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Road to the chromosome 9p-linked ALS/FTD locus

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We recently published that a large hexanucleotide repeat expansion within the *C9ORF72* gene is the cause of chromosome 9p-linked amyotrophic lateral sclerosis (ALS)/fronto-temporal dementia (FTD). Here, I describe how the chromosome 9p21 locus was first identified, the growing excitement (and frustration) over attempts to pinpoint the underlying genetic lesion, and how we and our collaborators ultimately cloned it. In many ways, this behind-the-scenes account showcases the various technological advances that have been driving the genomics field over the last few years, and which ultimately were responsible for finding the pathogenic repeat expansion.

Our story begins in 2006 with the publication of two papers that used traditional linkage analysis to identify a region on the short arm of chromosome 9 in two large ALS/FTD families.^{1,2} The overlap between these studies (plus several similar studies) was about 7 million base pairs (bp). In genetics terms, this was equivalent in size to a large city, such as Washington D.C..

Moving on a few years, genomics technology has advanced at a rapid pace, and we are in the middle of the genome-wide association study revolution. Several such studies of both ALS and FTD began to show a consistent association signal in the middle of the same chromosome 9p locus identified by linkage analysis.^{3–5} The high density of SNP genotyping in these studies narrowed the region of interest to a ~200,000bp segment encoding just three genes, namely *MOBK2B*, *IFNK*, and *C9ORF72*. To extend our geographical metaphor, this meant we now knew which street within the city the genetic lesion lived on, but still did not know which house (gene) or which room (exon) was responsible. Standard sequencing of these three genes failed to identify a convincing mutation, and the underlying genetic lesion was beginning to appear intractable.

Our involvement started with our genome-wide association study of ALS in Finland, which identified a strong association signal in the same chromosome 9 region.⁶ To maximize our chances of cloning this difficult locus, we formed a consortium with other laboratories in Europe and the US working on the same region. This collaboration involved Stuart Pickering-Brown at the University of Manchester, John Hardy at Queen Square, London, Peter Heutink at the VU Medical University, Amsterdam, Ian Mackenzie at the University of British Columbia, Huw Morris and Nigel Williams at the University of Cardiff, Rosa Rademakers at the Mayo Clinic Jacksonville, and Pentti Tienari at the University of Helsinki. Our intent was to leverage the sequencing resources of the NIH intramural research program to generate data that could be rapidly distributed to the other collaborators for analysis. Monthly phone calls helped to maintain the focus of the consortium.

We employed an array of genomics techniques to dissect chromosome 9p21. Key among these was massive parallel sequencing, a new technology that could rapidly generate genetic sequence data. Together with advances in sequence capture technology, we undertook deep re-sequencing of the region. Our initial lack of success with this approach made us suspect that the underlying lesion was either an inversion disrupting local genetic architecture, or a

pathogenic repeat expansion. Neither type of genetic defect would have shown up with sequence-capture technologies.

To pursue these possibilities, we massively sequenced DNA that had been flow sorted to enrich for chromosome 9, achieving deep coverage across our target area without relying on sequence capture. The key analysis step came from identifying novel SNPs within the 200,000bp region of interest. A number of these SNPs were located adjacent to the first exon of the *C9ORF72* gene. Closer inspection showed drop off in the sequence coverage, indicative of a problem in the area. Furthermore, the reference human genome contained a short run of GGGGCC repeats, consistent with the type of repeat that we thought might be responsible. Manual realignment of the sequences revealed that the case carried a greater number of repeats compared to the reference human genome. This observation was our first clue as to what was really happening on chromosome 9p21.

Over the subsequent weeks, we and our collaborators were busy screening samples to generate data for our publication. When I announced to the consortium that the mutation was a pathogenic hexanucleotide repeat expansion, our collaborator, Rosa Rademakers, said that she had also found it independently by exploring an aberrant segregation pattern within an ALS/FTD family linked to chromosome 9p21. We agreed to submit back to back papers to *Neuron*. The editorial review process at this journal proved to be fair and fast, and the papers were published online on September 21, 2011.^{7,8} This was the culmination to three years of effort by our laboratory and by our collaborators.

Much remains to be done to fully understand the pathogenic repeat expansion. How does it disrupt cellular processes to result in neurodegeneration? Why do some patients present with motor neuron dysfunction, while others develop cognitive defects? Is the region inherently unstable, and how does this instability contribute to disease? And, above all, is it possible to therapeutically target the region with small molecules or gene therapy? These questions are important to address, as we attempt to translate the genetic findings back to the clinic to help patients devastated by these neurodegenerative diseases. But at least the road ahead is a little bit clearer....

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