The Discovery of Alzheimer causing Mutations in the APP Gene and the Formulation of the "Amyloid Cascade Hypothesis".

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Abstract

The cloning of APP and genetic analysis of families with Alzheimer's disease were both reported in 1987 and much present work on the disease is based upon the foundations laid at that time. Progress was not smooth however, and many errors were made. In this memoir, I lay out both the progress and the errors.

My background was in neurochemistry and I had always wanted to work on the pathogenesis of diseases of the CNS. In Newcastle upon Tyne, UK and in Umea, Sweden I had worked on the transmitter biochemistry of Alzheimer's disease (1-3). I had enjoyed this work, but was very aware that by studying the neurochemical pathology, I was only looking at the end stage of the disease and it was impossible to make certain inferences of how disease started. In 1983, Gusella published his landmark paper (4) showing the gene for Huntington's disease was on the short arm of chromosome 4 and for me this was a critical piece of work. I realized that molecular genetics offered a route to finding how diseases started. Whilst a postdoc with Bengt Winblad in Sweden in 1985, we started to collect families with dementia to try the same approaches as Gusella had reported. However, in 1984, a job was advertised at St Mary's Hospital in London in the Biochemistry Department headed by Bob Williamson. Despite the name, this was actually an excellent research department of human genetics, where, for example, the first DNA-based linkage to human disease (Duchenne dystrophy) had been reported (5). Whilst, as a department, it was getting plaudits for its research, its teaching of biochemistry was being criticized by medical students and the job advert was to find someone to beef up their biochemistry teaching. I was extremely fortunate to get the job and while I initially started my lab continuing to work on the neurochemistry of Alzheimer's disease, with Bob's help, I started to learn molecular genetics. A year later, St. Mary's recruited Martin Rossor, who had also worked on the neurochemistry of Alzheimer's disease (6) to the Neurology Department and he and I, with Bob's support, began to advertise for families multiply affected by dementia both through Martin's clinical practice and the Alzheimer Society newsletter. We also wrote MRC and other grants together to start genetic work. Of note, the first 13 grant applications I wrote were unsuccessful and without the continuing support of Bob, our efforts would have foundered. However, in 1987 we were eventually successful and got charity and MRC funding to prosecute the work.

The letters from the families initially came in to me and Martin and I would discuss each of them and then Martin's nurse would go out and collect blood samples. The letters piled on my desk and, in fact, the crucial family, F23, was

the first to contact us and was numbered “Family 23” simply because letters from 22 others piled on top of it before we answered.

In 1984, Glenner had isolated “beta-amyloid” (now called Aβ) from the meningeal vessels of Alzheimer cases and got a partial sequence (7). People with Down syndrome nearly always develop Alzheimer’s disease (8) and later the same year, Glenner obtained a sequence from a Down’s case (9) and, realizing it was the same sequence, wrote in the abstract:

> *The cerebrovascular amyloid protein from a case of adult Down's syndrome was isolated and purified. Amino acid sequence analysis showed it to be homologous to that of the beta protein (Aβ) of Alzheimer's disease. This is the first chemical evidence of a relationship between Down's syndrome and Alzheimer's disease. It suggests that Down's syndrome may be a predictable model for Alzheimer's disease. Assuming the beta protein is a human gene product, it also suggests that the genetic defect in Alzheimer's disease is localized on chromosome 21.*

I regard this as the first implicit statement of the amyloid hypothesis since Glenner clearly thought that overproduction of Aβ leads to Alzheimer’s disease. The following year, Masters and Beyreuther separated and obtained partial sequence of plaque amyloid and realized that it was the same sequence (10). With these publications of the sequence, the race was on to clone to amyloid gene. We tried to clone the gene, but unfortunately followed Glenner’s view that this was likely to be a blood protein (7) and we spent most of our effort screening cDNA libraries made from human liver. Liver later turned out to be virtually the only tissue not to express the protein. The publication race to clone the gene was won by the Masters and Beyreuther team (11) although in fact a patent had been filed on the gene sequence earlier by Cordell and colleagues (12). Other groups also cloned the APP gene (13-15) and all realized that, as Glenner had predicted, its location was on chromosome 21. As this cloning was going on, the first genetic linkage analysis of large Alzheimer families was occurring in the Gusella lab and initial analysis suggested that they showed the Alzheimer locus was also on chromosome 21, close to the centromere (16) and apparently not far from the position of the APP gene (15). Immediately

thereafter, APP gene duplications were reported in a study from France in sporadic Alzheimer's disease (17).

In fact, it is clear with the benefit of hindsight that in the fevered atmosphere accompanying these observations in 1987, with groups rushing to be the first to make clear findings directly relating APP variants to Alzheimer's disease, a series of errors were made by many groups including ours.

The first error was in the report of the genetic linkage of Alzheimer's disease to the pericentromeric region of chromosome 21 (16). In fact, the 4 large families used in this report were later shown to have mutations in the presenilin gene on chromosome 14 (18). The second error related to the report of APP gene duplications (17) and this was quickly determined by a series of negative reports looking for duplications (19). We (20) and the Gusella lab (21) reported that, in many families there was no evidence for co-segregation of the amyloid gene with Alzheimer's disease. Importantly, the Gusella lab paper used the same families to which chromosome 21 linkage had been reported. This led to us making a third interpretive error. Because of these two negative papers about APP cosegregation, but in the light of the positive linkage reports to chromosome 21, we believed there was a gene for Alzheimer's disease on chromosome 21 that was distinct from APP. Indeed, one of the families labeled as family 5 (actually F23) in our report (20) independently showed evidence for linkage to chromosome 21 markers (labeled family 2 in ref 22).

At this time, I read a fascinating paper describing the pathology of Hereditary Cerebral Haemorrhage with Angiopathy, Dutch Type (HCHWA-D) from the Dutch clinical group (led by Raymond Roos and Joost Haan) and Blas Frangione (23). In this hereditary haemorrhage disorder the blood vessels were lined with the same Aβ as is found in Alzheimer's disease. I immediately wrote to the Roos and organized to visit Leiden with a view to collecting the family for genetic analysis. I involved Christine Van Broeckhoven and we started collection in Antwerp, 90 miles away. Christine's group started to run chromosome 21 markers. Her work showed complete linkage between disease and the genetic markers at APP. We started to sequence the gene and indeed Christine's lab found the mutation on the day we heard that the Frangione group had already identified APP E693Q as

a variant in their single case. Our paper and the Frangione paper describing the mutation and the APP cosegregation were published back to back in Science (24, 25).

While HCHWA-D and Alzheimer's disease are pathologically and clinically different, clearly the fact that mutations in APP could lead to amyloid deposition was important and this, together with our increasing realization that Alzheimer's disease was genetically heterogeneous (26, 27) started to make our group start to rethink our analytical approach. If the disease was heterogeneous and we wanted to find the gene on chromosome 21, we should only co-analyse those families in which we were sure there was linkage to chromosome 21. There were ostensibly 4 such families, our family F23, FAD4 in which both chromosome 21 linkage and APP exclusion had been reported (15, 16 and 21) and 2 Belgian families in Christine's collection (28). In FAD4 and in the two Belgian families, the APP gene had been published as being excluded, but in F23, it had not. Over the summer of 1990, our group, Mike Owen, Mike Mullan, Luis Giuffra, Alison Goate and I argued about our own data interpretation and the published data. Eventually we decided to rely only on our own data and to use the newly invented technology of PCR direct sequencing, which Marie Christine Chartier-Harlin had just got to work in our lab, to start to sequencing the APP gene in F23 alone. It worked, and we found the first APP mutation, APP V717I. Screening all the other families in the lab revealed a second family with the same mutation that Allen Roses had collected (29). Later the same year, we found a second family with linkage at the APP locus and found the second mutation at the same codon APP V717G (30): a third mutation was found contemporaneously also at the same position (31). We had, therefore, found the first molecularly defined causes of Alzheimer's disease.

I had always thought of genetics as an independent way of testing hypotheses of causation. There had been many competing theories of for Alzheimer's disease and I simply believed that genetics would allow a decision about these competing theories to be made. Genetic analysis told us that amyloid was the cause of Alzheimer's disease in these families, and also in Down syndrome. Without much thought, I wrote out my verdict on this work first with David

Allsop and then with Gerry Higgins (32, 33). Contemporaneously Dennis Selkoe came to the same conclusion (34), and these 3 papers, which Dennis and I have subsequently updated (35, 36), form the basis for the amyloid hypothesis of the disease. Together these papers have been cited more than 10,000 times. Subsequently, APP gene duplications were correctly reported to occur in Alzheimer families, also from France (37). I reviewed this latter paper for Nature Genetics and my only question as a reviewer was to request the authors make sure these families were not in any way related to those in the previous report of French APP gene duplications (17). They were not.

What have I learnt from these events? With regard to experimentation and data analysis the main lesson I have drawn is that if data is critical, make sure you see the raw data yourself. We were misled by the reports that FAD4 and the Belgian families seemed to be chromosome 21 linked but without APP co-segregation (they all later turned out to have presenilin 1 mutations) and this delayed us sequencing our own chromosome 21-linked family (DNA gene sequencing was much more difficult in 1989/1990 than it is now). With regard to expressing my views on pathogenesis: I have always thought it is very important to write what you think clearly. Sometimes you will be wrong, and that is fine, but you should always be clear. A third lesson: given all the mistakes that we and others made in the hotheaded analyses of 1987, is try not to be swept along. Speaking for myself, but also I suspect for the other groups involved in the Nature and Science papers in that year: we were too fast to be careful and, I suspect, the journal editors and reviewers were equally careless.

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Table 1

Errors and Excitement in Alzheimer's Disease in 1987

Report	Finding	Error	Comment
St George-Hyslop et al. 1987 (16)	Alzheimer linkage to chromosome 21	Families in which linkage was reported later shown to be chromosome 14 linked (37).	And yet some families were genuinely linked at this locus (29).
Tanzi et al. (1987a)	APP gene on chromosome 21 "near Alzheimer locus"	Since original linkage report was wrong, this paper was misinterpreted	
Van Broeckhoven et al. (1987)	APP gene does not co-segregate with Alzheimer's disease	This paper was misinterpreted. Most families had late onset disease and did not show co-segregation.	The large early onset family (labeled '5') did have evidence of cosegregation and had a mutation (29)
Tanzi et al. (1987b)	APP gene does not co-segregate with Alzheimer's disease	The families used in this report were believed to show chromosome 21 linkage so this paper was interpreted as revealing that APP and the AD loci were separate but both on chromosome 21	These families later were shown to have presenilin mutations
Delabar et al. 1987	APP gene duplications found in French Alzheimer families	Simply wrong	And yet, APP gene duplications later found in French Alzheimer families (37)