Y, Xu Y, Scearce-Levie K, Ptacek LJ, Fu YH. 2010. COL25A1 triggers and promotes Alzheimer's disease-like pathology in vivo. *Neurogenetics* 11(1): 41-52.
- Uitto J, Pulkkinen L. 1996. Molecular complexity of the cutaneous basement membrane zone. *Mol Biol Rep* 23(1): 35-46.
- Urabe N, Naito I, Saito K, Yonezawa T, Sado Y, Yoshioka H, Kusachi S, Tsuji T, Ohtsuka A, Taguchi T, Murakami T, Ninomiya Y. 2002. Basement membrane type IV collagen molecules in the choroid plexus, pia mater and capillaries in the mouse brain. *Arch Histol Cytol* 65(2): 133-143.
- Walchli C, Koch M, Chiquet M, Odermatt BF, Trueb B. 1994. Tissue-specific expression of the fibril-associated collagens XII and XIV. *J Cell Sci* 107 ( Pt 2): 669-681.
- van Horssen J, Wilhelmus MM, Heljasvaara R, Pihlajaniemi T, Wesseling P, de Waal RM, Verbeek MM. 2002. Collagen XVIII: a novel heparan sulfate proteoglycan associated with

vascular amyloid depositions and senile plaques in Alzheimer's disease brains. *Brain Pathol* 12(4): 456-462.

Watanabe S, Yamada K, Ono S, Ishibashi Y. 1987. Skin changes in patients with amyotrophic lateral sclerosis: light and electron microscopic observations. *J Am Acad Dermatol* 17(6): 1006-1012.

Veit G, Kobbe B, Keene DR, Paulsson M, Koch M, Wagener R. 2006. Collagen XXVIII, a novel von Willebrand factor A domain-containing protein with many imperfections in the collagenous domain. *J Biol Chem* 281(6): 3494-3504.

Wieland CN, Comfere NI, Gibson LE, Weaver AL, Krause PK, Murray JA. 2010. Anti-bullous pemphigoid 180 and 230 antibodies in a sample of unaffected subjects. *Arch Dermatol* 146(1): 21-25.

Wijeratne C, Webster P. 1996. Risperidone and bullous pemphigoid. *Am J Psychiatry* 153(5): 735.

Vitale P, Braghetta P, Volpin D, Bonaldo P, Bressan GM. 2001. Mechanisms of transcriptional activation of the col6a1 gene during Schwann cell differentiation. *Mech Dev* 102(1-2): 145-156.

Xiao T, Baier H. 2007. Lamina-specific axonal projections in the zebrafish tectum require the type IV collagen Draqnet. *Nat Neurosci* 10(12): 1529-1537.

Yamada T, Endo R, Tsukagoshi K, Fujita S, Honda K, Kinoshita M, Hasebe T, Hirohashi S. 1996. Aberrant expression of a hemidesmosomal protein, bullous pemphigoid antigen 2, in human squamous cell carcinoma. *Lab Invest* 75(4): 589-600.

Yang YW, Chen YH, Xirasagar S, Lin HC. 2011. Increased risk of stroke in patients with bullous pemphigoid: a population-based follow-up study. *Stroke* 42(2): 319-323.

Zeevi N, Pachter J, McCullough LD, Wolfson L, Kuchel GA. 2010. The blood-brain barrier: geriatric relevance of a critical brain-body interface. *J Am Geriatr Soc* 58(9): 1749-1757.

**ALLAN SEPPÄNEN**

*Collagen XVII  
in the Human Brain*

Collagens have previously been overlooked for roles in the brain since fibrillar collagens, the best known and most widely studied example of collagens, are not present in the mature central nervous system. However, over the last decade it has become increasingly apparent that collagens are not merely structural proteins giving strength to tissue, but bio-active molecules with a dynamic role within the nervous system. Collagen XVII particularly has been emerging as a putative antigen common to both dermatological and neurological disease. In this thesis these lines of thought found support as collagen XVII was found to be widely expressed in neurons of the human brain. Its exact function in the brain, however, remains unknown.



UNIVERSITY OF  
EASTERN FINLAND

PUBLICATIONS OF THE UNIVERSITY OF EASTERN FINLAND  
*Dissertations in Health Sciences*

ISBN 978-952-61-0583-3