GGC Repeat Expansion in *NOTCH2NLC* is rare in European Patients with Essential Tremor

Short running title: The novel GGC expansion is not found in European ET

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Sir,

We read with interest that Sun *et al*. identified GGC repeat expansion in the 5' region of *NOTCH2NLC* gene in Chinese patients with essential tremor (ET)[1]. Recently, two independent research teams have shown that this novel repeat expansion is the causative mutation for neuronal intranuclear inclusion disease (NIID)[2, 3]. NIID is a progressive neurodegenerative disease with the pathological hallmarks of eosinophilic ubiquitin-positive and p62-positive intranuclear inclusions in both central and peripheral nervous systems, and

other tissue organs[4-6]. Patients can present with either dementia-dominant or weakness-dominant subtypes[6]; a Parkinson Disease phenocopy has also been recently described[7]. Tremor is present in approximately one-third of these reported cohorts carrying the GGC repeat expansion[3, 8-10], although it invariably manifests with other neurological features such as leukoencephalopathy and cognitive impairment.

In contrast to NIID, ET is a clinical syndrome defined as an isolated tremor syndrome of bilateral upper limb action tremor of at least three years' duration[11]. A new addition to the classification is essential tremor plus (ET-plus)[11]: ET with additional neurological signs of uncertain significance such as impaired tandem gait, ambiguous dystonic posturing or memory impairment. There is no definitive radiological or pathological marker for the ET syndromes; and only a handful of genetic variants are reported in single families and none has been reproducible[12]. The finding of 11/197 (5.58%) Chinese ET pedigrees carrying the GGC repeat expansion suggests that this mutation may play a significant role in the genetics of ET[1]. Given all the patients with the GGC repeat expansion in *NOTCH2NLC* reported in the literatures are East Asians, we are interested to establish the prevalence of this mutation in a European ET cohort.

We analysed 111 index ET patients of European descent who were recruited from the National Hospital for Neurology and Neurosurgery (NHNN). All patients were clinically assessed by neurologists who have interests in neurogenetics and movement disorders. The distinction between ET and ET-plus was made via retrospective review of patients' clinical records. The study was approved by the joint ethics committee of UCL institute of

Neurology and NHNN, UK (UCLH: 04/N034). To test for the presence of GGC repeat expansion at *NOTCH2NLC*, we carried out repeat primed PCR (RP-PCR) on all patients' genomic DNA based on published protocol[2], followed by fragment length analysis on an ABI 3730xl DNA analyser with a GeneScan 500 LIZ Size Standard (Thermo Fisher Scientific) and GeneMapper software (version 5.0, Scientific)(Sup Table 1)(Sup Fig 1A,C). To avoid false negative results, we repeated the RP-PCR in duplicate with two 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*[2] (Sup Fig 1B,D).

In our cohort of 111 index European patients with ET, 35 patients had a family history of autosomal dominant inheritance. "Pure" ET subgroup comprised 74 patients whilst ET-plus subgroup comprised 37 patients. In the latter subgroup, 62% had ambiguous dystonic posturing, 32% had impaired tandem gait, and 5% had equivocal parkinsonism. Table 1 outlines the demographic data of the cohort. In our screening using RP-PCR, we did not identify any patient carrying the GGC repeat expansion in *NOTCH2NLC*. The repeat sizes of our patients ranged from 9 to 33, with an average of 19.44 ± 4.02 (Fig 1).

The absence of a positive screening result in our cohort infers that this mutation is unlikely a major contributor to the genetic aetiology of ET in the Europeans. The ethnic difference in mutation prevalence may be a result of a founder effect in East Asian populations. Another example of this is dentatorubral-pallidoluysian atrophy, a CAG repeat expansion disorder, which has a relatively high prevalence in Japan but rare elsewhere[13]. The GGC repeat sizes

of our cohort is similar to those in the Chinese ET patients without expanded GGC repeats. The weaknesses of our study include: (1) our cohort size may not be sufficiently large, especially for familial ET patients; (2) we retrospectively classified patients into the ET and ET-plus subgroups. However, we would still expect to identify patients carrying the GGC repeat expansion in *NOTCH2NLC* if the prevalence of this mutation is similar in Chinese and European cohorts of ET patients. Further studies with larger cohorts of European patients will help us to better define the role of this mutation for ET, NIID and other movement disorders outside of Japan and China.

	All Probands	ET	ET-plus
n	111	74	37
Sex, female, n (%)	55 (49.5)	42 (56.8)	13 (35.1)
Age at onset, mean (ranges) in years	42.4 (10-70)	45.5 (15-52)	36 (13-70)
Tremor			
Upper limbs, n (%)	111(100)	74 (100)	37 (100)
Lower limbs, n (%)	12 (10.8)	7 (9.5)	5 (13.5)
Head, n (%)	62 (55.9)	35 (47.3)	27 (73)
Voice, n (%)	21 (18.9)	15 (20.3)	6 (16.2)
Ataxia, n (%)*	12 (10.8)	0	12 (32.4)
Parkinsonism, n (%)*	2 (1.8)	0	2 (5.4)
Dystonia, n (%)*	22 (19.8)	0	22 (59.5)
Memory impairment, n (%)*	0	0	0
Family History, n (%)	33 (29.7)	23 (31.1)	10 (27)

Table

Table 1. Demographic data of European essential tremor cohort screened.

Figure

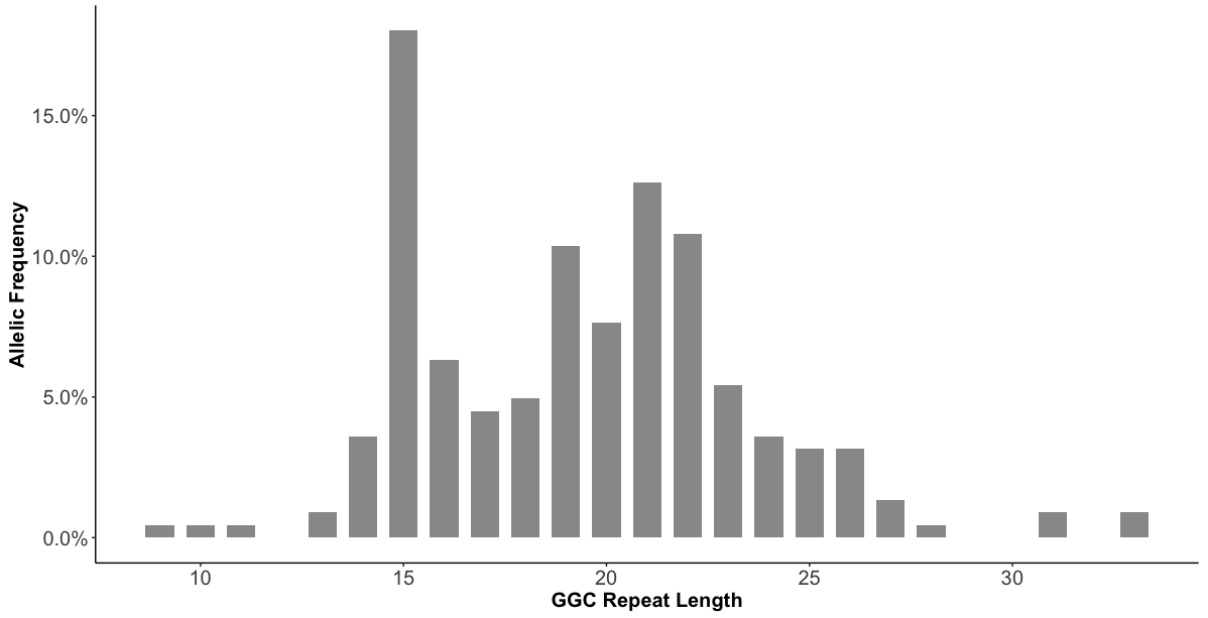

Figure 1. Distribution of the GGC repeat length of *NOTCH2NLC* in the 111 patients with essential tremor from the UK.

Data availability

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

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Competing interests

The authors declare no competing interests.

Supplementary materials

Supplementary figure 1. (A, C) Representative electropherograms of RP-PCR analysis in a positive control shows a characteristic sawtooth appearance as compared to a patient without the expansion. (B, D) Fragment analysis of a positive control only captured one non-expanded allele (~19 GGC repeats). In contrast, two alleles were captured in a patient in our UK cohort (~15/28 GGC repeats).

Supplementary table 1. Primers sequences and thermocycling conditions

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