Dr Rosa Rademakers is using molecular genetics analyses to study disorders such as frontotemporal dementia and amyotrophic lateral sclerosis, with a view to developing new patient therapies.

Could you briefly outline your academic background and research ambitions?

My PhD focused on the genetics of dementias and was carried out in Dr Christine van Broeckhoven’s Molecular Neurogenetics laboratory at the University of Antwerp, Belgium. Since I was trained in human genetics, I subsequently pursued a postdoctoral fellowship at the Mayo Clinic in Jacksonville in Dr Mike Hutton’s laboratory to further my knowledge of the cell biology and animal modelling of these diseases. In 2007, I was fortunate to be given the opportunity to lead an independent research laboratory at the Mayo Clinic.

By what means do you identify novel causal genes and genetic risk factors?

We have used classical linkage analyses in extended families with a clear inheritance of frontotemporal lobar degenerations (FTLDs) or amyotrophic lateral sclerosis (ALS) for the identification of novel causal genes, while we have focused on larger populations of unrelated patients and healthy individuals in so-called genetic association studies to identify genetic risk factors. This approach has been very effective and led us to identify major causes of FTLD and ALS including mutations in the progranulin gene (GRN) and repeat expansions in chromosome 9 open reading frame 72 (C9ORF72).

During your studies you used in vivo and in vitro molecular approaches to better understand the function of newly identified genes and mutations. What was your methodology?

The molecular studies in our laboratory are focused on providing an understanding of why or how specific gene mutations lead to disease. A major benefit of being at the Mayo Clinic is our access to unique patient material, including DNA, plasma and human brain tissue samples from large cohorts of very well-phenotyped patients affected by FTLD and related disorders. We also perform cell-based assays to further determine the function of novel disease genes and their associated mutations.

Could you provide an insight into your research into microRNAs (miRNAs)?

miRNAs are small non-coding RNA molecules with an important function in the regulation of gene expression. In 2008, we showed that the expression of the progranulin protein (PGRN) encoded by GRN can be regulated by miRNAs in vitro. Moreover, we provided evidence to suggest that some individuals were at a significantly increased risk of developing FTLD because of the presence of a variant in their GRN gene affecting a predicted miRNA-binding site, resulting in lower levels of PGRN.

What is the importance of PGRN?

The identification of loss-of-function mutations in GRN was a major breakthrough. Why not having enough PGRN causes FTLD is still unknown, but it’s the question driving research in FTLD laboratories around the world. If not having enough PGRN causes FTLD, restoring PGRN levels should prevent or potentially cure FTLD. This straightforward possibility has inspired researchers to focus on developing ways to increase PGRN levels as potential therapeutics and has led to the establishment of the Consortium for Frontotemporal Dementia Research.

Could you discuss your current research on C9ORF72?

A major goal of our current research is to understand why some individuals with C9ORF72 repeat expansions develop FTLD, while others develop ALS (or both) and what determines at what age the first symptoms occur. For this purpose, we have collected DNA samples of a cohort of more than 300 individuals with C9ORF72 repeat expansions. While we eventually plan to perform exome and whole-genome sequencing to identify the complete spectrum of genetic modifiers in these patients, we started with the study of genetic variants known to be implicated in FTLD or ALS. One such gene is TMEM106B, which we initially identified as a risk factor for FTLD but now also implicated as a disease modifier in patients with GRN and C9ORF72 mutations. Other ongoing studies related to C9ORF72 in our laboratory are focused on the detailed study of brain and spinal cord tissue that we have available on a large number of mutation carriers. By systematically analysing the repeat length, number of RNA foci, expression of C9ORF72 transcripts and associated TDP-43 and dipeptide pathologies, we hope to gain further insight into the role of each aspect of the pathology on disease presentation and progression.

Why did you ultimately decide to focus on FTLD and ALS?

I had always been fascinated by human genetic research and the laboratory at the University of Antwerp focused on the genetics of neurodegenerative diseases, especially Alzheimer’s disease and FTLD, so it was an easy choice when I selected a laboratory for my PhD studies. While this choice was not initially driven by a specific interest in neurodegenerative disease, it fascinates me to attempt to unravel how the human brain makes us who we are, how it controls our personalities and our memories, and how these important processes are affected by disease.
Researchers at the Mayo Clinic, Florida, USA, are uncovering the genetic risk factors behind neurodegenerative disorders. Their work promises to herald new therapeutic approaches.

**FRONTOTEMPORAL LOBAR DEGENERATION (FTLD)** describes a group of disorders in which the frontal and temporal lobes of the brain atrophy to cause dementia. The predominant clinical manifestation of FTLD is frontotemporal dementia (FTD), a form of early onset dementia. Unlike Alzheimer’s disease (AD), where memory problems tend to be the first symptom, FTD patients are usually diagnosed after displaying personality and behavioural changes or, occasionally, language impairments. Around 10-15 per cent of patients with FTLD present with amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig’s disease, in addition to their FTD symptoms (FTD-ALS). ALS is caused by the loss of certain cells in the brains and spinal cords of patients – motor neurons – that control muscle activity. As a result, the disease can affect the patient’s walking, breathing and swallowing. It is currently estimated that there are 300,000 people living with FTD or ALS in the US, and yet, at present, there are no treatments available to either stop or slow down the progression of these devastating dementias.

**MOLECULAR GENETICS**

At the forefront of research in this field is Dr Rosa Rademakers, who heads a laboratory of a dozen researchers at the Mayo Clinic, Florida, USA. With multiple grants from the US National Institutes of Health (NIH), the team’s research focuses on the molecular genetics analyses of neurodegenerative diseases including FTD and ALS. The first gene to be associated with FTLD was the microtubule-associated protein tau (MAPT) gene discovered in 1998, which causes FTLD with tau pathology. In 2006, Rademakers played a key role in the discovery of the progranulin gene (GRN) and subsequent study of the progranulin protein (PGRN). “Through my PhD research in Belgium in the laboratory of Dr van Broeckhoven and postdoctoral work at the Mayo Clinic with Dr Mike Hutton I directly contributed to the discovery of mutations in GRN as the first cause of FTLDs with TAR DNA-binding protein 43 (TDP-43) pathology,” she explains.

In 2011, Rademakers discovered the chromosome 9 open reading frame 72 (C9ORF72) repeat expansion – a novel genetic mutation that is the most common cause of FTD and ALS identified to date. Today, Rademakers’ laboratory has three main research areas: finding more novel genes for neurodegenerative diseases; continued research into GRN; and further study of the C9ORF72 repeat expansion.

**IDENTIFYING NOVEL GENES**

In the lab’s first focus area, Rademakers is working to identify further novel genes responsible for the FTLD and ALS disorders. The researchers conduct classical linkage analyses using DNA samples from extended families. In common with other neurodegenerative diseases such as AD, FTD and ALS can be either familial (ie. inherited through the genes) or sporadic (ie. without a family history of disease). Despite the remarkable progress made in identifying novel FTD and ALS disease genes in the last decade, the cause of 50 per cent of familial FTLD cases, 40 per cent of familial ALS, and nearly all of the sporadic FTLD and ALS cases are still unknown. To address this, Rademakers’ lab has started to employ next-generation sequencing techniques to study the coding regions of the genome in a process known as exome sequencing, which will allow the researchers to identify the novel genetic causes underlying these diseases. The researchers will also compare the sequencing data from larger sets of unrelated patients.

**PROGRANULIN MUTATIONS**

The laboratory’s second research area focuses on GRN, a novel gene discovered by Rademakers in 2006. The team’s studies have shown that individuals with GRN mutations have half the usual amount of functional PGRN. Rademakers’ team is unravelling the role of this protein by defining the genetic spectrum of GRN mutations and uncovering other genetic factors that modify the disease presentation of GRN mutations such as the uncharacterised trans-membrane protein 106B (TMEM106B). “We showed that individuals with a GRN mutation who also carry specific genetic variants in TMEM106B can be protected from developing FTLD symptoms and we provided evidence that this protective effect may be driven by changes in TMEM106B protein levels,” she explains.

More recently, thanks to a network of collaborators, Rademakers has been able to compile a cohort of DNA samples of all possible symptomatic GRN mutation carriers. “Worldwide, more than 550 mutation carriers are now available for study and we plan to perform exome sequencing and genome-wide association studies in this large cohort to identify novel genetic modifiers of the disease onset, penetrance and presentation of these GRN mutation carriers,” she elucidates.

The Mayo Clinic team has also demonstrated that an enzyme-linked immunosorbent assay (ELISA) for PGRN can detect all symptomatic and pre-symptomatic loss-of-function GRN mutation carriers in a patient’s blood. The researchers also identified the multiligand receptor sortilin (SORT1) as a regulator of PGRN levels in human plasma.

Sufferers of both FTLD and ALS show deposits of the TDP-43 in the brain and spinal cord. The TDP-43 pathology, or FTLD-TDP, seen in all GRN mutation carriers, suggests that low PGRN levels could be behind the TDP-43 dysfunction seen in neurodegenerative disorders. TDP-43 pathology is also found in sufferers of other disorders including AD and Parkinson’s disease; hence all of these neurodegenerative disorders have been linked together as TDP-43 proteinopathies.

**REPEAT EXPANSIONS**

Rademakers’ identification of a repeat expansion in the non-coding region of C9ORF72 in 2011 solved the long-standing question of how FTLDs and ALS were linked to chromosome 9. Patients with FTLD or ALS were found to have hundreds to thousands of copies of a GGGGCC DNA repeat sequence near the beginning of the C9ORF72 gene, while healthy individuals only showed between two and 23 repeats. Rademakers and her colleagues rely heavily on a brain bank of more than 5,500 tissue samples maintained by Dr Dennis Dickson at the Mayo Clinic. “These samples are critical in our ongoing studies as they allow us to measure the length of C9ORF72 repeat expansion in different
areas of the brain and to study the effects on the expression of the \textit{C9ORF72} gene and protein and visualise the mutation-specific RNA foci generated by repeat expansion,” explains Rademakers.

The team now believes that this repeat expansion is the most common cause of familial cases of FTD and ALS, while the mutation is also believed to be responsible for up to 5 per cent of sporadic cases. “Although it has only been a little over two years since the discovery of \textit{C9ORF72} repeat expansions, the progress to date has already been impressive and it is very exciting to see that there is true hope for the development of a therapy for ALS and FTLD based on our findings,” enthuses Rademakers.

LARGER COHORTS

By identifying the molecular pathways underlying the genetic causes of FTD and ALS, Rademakers’ work is opening the way for the development of new diagnostic tests

Looking to the future, Rademakers and her colleagues are acutely aware of keeping their research strategies up to date: the rapid development of next-generation sequencing techniques means it is now affordable to study all of the coding regions of the genome or even the entire genome. As a result, the Mayo Clinic scientists are expanding their work to include larger cohorts of well-phenotyped unrelated patients who are exome sequenced with the aim of identifying novel disease genes. “Bioinformatic analysis of these vast amounts of data will surely become a new focus and challenge in the years to come,” concludes Rademakers.