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Title: A new family with *GLRB*-related hyperekplexia showing chorea in homo- and heterozygous variant carriers

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Main text:

The startle reflex consists of a rapid and generalized motor response to a sudden, unexpected stimulus, which is thought to arise in the caudal reticular formation [1]. Exaggerated startle reflexes, consisting of excessive motor response, no habituation to stimuli and sometimes generalized stiffness, is defined as hyperekplexia.

Although exaggerated startle reflexes can be part of neurodevelopmental syndromes with intractable epilepsy and psychomotor retardation, hereditary hyperekplexia (HPX) is strictly considered a genetically determined glycinergic synaptopathy showing increased startle and continuous or episodic muscle stiffness, with neonatal onset and response to clonazepam [2].

HPX is caused by mutations in genes encoding the α_1 (*GLRA1*) and β (*GLRB*) subunits of the postsynaptic glycine receptor (GlyR) or the presynaptic glycine transporter 2 (*SLC6A5*) [2]. Monoallelic or, less frequently, biallelic *GLRA1* mutations account for the majority of cases of classic HPX. Autosomal recessive (AR) or dominant (AD) mutations of glycine transporter 2 (*SLC6A5)* are the second most common cause of HPX, often combined with delayed development and apneic spells. *GLRB*-related HPX is the rarest and clinically similar to *SLC6A5*-HPX apart from ocular misalignment, which is a frequent characteristic [3].

Here, we present a family where the index cases developed HPX and chorea associated with a homozygous pathogenic variant in *GLRB*, whereas both parents and one brother, all heterozygous carriers, manifested only chorea without hyperekplexia.

Two female monozygotic twins (subjects II-3 and II-4) born to non-consanguineous parents (Figure 1A) developed, since three weeks after birth, constant hypertonia and daily episodes of hyperekplexia with associated stiffness and falls in response to auditory or tactile stimuli. Both twins also presented occasional cyanotic attacks. Clonazepam decreased the frequency of the startle response. Subject II-4 had corrected ocular misalignment. They were first examined at our center at 18 years of age reporting weekly episodes of falls in response to unexpected stimuli together with hand cramps, leg stiffness and jaw opening difficulty. Neurological examination showed trismus, facial weakness and positive head retraction reflex (HRR). In addition, both twins had distal jerky choreic movements in the hands (Video 1). These movements were flitting, unpredictable and not stimulus-sensitive. There were no cerebellar features. Brain MRI and EEG were normal. Neurophysiological evaluation of subjects II-3 and II-4 disclosed pathological startle in response to auditory stimuli (characterized by typical cranial and limbs muscles pattern of activation, short-onset latency of EMG responses, long duration of EMG bursts and no habituation after 20 auditory stimuli, at the interval of 45-60s). Surface EMG (ECR, FCR, ADM, FDI muscles) showed intermittent random bursts of EMG muscular activity, as seen in chorea. There was no evidence of short duration and cranial-caudal progression of the EMG burst, SEP were normal and C-reflex absent. All this evidence makes it unlikely that the involuntary movements are cortical myoclonic in origin. We examined family members; the older brother (subject II-1) was asymptomatic, whereas the middle brother (subject II-2, Video 1), the father (subject I-1, Video 1) and the mother of the probands (subject I-2) showed mild distal chorea without HRR. In all these family

members, movements were not stimulus-sensitive and did not increase with action. Subject I-1 also had chorea of toes (Video 1).

Sequence analysis of genes *GLRA1*, *SLC6A5*, and *GLRB* in subject II-3 detected the homozygous intronic variant NM_000824.4:c.610+5G>A in *GLRB* which was previously reported as pathogenic [4]. Sanger sequencing revealed that her twin sister (subject II-4) was homozygous for the variant while their father (subject I-1), mother (subject I-2), and the younger brother (subject II-2) were heterozygous. The unaffected eldest brother, without any chorea, was the only one that did not carry the mutation (Figure 1). While we cannot completely exclude a distinct genetic cause of chorea, and did not perform additional genetic analyses, there were no additional neurologic, systemic, psychiatric or radiological features of autosomal dominant or X-linked conditions associated with chorea.

Less than 30 patients with *GLRB*-HPX have been reported, with five cases carrying the c.610+5G>A variant [3]. Although both AR and AD patterns of inheritance have been described in *GLRB*-HPX, an AR mode of inheritance is much more common [2]. Moreover, altered function of the GlyR channel with reduced current amplitude has been reported in heterozygous carriers of a mutation in *GLRB* presenting with mild startle symptoms, supporting incomplete dominance as one of the explanations for the minor form of hyperekplexia [5]. However, heterozygous carriers in our family did not present startle, but only chorea, denoting the contribution of the GlyR in the pathophysiology of this movement disorder.

Although GlyR is widely expressed in the brainstem and spinal cord, immunohistochemical investigations have also demonstrated the presence and distinctive distribution of GlyR in the human striatum, globus pallidus and sustantia nigra[6]. Moreover, application of glycine in the striatum of rats causes a release of dopamine which is blocked by strychnine[7], supporting a role of GlyR in modulating dopaminergic neuronal connections in the basal ganglia and possibly explaining the presence of chorea in our patients.

In summary, we reported a family investigated for HPX in which distal chorea was observed in both homozygous and heterozygous carriers of a pathogenic variant in *GLRB*. It is uncertain why patients present with this phenomenology, which might expand the phenotype related to *GLRB* mutations, thus prompting clinicians to be on the lookout for additional movement disorders in *GLRB*-HPX pedigrees.

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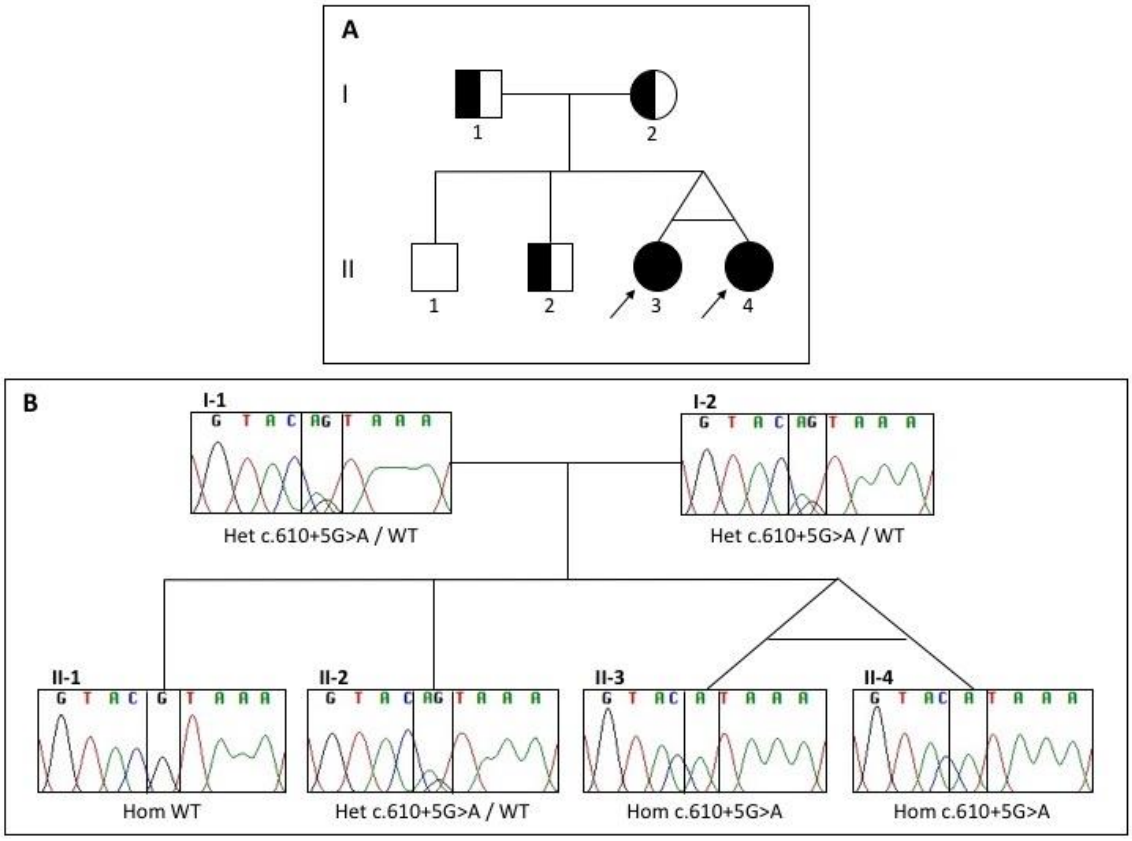
Figure 1 – Family tree and Sanger sequencing chromatograms of the family reported.



(A) Pedigree of the family with the *GLRB* mutation. Arrows = probands. Filled-in symbols indicate homozygous subjects presenting with hyperekplexia and chorea, while half-filled symbols represent heterozygous subjects showing chorea only. (B) Sequencing chromatograms demonstrate the homozygous NM_000824.4:c.610+5G>A variant of *GLRB* in the probands (II-3 and II-4) and the heterozygous state in their parents (I-1 and I-2) and one of their brothers (II-2). The region was amplified using the following primers: 5'-actctttcatgcccttggac-3' and 5'-caaagcactagttttaccactgt-3'. Electropherograms were analyzed using the Sequencher software package. Het = heterozygous; Hom = homozygous; WT = wild type.

Video 1

The subjects included in the video (II-4, II-3, II-2 and I-1) gave signed consent to be videoed for publication including online publication. Subject I-2 did not consent to be filmed.

Video 1 – Subject II-4 shows choreic movements in both arms, exaggerated head-retraction reflex to tactile stimuli in the face and the nose without habituation.

Video 1 – Subject II-3 has very similar neurological exam to subject II-4.

Video 1 – Subject II-2 presents mild chorea of the upper limbs

Video 1 –Subject I-1 presents mild chorea in upper and lower limbs.