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GGC Repeat Expansion in *NOTCH2NLC* is rare in European Leukoencephalopathy

Running head: No novel GGC expansion in European Leukoencephalopathy

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We read with interest that Okubo et al. identified GGC repeat expansion in *NOTCH2NLC* as the most frequent genetic cause of adult-onset leukoencephalopathy in Japan[1]. After the initial discovery of the causative mutation for neuronal intranuclear inclusion disease (NIID)[2, 3], all additional reported NIID patients with this mutation are East Asians. Adult-onset genetic leukodystrophy is clinically and radiologically heterogeneous, making definitive diagnosis challenging. We aim to screen for this novel expansion in a European cohort of adult-onset leukoencephalopathy.

Our cohort consists of 52 adult-onset leukoencephalopathy patients with unknown genetic diagnosis. The patients underwent appropriate investigations to exclude acquired causes, severe small vessel diseases and neuro-metabolic disorders. This was followed by whole-exome sequencing, the protocol of which was previously described[4]; no pathogenic mutation was identified. They underwent screening using repeat-primed polymerase chain reaction (RP-PCR) following published protocol[3] (Fig 1 B, D). To avoid false negative results, we repeated the RP-PCR in triplicate with three different positive controls. In patients without the expansion, we estimated their GGC repeat sizes by amplifying the genomic DNA region containing the repeat using PCR primers specific for *NOTCH2NLC*[3] (Fig 1 C, E). All patients were from the National Hospital for Neurology and Neurosurgery (NHNN) in the United Kingdom (UK) and were clinically assessed by neurologists with interest in leukodystrophy.

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The study was approved by the joint ethics committee of UCL institute of Neurology and NHNN, UK (UCLH: 04/N034).

In our leukoencephalopathy cohort, we did not identify any patient with the GGC repeat expansion in *NOTCH2NLC*. The estimated repeat sizes ranged from 12 to 26 in our patients (Fig 1A). The main weaknesses of our study include: (1) our modest sample size; (2) single centre recruitment in the UK. Despite of these limitations, our study demonstrates that this novel expansion is likely to be rare in European leukoencephalopathy patients. NIID may have a founder effect in East Asian population, similar to spinocerebellar ataxia type 31, which is a rare form of ataxia with repeat expansion worldwide but a very frequent cause in Japan[5]. *CSF1R* remains the most common genetic cause of adult-onset leukoencephalopathy with European descent[4]. Further studies are required to establish the exact prevalence of *NOTCH2NLC* repeat expansion and its role in the aetiology of progressive dementing disorders with white matter changes in the European and other ethnic populations.

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Author contributions

WYY, RS, NWW and HH contributed to conception and design of the study; WYY, RS, DSL, JV and ZC contributed to the acquisition and analysis of data; WYY and RS contributed to drafting the text and preparing the figures.

Potential conflicts of interest

The authors declare no competing interests.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figure legend

Figure 1. (A) Distribution of the GGC repeat length of *NOTCH2NLC* in the 52 adult-onset leukoencephalopathy patients from the UK. (B, D) Representative electropherogram of RP-PCR analysis in a positive control showed a characteristic sawtooth appearance compared to a patient without the expansion. (C, E) Fragment analysis of a positive control only captured

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one non-expanded allele (~19 GGC repeats). In contrast, two alleles were captured in a patient from our UK cohort (~15/23 GGC repeats).

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