

# From counting neurons to the preclinical diagnosis of Alzheimer's disease

*The fruits of collaborative work with Dr. Henryk M. Wisniewski*

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This article is a review of scientific work on Alzheimer neurofibrillary degeneration and A $\beta$ -amyloidosis that was done in collaboration with Dr. Henryk Wisniewski, in part at the Institute for Basic Research in Developmental Disabilities. Our work on paired helical filaments and the tau protein spans from basic immunocytochemical analyses of brain tissue to clinical application as a biological marker used in diagnostic tests. Even though only a small part of Dr. Wisniewski's scientific oeuvre, these data illustrate how a great scientist opens the field to his student, collaborator and friend, how basic science can evolve, and how results can be applied in clinical practice to the benefit of our patients.

This article is not an original scientific paper reporting new data, nor is it meant as a timely in-depth review of a topic related to dementia, nor is it my intention to compile the publication list of the late Dr. Henryk Wisniewski. The purpose of this paper is to review some of the data we had acquired in collaboration with Dr. Wisniewski within the decade prior to his too early death as an example of how new knowledge acquired by basic research can evolve and eventually be applied in clinical routine to the benefit of the patients. Those who will find the citation biased will forgive me; the list is to illustrate our common work, not to reflect the complete literature. To all of the data reviewed here, Henry contributed both, intellectually and physically; probably none of the work would have been possible without him. And it should be emphasized that the data

presented here represent only a small fraction of the scientific heritage he has left us with.

*Vienna, 1986–1987: NFT, PHF and the tau protein*

When I first met Dr. Wisniewski, I had merely graduated from medical school and it was just for a few weeks that I had joined Dr. Lassmann's laboratory at the Neurological Institute of the University in Vienna. From a cooperative project, we had obtained antibodies against different cytoskeletal proteins from the Institute of Basic Research in Developmental Disabilities in order to study their immunoreactivity on human brains with different neurodegenerative conditions. The contact between Henry and the Neurological Institute had actually been established long before, and it was at the occasion of a scientific meeting organized in honor of Professor Seitelberger's 70th birthday that I was given the very important duty to pick up from the airport one of the most distinguished guests: Dr. Henryk M. Wisniewski. Contrary to all my expectations, the important guest from the US turned out to be a very friendly, open minded, sympathetic man who immediately impressed me with his incredible amount of energy and power – none of the old gray-haired professors a freshly graduated 25-year-old guy would expect. Right away in the car, we started talking about science, and I came back with the newly acquired knowledge, that scientific celebrities – as Dr. Wisniewski was – do take serious the ideas of young Austrian scientists. And somehow I had the impression that the big professor had enjoyed our discussion.

I learned many new things during the meeting, and my interest, guided by Dr. Lassmann's help and instructions, started to turn towards those neurons bearing strange fibrillar inclusions that could best be seen in the hippocampus. It did not take long until I learned that these neurofibrillary tangles (NFT) within neurons of the hippocampus and neocortex are major histopathologic hallmarks of Alzheimer's disease (AD). Even though not specific to the disease [33], the numbers of

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NFT correlate with clinical severity of dementia, and the formation of paired helical filaments (PHF) [30, 32] within neurons is one cause of nerve cell death in AD [14]. Dr. Wisniewski had compared them to a tumor growing within the cell, what indeed they looked like, so I could easily understand why it was important to study these structures. In addition to NFT, PHF are also found in dystrophic neurites within the corona of senile plaques and in small bundles "free" in the neuropil as so-called neuropil threads. When I started working with the electron microscope, the first thing I discovered was actually a tangle of PHF.

The biochemical study of PHF had long been hampered by their relative insolubility [29], until it was discovered at Dr. Wisniewski's Institute, simultaneously with other research groups, that the abnormal fibres contain an abnormally phosphorylated form of the microtubule-associated protein tau [11,12]. Other antigenic components of NFT include ubiquitin and phosphorylated epitopes of high molecular weight neurofilament proteins [1,2]. Indeed, the antibodies that had been raised to PHF at the Institute of Basic Research [26] recognize epitopes of ubiquitin [21].

This finding was the basis of the cooperative project between the Institute for Basic Research and our Institute in Vienna: Henry provided us with antibodies, the brain specimens were from the Vienna collection, and I was to count several thousand NFT immunostained with different antibodies in order to quantify the results.

Dr. Wisniewski had told me that, in addition to AD, NFT can also be found in a variety of unrelated diseases of the brain [33]. In these conditions, they occur generally at other predilection sites than in AD. Progressive supranuclear palsy (PSP) is one of them. By histopathology, this condition is characterized by the presence of numerous NFT in subcortical structures, including the basal ganglia, the brain stem and the cerebellum. Even though closely resembling NFT of AD, NFT in PSP are not made up of PHF, but of 20nm straight filaments. Using the antibodies we had obtained, we found that the NFT in PSP had antigenic similarities and differences compared to those found in AD. Both tangles contain phosphorylated tau protein, whereas ubiquitin was basically absent from the tangles in PSP [5]. The inverse was true for Lewy bodies, that reacted strongly with the antibodies to ubiquitin and phosphorylated neurofilament proteins, but not did not contain tau epitopes [6].

During the study of PSP and AD brains, we observed that the antibodies to tau we had obtained from Dr. Wisniewski's Institute did not only stain NFT, but also

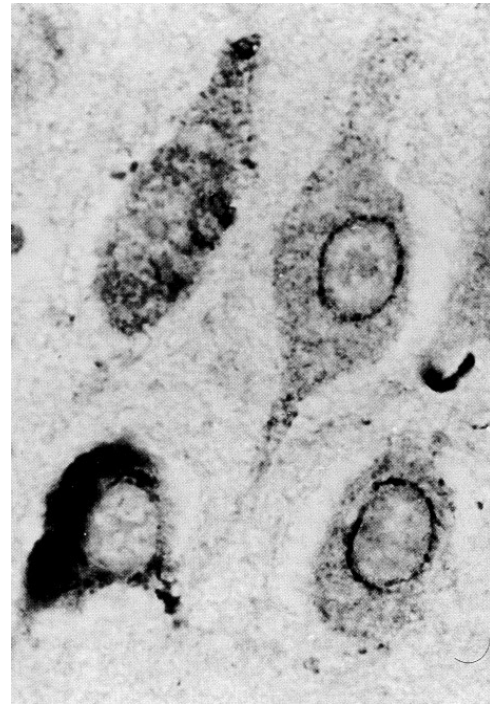


Fig. 1. Pretangle neurons in sector CA 2 of the hippocampus of a 74-year-old individual with profound mental retardation. Immunostaining with monoclonal antibody Tau-1 on dephosphorylated tissue. Reprinted from Ref [2].

a number of neurons that did not contain tangles as well as neurons containing minute bundles of filaments (Fig. 1). Such staining could not be observed with the other antibodies reacting with NFT. Dr. Lassmann proposed a morphological analysis classifying the tangles into 4 stages according to their degree of maturation. From examining the staining properties of the tangles at these different stages, we deduced that the accumulation of abnormally phosphorylated tau precedes the formation of NFT within neurons, and that this accumulation is the earliest detectable event in the process of neurofibrillary degeneration (Stage 0 tangles, pretangle neurons) [1,2]. This finding turned out to be important, since it became clear that tau is the major molecular component of PHF and that its pathological posttranslational modification, including abnormal phosphorylation, is a key event in the pathogenesis of neurofibrillary degeneration.

#### *New York, 1987–1990: Plaques, amyloid and A $\beta$*

It was with that background and some experience in immunocytochemistry that I was given the opportunity to join Dr. Wisniewski's Institute in the fall of 1987.

It was not only a very warm welcome that I received, but it was also a very good time to join, since it had not been long that the gene of the amyloid  $\beta$ -protein precursor (A $\beta$ PP) had been cloned [23,24] and monoclonal antibodies to amyloid- $\beta$  (A $\beta$ ) [17,18] and other regions of the A $\beta$ PP had been raised and were available at the Institute. Using these antibodies on brain tissue of AD patients and on aged animals, we could show that deposits of A $\beta$  in the brain were much more widespread than appreciated so far. Beyond the different types of senile plaques, A $\beta$  deposits in AD can adopt a spectrum of morphological forms, basically according to the cyto- and fibroarchitectonics of the brain region involved. These included large areas of diffuse infiltration of the neuropil reaching several mm in size in the entorhinal cortex, ribbon-like infiltration of the subpial layer of the cerebral cortex (Fig. 2), granular deposits in the white matter and star-shaped deposits in the cerebellar Purkinje cell layer [28]. Only a small number of these amyloid deposits were surrounded by dystrophic neurites, and it turned out that PHF were only seen in plaque-neurites when NFT occurred in the same brain region [9]. When examining the brains of mentally retarded people that had suffered from other conditions than Down's syndrome, we found that there was a very high prevalence of amyloid deposits and that most of these did not have a neuritic component containing PHF [22].

At the same time, we applied the A $\beta$  antibodies to the brains of aged dogs, and were quite surprised to see that some of them contained very large amounts of amyloid and numbers of plaques sufficient to warrant a diagnosis of AD according to the histopathologic criteria in use at that time [31]. This was of importance, since there was no animal model of AD at that time. Of course, Dr. Wisniewski who had known some of the animals in person, was of the opinion that they had exhibited some symptoms of dementia before their death. For an experimental neuropathologist as Dr. Wisniewski was – and he used to hope “that God is not a rabbit” for he had worked with small animals for years – one of the things he was proud of was the lion head in the staircase of his home on Staten Island. It was not a hunters trophy, but it came from the Bronx Zoo. Apparently, Henry had been the only qualified person to put an end to the life of the savage animal chronically suffering from diverse illnesses of advancing age – of course not without taking home the brain and the trophy.

While studying amyloidosis in AD brains, we noticed that one antibody to A $\beta$  did not only stain amyloid deposits, but also granular material within nerve



Fig. 2. Spectrum of morphological appearance of amyloid deposits in the parahippocampal gyrus. Amyloid deposits are not only seen in the form of plaques, but also as diffuse infiltration covering almost the entire width of the entorhinal cortex, as ribbon-like subpial infiltration and as loose amyloid deposits in the subcortical white matter. Note staining of lipofuscin containing nerve cells in the lateral geniculate body (lower left). Asterisk: CA 4 sector of the hippocampal formation. Amyloid- $\beta$  immunostain (monoclonal antibody 4G8 on tissue pretreated with formic acid). Reprinted from Ref [28].

cells. By its distribution in the brain and by immune electron microscopy, it turned out that the stained material was lipofuscin, an end product of lysosomal protein breakdown; in other words “the garbage bin of the cell”, as Henry Wisniewski used to explain. Further biochemical analysis of the immunoreactive material in Dr. Iqbal's laboratory disclosed a polypeptide of 31 kDa of yet unknown function [4].

#### *New York, 1987–1990: tau, ubiquitin and CSF*

Even though getting involved in the amyloid story, there was also ample opportunity to follow-up on NFT, PHF and the tau protein. Further immunocytochemical work corroborated the finding that the accumulation of tau in the neuron precedes the formation of NFT (pre-tangle neurons). On the other hand, at late stages of maturation, epitopes of tau are lost and NFT become positive for ubiquitin, a polypeptide involved in the non-lysosomal protein breakdown [2]. The importance of tau was further underlined when it became clear that the abnormal phosphorylation of tau precluded its biological activity promoting the polymerisation of tubulin into microtubules [16].



Fig. 3. Dr. Henryk Wisniewski with the author at the First International Conference on Alzheimer Disease and Related Disorders in Las Vegas (September 1988). Dr. Wisniewski was co-convenor of this conference, that was the first of a series of biennially held congresses. These meetings have grown quickly and represent today the world's largest platform for scientific discussion on basic research on dementia and Alzheimer disease.

It was at that time that some interest came up in using PHF-specific antibodies in ELISA systems on the CSF to see if such measurements would be helpful in diagnosing AD in living patients. Knowing that I had planned to get into clinical neurology, Dr. Wisniewski asked me to review the topic. Antibodies to PHF were available [26], some data had been collected and looked promising [15,20,27]. However, ELISA systems to measure tau in the CSF were not available at that time. Everything taken together, measuring antigens derived from the neuropathologic lesions in the AD brain seemed the most promising approach for an *in vivo* marker of the disease [8]. For that purpose, tau was the obvious candidate.

It was a few years later that a number of clinical studies have shown that tau actually is elevated in the CSF of patients with AD and that lumbar puncture for determining tau levels contributes to diagnostic accuracy [25]. Recent data point to the fact, that measuring tau in CSF might even allow a preclinical diagnosis of the disease [3]. Indeed, morphological studies have shown that the first neurons with NFT develop in the transentorhinal/entorhinal cortex as early as decades before the onset of dementia. Today, CSF analysis of tau, in combination with A $\beta$ 42, has entered clinical practice in many countries, including Austria [13].

Even though the provenience of tau in the CSF is not resolved as yet (normal tau liberated from degenerating axons? PHF-tau diffusing into the CSF?), a decade of basic and clinical research on NFT, PHF and the tau protein has fruited in a diagnostic tool that is helpful

to clinicians in diagnosing AD and beneficial to our patients with dementing diseases.

Of course, my planned one year stay at the Institute of Basic Research was extended, and it was after more than two years of intense and rewarding science (Fig. 3) that I returned to Vienna to join Professor Jellinger's department. We kept contact, and kept collaborating [7, 10,19]. For my good bye, Henryk Wisniewski offered me two books and a crystal that I keep on my desk. He told me that I should go on doing research, what I am doing; and definitely he has shown me how enjoyable science can be. You just have to work with the right people.

I knew that Dr. Wisniewski had health problems, and that he did not slow down his pace – this would not have been Henryk Wisniewski. When I heard about his sudden death, it was totally unexpected, and I knew right away that I had lost one of the very few men that I consider to be my teachers. I would have wished that he had learned about the new position I am getting now, and that I am doing well. And if this is so, it is for the scientific background of which I owe so much to Henry.

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