ExPLAining early synucleinopathies

This scientific commentary refers to ‘Direct visualization of alpha-synuclein oligomers reveals previously undetected pathology in Parkinson’s disease brain’, by Roberts et al. (doi: 10.1093/brain/awv040).

Parkinson’s disease is the commonest movement disorder, with an estimated life-time risk of 1.5% (de Rijk et al., 1995). It is characterized by severe cell loss in the substantia nigra and neuronal/neuritic accumulation of aggregated α-synuclein (αSyn) (Spillantini et al., 1997), a 140-amino acid protein of unclear function. Point mutations lead to early-onset Parkinson’s disease (Polymeropoulos, 1997), and importantly, total αSyn levels—which can be increased following duplication or triplication of the alpha-synuclein gene SNCA—help to determine time of onset and rate of progression (Fuchs et al., 2007). The causes of the ~90% of Parkinson’s disease cases that are sporadic are not well understood. Overall, there is abundant evidence linking αSyn and its aggregation to the pathogenic mechanism of Parkinson’s disease, dementia with Lewy bodies and other neurodegenerative diseases collectively termed ‘synucleinopathies’. However, which forms of αSyn are cytotoxic and how these are distributed within the human brain are largely unknown, due in no small part to the lack of sensitive techniques for their specific detection. Classical immunohistochemistry cannot differentiate between physiological and pathological αSyn assemblies, and differentiation based on morphology of the αSyn signal—diffuse or punctate—is not helpful in detecting pathological oligomers that are dispersed inside or even outside of cells. Aggregate-binding dyes such as thioflavin, on the other hand, detect only very large β-sheet-rich inclusions of αSyn and are not protein-specific, simultaneously detecting other aggregation-prone proteins such as tau and amyloid-β in Alzheimer’s disease. Detecting intermediate-sized and potentially diffusible aggregates of αSyn in human tissue has therefore been a major challenge in the field. The study by Roberts et al. published in this issue of Brain provides a significant step towards solving this problem by establishing a method that promises to make small and intermediate αSyn aggregates visible (Roberts et al., 2015).

Currently it is believed that neither the soluble monomeric nor the soluble tetrameric forms of αSyn (both presumed to be physiological) are cytotoxic. The monomeric form, however, is known to readily aggregate in vitro into soluble higher oligomeric forms and thence into amyloid-like fibrils, with the latter found in the pathological hallmarks of synucleinopathies, Lewy bodies and Lewy neurites (Spillantini et al., 1997). The fibrils appear to be devoid of neurotoxicity themselves, but they are thought to work as a temporarily protective reservoir of putatively active intermediates that can be released into the tissue, thereby contributing to the pathological mechanism (Fig. 1). While a variety of studies have focused on which toxic αSyn aggregates can form in vitro on the way from physiological monomers to the biologically inactive amyloid fibril, no evidence has emerged so far as to which of these intermediate forms are present in human disease-associated tissue. Roberts et al. now use an elegant technique to answer this question directly in frozen brain slices from patients with Parkinson’s disease. The authors adapted a method originally developed to detect interaction between two proteins, the proximity ligation assay (PLA) (Söderberg et al., 2006). The assay utilizes two antibodies coupled to specific short nucleotide sequences that, when in close proximity, undergo complementation and are ligated to form complete DNA circles, which in turn are amplified by ‘rolling circle’ DNA replication. The amplified DNA then binds fluorescent probes, hence enabling the detection of protein-protein complexes. Here, Roberts et al. chose to use two identical monoclonal anti-αSyn antibodies, thereby visualizing any protein aggregates composed of two or more αSyn molecules in tissue sections, and were thus poised to detect the earliest aggregation forms and give us a glimpse of the first aggregation events in the αSyn mechanism of Parkinson’s disease.

This new method appears to preferentially detect intermediate sized αSyn oligomers while having much lower sensitivity for fibrillar material and monomeric αSyn in vitro. In tissue sections, large aggregates are only very occasionally (and then only peripherally) stained by this method, and physiological tetramers-multimers—for the existence of which there is growing evidence (Bartels et al., 2011; Dettmer et al., 2013; Burre et al., 2014; Wang et al., 2014)—also appear to be spared. In their Fig. 1C, for example, Roberts et al. show how wild-type αSyn-mediated YFP-complementation occurs diffusely in the cytosol, suggesting the presence of physiological tetramers/multimers, whereas αSyn-PLA in the same cells detects only local punctate staining indicative of an abnormally aggregated subpopulation, perhaps caused by overexpression/tagging of αSyn. It is tempting to speculate that this unexpected specificity may turn out to be one of the method’s biggest strengths: using other αSyn antibodies besides syn211 in the same assay could lead to a preferential detection of alternative pathological oligomers, potentially deepening our understanding of different αSyn aggregation species and possibly identifying species that are highly typical of the disease and completely absent in healthy brains. Combining different antibodies in this new method might lead to an even bigger tool set, which could indeed turn out to be very useful for the αSyn field. This, however, will require detailed controls that, for example, exclude signals caused by two antibodies...
binding to the same αSyn molecule. Roberts et al. provide such controls and appropriately discuss certain ambiguities that they observed.

The abovementioned detection, limited to early and not mature aggregates, is useful for addressing several open questions in the αSyn field. First, is disease correlated with the accumulation of oligomers rather than mature Lewy bodies? Second, which brain regions are affected in the earlier, probably soluble oligomer-driven stage of the disease? Third, how common are these oligomers in the advanced stage of the disease?

Robert et al.'s use of their new method to compare eight patients with Parkinson's disease to an equal number of age- and sex-matched healthy controls revealed the presence of extensive, disease-associated oligomer staining in the medulla, midbrain and cingulate cortex. These brain regions are of particular interest, given their hypothesized involvement in the early stages of Parkinson's disease and the presumed ‘spreading’ of cytopathology from the dorsal motor nucleus through the substantia nigra to the neocortex, as first proposed by Braak et al. (2004). Analogous to the aggregation time course of synthetic αSyn in vitro, one would expect a variety of aggregates among the different regions that are affected in a post-mortem Parkinson's brain, with more mature aggregates in the regions that had been affected earliest, and more diffuse staining in areas of pathology emerging closer to the time of the patient's death. While Roberts et al. focus only on regions that should be heavily affected according to the Braak hypothesis, the distinct staining with conventional immunohistochemistry (which detects mature Lewy bodies) versus PLA (which apparently detects early intermediates) is intriguing. In the study, mature Lewy bodies in the brainstem, thought to be the ‘oldest’ aggregates in patients according to Braak staging, are picked up by standard immunohistochemistry but rarely by PLA. Other aggregates, like extrasynaptic Lewy bodies and pale bodies on the other hand, are indeed detected with high sensitivity by PLA, indicating that they represent the less dense aggregates presumably characteristic of early pathology. Interestingly, Lewy bodies in areas that are putatively affected later in Parkinson’s disease, like the cingulate cortex, are detected more strongly by PLA than by conventional immunohistochemistry. This result further strengthens the claim that these regions have a later temporal involvement in the apparent pathological spread of αSyn aggregation. Excitingly, the authors also find diffuse cytoplasmic staining by PLA in neurons of patients but not healthy controls, which may give us a glimpse into the earliest events in disease pathogenesis. This diffuse staining extended to the neuropil and the white matter of the medulla and cingulate cortex. One could speculate that this points towards the involvement of the axons of the respective neurons, or perhaps even glial cells, in the earliest pathogenic events. For future studies, it would be very interesting to analyse a wider array of different brain regions to search for lesions detected with PLA. This might sketch out a timeline of αSyn aggregation in Parkinson’s disease and might even offer clues as to why certain neuronal populations, i.e. in the substantia nigra, are more severely affected than other αSyn-expressing regions in the brain. Finally, the authors show the general applicability of the PLA method in brains from other synucleinopathies, including dementia with Lewy bodies and multiple system atrophy. The technique used by Roberts et al. therefore holds great promise for elucidating the temporal hierarchy behind a variety of neurodegenerative diseases associated with αSyn aggregation.

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References


Figure 1 Possible pathways of αSyn aggregation in synucleinopathies. Destabilization of the physiological forms of αSyn initiates aggregation and ultimately Lewy body formation. Detection of the respective intermediates is important for understanding the pathomechanism of Parkinson's disease and as a valuable neuropathological marker of disease progression. Lewy body/neurite images reproduced from Spillantini et al. (1997).
Nuclear-mitochondrial proteins: too much to process?

This scientific commentary refers to ‘PMP22 mutations cause abnormal mitochondrial protein processing in patients with non-progressive cerebellar ataxia’, by Jobling et al. (doi:10.1093/brain/awv057).

In the not too distant past, non-progressive focal neurological deficits emerging during child development were all labelled as ‘cerebral palsy’, and assumed to be caused by an insult during pregnancy or in the perinatal period. This view has changed dramatically over the last two decades, in large part as a result of advances in structural brain imaging, which failed to identify the characteristic patterns of hypoxic-ischaemic brain injury or perinatal infection in every case. Metabolic and genetic studies subsequently unravelled a diverse and unexpected range of mechanisms responsible for these ‘static’ neurological deficits, and the pace has accelerated with the application of exome sequencing (Srivastava et al., 2014). Patients with non-progressive cerebellar ataxia have been particularly difficult to explain. The number of known causes has remained limited, and includes extreme rarities such as congenital disorder of glycosylation type Ia, caused by phosphomannomutase 2 deficiency (Boddaert et al., 2010). However, the differential diagnosis and mechanisms responsible for this phenotype have now broadened with the findings of Jobling et al. (2015), described in this issue of Brain.

Initially studying a family from the Bekaa valley in north east Lebanon, Jobling et al. looked for regions of shared homozygosity using microsatellite markers in 12 individuals with non-progressive ataxia, spasticity, and intellectual disability (Delague et al., 2002). All shared a 12.1-cM homozygous region on chromosome 9q34-qter between markers D9S67 and D9S312. Exome sequencing identified homozygous mutations in two genes within the mapped candidate region: PMP22 and CAMSAP1, neither previously associated with disease. These findings presented a challenge—which mutation was responsible for the disorder?

In the early days (2010–11), exome sequencing identified a huge range of apparently unique genetic variants in every individual sequenced. This led many of us down the garden path, chasing variants that turned out to be harmless polymorphisms restricted to the local population. However, with increased global efforts, and (most importantly) an understanding of local-sequenced exomes, our knowledge of the genetic architecture and machine-specific artefacts now allows instant recognition of these variants, which can be ‘filtered out’ at an early stage of the analysis. Unfortunately the challenge still remains when studying families from an ethnic minority group, or from outside the locale, where the background genetic variation may not be well known. Moreover, although consanguinity makes the initial genetic mapping somewhat straightforward, several rare polymorphisms are usually ‘trapped’ in the same homozygous region, making it impossible to sort the wheat from the chaff. Under these circumstances, a second family is key, and most important of all, the neurologist must be confident that the phenotype is the same. In this way, Jobling et al. identified an identical homozygous nucleotide substitution in exon 10 of PMP22 (c.1129G>A), predicted to alter the amino acid sequence (p.Ala377Thr), in a second family without the co-segregating CAMSAP1 variant. The final genetic evidence came when homozygous c.1129G>A/p.Ala377Thr variants were identified in a French patient of Lebanese extraction, subsequently shown to be related to the other two families through a founder effect; and when different, and very rare, compound heterozygous variants in PMP22 were identified in an unrelated French family.

Having defined the likely causal variants, Jobling et al. then went on to study the functional consequences of