Parkinsonism and Related Disorders 105 (2022) 128–131

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Short communication

Four Turkish families with hyperekplexia: A missense mutation and the exon 1–7 deletion in the *GLRA1* gene

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ARTICLE INFO

Keywords:
Hyperekplexia
GLRA1
Genetic counseling
Turkish mutations

ABSTRACT

Background: Hyperekplexia is a disease that progresses with excessive startle attacks and is included in the differential diagnosis of epilepsy and many movement disorders.
Methods: The WES results were validated in available family members by Sanger sequencing, or in the case of deletion, PCR followed by agarose gel electrophoresis was performed.
Results: WES analysis revealed the previously reported homozygous c.277C>T p.Arg93Trp variant in the *GLRA1* gene (ENST00000455880.2) in Family 1. In all other three families, the previously reported homozygous deletion of exons 1–7 of the *GLRA1* gene was identified using CNV analysis based on the WES data.
Conclusions: The homozygous exon1-7 deletion has been described several times in different populations and may be a founder mutation in the Kurdish people in Turkey. The family with Arg93Trp variant stems from the Black Sea region of Turkey where close consanguinity is common. These analyses are important to provide genetic counseling to families and for a better understanding of the pathophysiology of the disease.

1. Introduction

Hyperekplexia is a rare neurological disorder clinically characterized by an exaggerated startle reaction, neonatal hypertonicity, or generalized stiffness [1]. Hyperekplexia is classified as hereditary, sporadic or symptomatic. The hereditary form of hyperekplexia is caused by various mutations in glycine metabolism. In the normal startle reaction, the receptors involved are located in the brainstem and spinal cord. Hereditary hyperekplexia is divided into major and minor forms. The major form is characterized by generalized stiffness after birth, and can be defined as an exaggerated startle reflex accompanied by temporary falls, whereas in the minor form only excessive startle and hypnic jerks have been described [2]. Five genes have been shown to be associated with hyperekplexia so far, namely *GLRA1*, *GLRB*, *SLC6A5*, *GPHN* and *ARHGEF9*. Both autosomal dominant and recessive modes of inheritance have been demonstrated in *GLRA1* and other genes [2]. We here report four independent Turkish families with hyperekplexia, which stems from *GLRA1* mutations.

2. Methods

Twelve patients from four different families who suffer from hyperekplexia are the subject of this report. Five patients from Family 1 and 2 were examined in Cerrahpasa School of Medicine, the patient in Family 3 was examined in Istanbul Faculty of Medicine. All patients in generations IV. and V. of Family 4 were examined in the Haseki Training and Research Hospital, but only the patients who consented to genetic testing are listed in Table 1. Laboratory and imaging studies of the patients were performed in the above hospitals, and their follow-up continues except patient IV-1 in Family 1. Genomic DNA was extracted from

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https://doi.org/10.1016/j.parkreldis.2022.11.011
Received 30 June 2022; Received in revised form 1 November 2022; Accepted 7 November 2022
Available online 15 November 2022
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Table 1
Compiles the clinical data of the affected individuals in four families. Good response: quite satisfactory, not disturbing the routine life; moderate response: easy to watch from the outside, but not causing dramatic problems; minimal response: not satisfactory; no response.

Family	Patient number	Age/sex	Type of birth	Perinatal problem	Symptoms	Symptom onset	Neurological examination	Consanguinity in parents	Motor development	Cognitive level	Psychiatric condition	Seizure type and frequency	EEG findings	Imaging findings	Treatment dosage/time	Benefit of treatment	Other treatments	Follow up time in clinic	Duration of illness	Current symptoms	Candidate Gene/Mutation
1	IV-9	27/M	C-section	No	Generalized stiffness, excessive startle and drop attacks with sudden noise	First day of neonatal term	Increased deep tendon reflexes Spontaneous horizontal nystagmus	Yes	Normal	Normal	Social withdrawal for fear of falling	No seizure	Normal background activity	Sequelae of right parietooccipital subdural hemorrhage	1.2 mg/d of clonazepam/2 years	Good response	Valproate, levetiracetam	2 years	27 years	symptom free under treatment	GLRA1 (ENST00000455880.2) p.Arg93Trp (c.277C>T)
	IV-10	22/M	C-section	No	Generalized stiffness, excessive startle and drop attacks with sudden noise	First day of neonatal term	Increased deep tendon reflexes Spontaneous horizontal nystagmus	Yes	Normal	Normal	Social withdrawal for fear of falling	No seizure	Normal background activity	Normal MRI	1 mg/d of clonazepam/2 years	Good response	Valproate, levetiracetam	2 years	22 years	symptom free under treatment	GLRA1 (ENST00000455880.2) p.Arg93Trp (c.277C>T)
	IV-1	40/M (No other information due to not following)						Yes													GLRA1 (ENST00000455880.2) p.Arg93Trp (c.277C>T)
2	IV-2	14/M	Spontan vaginal	No	Generalized stiffness, excessive startle and drop attacks with sudden noise	First day of neonatal term	Increased deep tendon reflexes	Yes	Normal	Normal	Social withdrawal for fear of falling	No seizure	Not done	Not done	0.6 mg/d of clonazepam/14 years	Moderate response	None	14 years	14 years	non-injurious falls, triggered by very loud noise, at an average frequency of once a month	GLRA1 (ENST00000455880) homozygous exon1-7 deletion
	IV-1	16/F	Spontan vaginal	No	Generalized stiffness, excessive startle and drop attacks with sudden noise and tactile stimulus	First day of neonatal term	Increased deep tendon reflexes	Yes	Normal	Normal	Normal	No seizure	Normal background activity	Not done	0.2 mg/d of clonazepam/16 years	Good response	Clobazam	16 years	15 years	symptom free under treatment	GLRA1 (ENST00000455880) homozygous exon1-7 deletion
	IV-3	5/F	Spontan vaginal	No	Generalized stiffness, excessive startle with sudden noise	First day of neonatal term	Normal	Yes	Normal	Normal	Normal	No seizure	Not done	Not done	0.1 mg/d of clonazepam/5 years	Good response	None	5 years	5 years	symptom free under treatment	GLRA1 (ENST00000455880) homozygous exon1-7 deletion
3	V-3	24/F	C-section	No	Excessive startle reaction and generalized stiffness with sudden noise or touch	First 15 days of life	Normal	Yes	Normal	Normal	Normal	No seizure	Normal background activity, and rare hyperexcitability in the left central region	Benign subarachnoid widening in MRI	1.5 mg/day clonazepam	Moderate response	None	23 years	24 years	Especially if the middle part of the face is touched suddenly, a mild startle response is obtained in the head and upper body (nose, mustache area is remarkable)	GLRA1 (ENST00000455880) homozygous exon1-7 deletion
4	V-1	21/M	Spontan vaginal	No	Generalized stiffness with sudden tactile and auditory stimuli, many times a day until medication starts Drop attacks when medication is interrupted Excessive startle reaction	First day of neonatal term	Increased deep tendon reflexes	Yes	Mild retardation in motor development in childhood	Mild cognitive deficite	Unable to work in a long-term job due to his aggression	No seizure	Normal background activity	Normal MRI	2 mg/day clonazepam	Good response	Valproate in infancy	21 years	21 years	symptom free under treatment	GLRA1 (ENST00000455880) homozygous exon1-7 deletion
	V-2	17/F	Spontan vaginal	No	Excessive startle and drop attacks with sudden noise	around 1 year old	Normal	Yes	Normal	Normal	Normal	No seizure	Normal background activity	Not done	0.3mg/day clonazepam	Good response	None	16 years	16 years	symptom free under treatment	GLRA1 (ENST00000455880) homozygous exon1-7 deletion
	V-3	14/M	Spontan vaginal	No	Excessive startle and drop attacks with sudden noise	around 1 year old	Normal	Yes	Normal	Normal	Normal	No seizure	Normal background activity	Not done	0.6mg/day clonazepam	Good response	None	13 years	13 years	symptom free under treatment	GLRA1 (ENST00000455880) homozygous exon1-7 deletion
	IV-14	40/F	Spontan vaginal	No	Excessive startle with sudden noise	childhood	Normal	No	Normal	Normal	Normal	No seizure	Normal background activity	Not done	No treatment startles do not affect daily life		None	21 years	21 years	startles that do not affect daily life	GLRA1 (ENST00000455880) homozygous exon1-7 deletion
	IV-16	20/F	Spontan vaginal	No	Excessive startle and drop attacks with sudden noise	around 1 year old	Normal	No	Normal	Normal	Normal	No seizure	Normal background activity	Not done	0.6mg/day clonazepam	Good response	None	19 years	19 years	symptom free under treatment	GLRA1 (ENST00000455880) homozygous exon1-7 deletion

peripheral blood using the MagNA Pure DNA extraction kit. WES analysis was applied to index cases only (Macrogen, Korea) and the SEQ platform (Genomize, Istanbul, Turkey) was used for bioinformatic evaluation. WES results were validated in available family members by Sanger sequencing, or in the case of deletion, PCR followed by agarose gel electrophoresis was performed. Written consent was obtained from all patients (see Fig. 1).

3. Results

WES analysis revealed the previously reported homozygous c.277C>T p.Arg93Trp variant in the *GLRA1* gene (ENST00000455880.2) in Family 1. Sanger sequencing showed that all the affected offspring have the mutation in the homozygous state while the parents are as expected heterozygous carriers. In all other three families, the previously reported homozygous deletion of exons 1–7 of the *GLRA1* gene was identified using CNV analysis based on the WES data. The deletion of exons 1–7 was shown by the absence of a PCR product in agarose gel electrophoresis (see Fig. 2).

4. Discussion

All our patients show the classic triad of hyperekplexia. In the wider sense, the differential diagnosis of hyperekplexia includes epilepsy, paroxysmal movement disorders, some cortical myoclonic attacks, syncope and somatoform disorders. The attacks may falsely be interpreted as epilepsy and wrong treatment can be prescribed [1]. Particular attention should be paid to widespread stiffness in the neonatal period and exaggerated startles in childhood as well as in adulthood. Nose tapping test may be useful for the diagnosis of the condition in the neonatal period. Diffuse hypertonia seen in the neonatal period may cause apnea attacks and the startle reaction that occurs after the tactile stimulus, while feeding, may lead to aspiration. During an attack, the Vigevano maneuver, forcefully flexing the baby, may be effective in preventing the startle reaction.

One of our patients (Family 4, patient IV-14) had neurodevelopmental delay. However, tests and examinations carried out on this patient did not reveal the presence of any other disease manifestation. This situation may be associated with an additional disease, related to multiple consanguineous marriage in the family or this picture may be the result of the *GLRA1* mutation. *GLRA1* cases may be accompanied by speech delay or learning difficulties [3]. In a recent review, it was reported that developmental delay caused by homozygous mutations is seen in fewer cases than in heterozygotes, but severe cognitive involvement is seen in homozygotes [2]. There was no difference between the phenotypes of our patients neither in Family 1 with a missense mutation, nor in the other three families with the exon 1–7 deletion. Although complete loss of the glycine receptor has been shown to be fatal in animal studies, there was no clinically significant difference with partial loss of function of the receptor in humans [4].

Serious injuries because of falls due to startle reaction and psychological problems, such as anxiety or social isolation in adulthood, show the importance of treating hyperekplexia. Though rare, hyperekplexia is a treatable disorder. Clonazepam, which acts by facilitating the binding of the inhibitory neurotransmitter GABA, probably by compensating defective glycine receptors, is the main and most effective treatment for the patients with hyperekplexia [1]. We were not able to judge the effectiveness of the clonazepam in only two of our 12 patients. One of these patients could not be followed up, and the other did not use medication on the grounds that startles did not affect her daily life (Table 1). The symptoms of other patients were found to be acceptably under control (good and moderate response) with clonazepam treatment, which was consistent with two previous studies [1,3].

The homozygous exon 1–7 deletion has been described several times in different populations and may be a founder mutation in the Kurdish people in Turkey [5,6]. Also, our three families described in this report with the exon 1–7 deletion have Kurdish origin. 500 individuals from the southeastern Mediterranean region, belonging to four distinct ethnic groups were screened, and no heterozygosity was found for this mutation [5]. The ethnicity distribution of the dataset were Caucasian (37.8%), Turkish (27.8%), Asian (15.5%), Arabic (13.3%), Jewish (4.4%) and African-American (1.1%); 68% had recessive GLRA1 hyperekplexia [7]. The homozygous deletion is classified as pathogenic in Varsome.

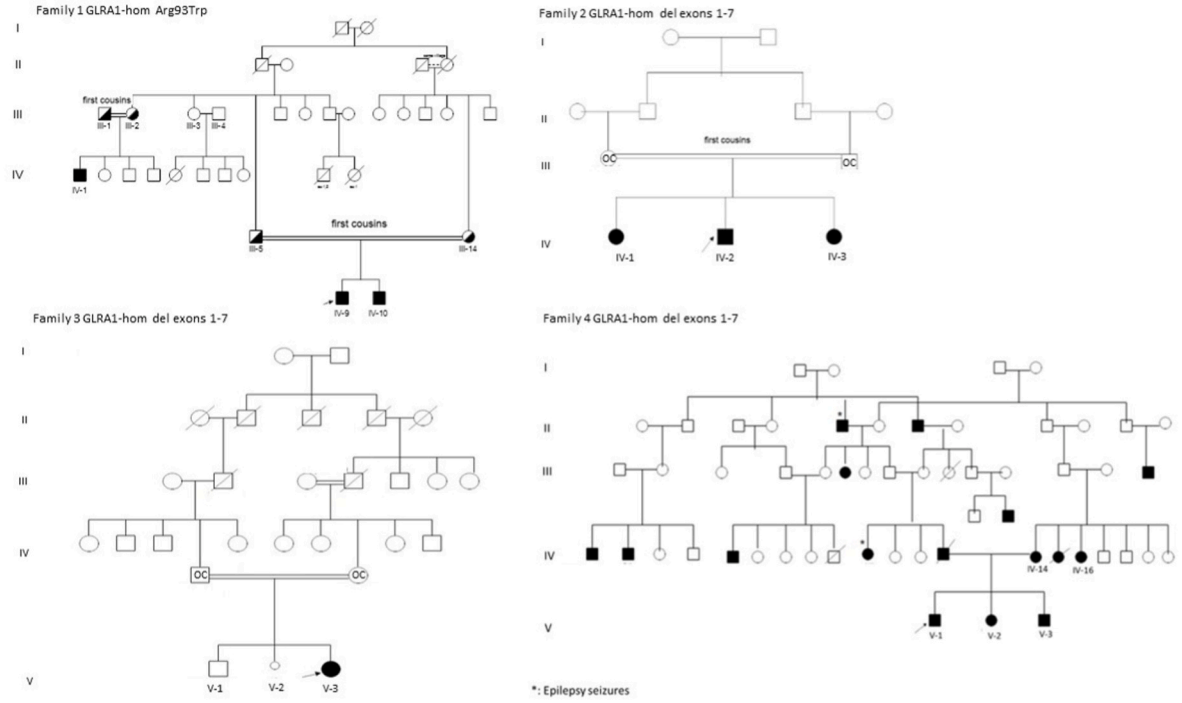


Fig. 1. Pedigrees of families.

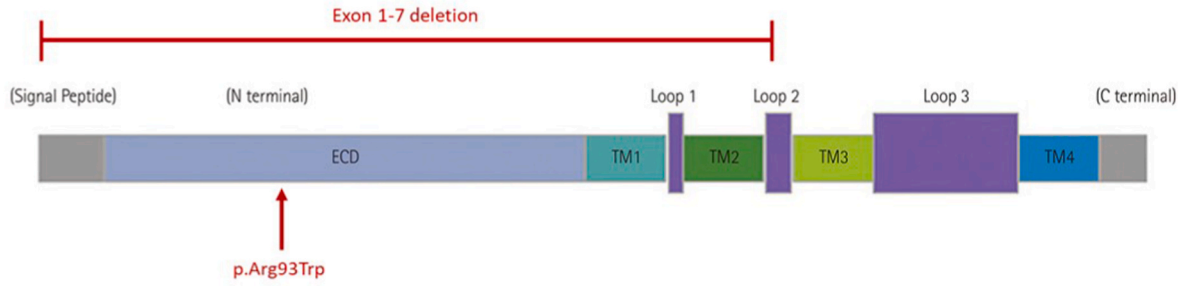


Fig. 2. A lollipop diagram depicting the mutations for our GLRA1 variants

The family with Arg93Trp variant stems from the Black Sea region of Turkey where close consanguinity is common. The Arg93Trp variant is classified as likely pathogenic in Varsome (www.varsome.com) and in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). The DANN score is 0.9986, while the conservation tool GERP generates 5.38. The CADD score for the variant is 24.7. MutationTaster, SIFT, and REVEL classify this variant as disease-causing, damaging, and pathogenic, respectively. This mutation has only been reported once in an autism cohort and the ethnicity and further phenotypic details of the apparently homozygous patient is not known [8]. Total allele frequency for this variant in GnomAD (https://gnomad.broadinstitute.org) is 0.00001193 although no homozygotes are reported. The total allele count is three, two being non-Finnish European and one South Asian. Thus, our homozygous p. Arg93Trp is the first description of this variant in association with hyperekplexia.

Investigation of the genetic defects in hyperekplexia patients is important in terms of giving genetic counseling to the families, understanding the pathophysiology and determining the prevalence of the disease in the population.

Author roles

DT: Organization, Writing of the First Draft. GŞ: Organization, Writing of the First Draft. ÖÇ: Conception, Organization, Execution, Review and Critique. VD: Conception, Organization, Execution, Review and Critique. ANB: Conception, Organization, Execution, Review and Critique. ZY: Conception, Organization, Execution, Review and Critique.

Financial disclosures

Authors declare they have no relevant conflict of interest.

Acknowledgement

The genetic part of this study was conducted using the service and infrastructure of Koç University Translational Medicine Research Center (KUTTAM). We thank Suna and İnan Kirac Foundation and Koc University for their great support.

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