POLY-GA IMMUNOHISTOCHEMISTRY IS A RELIABLE TOOL FOR DETECTING C9ORF72 GENETIC MUTATION AT THE SYDNEY BRAIN BANK

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BACKGROUND

The most common genetic mutation in familial frontotemporal lobar degeneration (FTLD) and motor neuron disease (MND) is a hexanucleotide repeat expansion (G_4C_2) in the C9orf72 gene. In 2013 a characteristic star-shaped neuronal inclusion pathology was found to contain dipeptide repeat proteins, including poly Gly-Ala (polyGA), which was later found to be the dominant form[1]. The commonly-used repeat-primed PCR method of testing has been shown to have limitations in sensitivity, specificity and inter-laboratory interpretation and not all brain banks perform routine genetic screening of their cases[2]

AIMS

To determine the accuracy of Poly-GA immunohistochemistry when detecting dipeptide repeat pathology in the C9orf72 gene.

METHODS

- Formalin Fixed paraffin embedded human cerebellum tissue from FTLD and MND cases (n=63, n=64).
- Using a commercially available poly-GA antibody (Millipore, MABN889, clone 5E9) at a concentration of 1:1000uL we immunohistochemically stained the cases using a Roche Benchmark GX autostainer in a research setting.
- Diaminobenzidine (DAB) was used as the substrate, and counterstained using Haematoxylin.

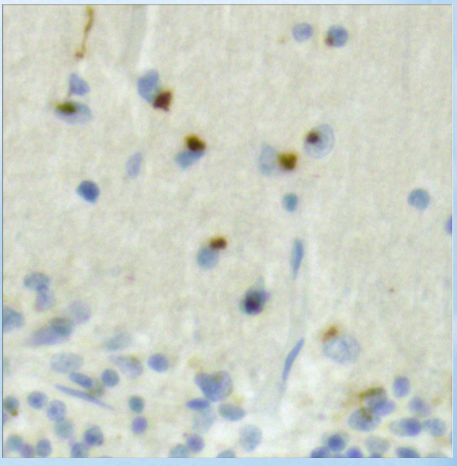

Figure 1: Star shaped neuronal cytoplasmic Poly-GA inclusions in the cerebellar cortex[3].

RESULTS

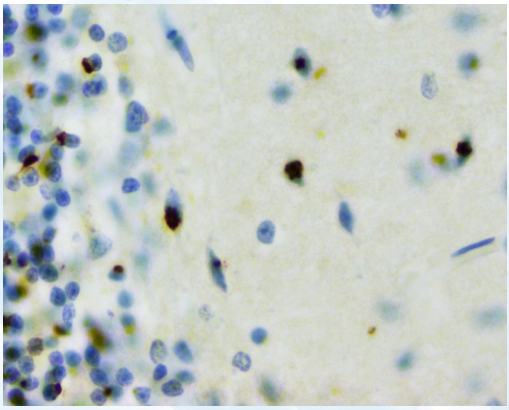

Figure 2: Star and ring shaped neuronal cytoplasmic Poly-GA inclusions in the cerebellar cortex.

- We found 100% accuracy confirming cases with known C9orf72 repeat expansion (n=26).
- A further five cases were found to be positive that had not yet undergone clinical genetic screening. Two cases were subsequently screened and demonstrated a positive result.
- Of the last three cases that did not undergo genetic screening, one had a sibling with C9orf72 mutation and the other two had no known genetic testing results or family history.

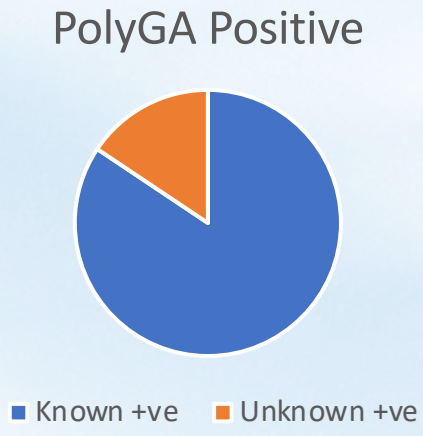

Figure 3: Representation of previously unknown +ve C9orf72 mutation.

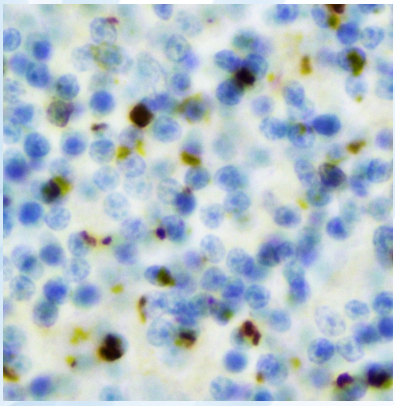

Figure 4: Star and ring shaped neuronal cytoplasmic Poly-GA inclusions in the granular cell layer of the cerebellum.

CONCLUSION

- PolyGA immunohistochemistry of postmortem human brain tissue serves as a useful tool to accurately identify the presence of dipeptide repeat pathology due to defects in the C9orf72 gene.
- We have demonstrated the polyGA antibody to be a reliable and sensitive method of detection in a research setting, with high utility in cases where there has been no opportunity for genetic testing during the life of the patient.

REFERENCES

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