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Biallelic Start Loss Variant, c.1A>G in *GCSH* is Associated with Variant Nonketotic Hyperglycinemia

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Abstract

The glycine cleavage system H protein (*GCSH*) is an integral part of the glycine cleavage system with its additional involvement in the synthesis and transport of lipoic acid. We hypothesize that pathogenic variants in *GCSH* can cause variant nonketotic hyperglycinemia (NKH), a heterogeneous group of disorders with findings resembling a combination of severe NKH (elevated levels of glycine in plasma and CSF, progressive lethargy, seizures, severe hypotonia, no developmental progress, early death) and mitochondriopathies (lactic acidosis, leukoencephalopathy and Leigh-like lesions on MRI). We herein report three individuals from two unrelated Indian families with clinical, biochemical, and radiological findings of variant NKH, harboring a biallelic start loss variant, c.1A>G in *GCSH*.

Keywords

Variant NKH; nonketotic hyperglycinemia; glycine cleavage system; lipoic acid; *GCSH*

Introduction

Nonketotic hyperglycinemia (NKH) is caused by defects in the glycine metabolism leading to accumulation of glycine in all body tissues, including the brain. Classic NKH (also known as glycine encephalopathy, MIM# 605899) is caused by biallelic pathogenic variants in the mitochondrial glycine cleavage enzyme system (GCS) genes, *GLDC* and *AMT*.[1] Variant NKH is known to occur due to defects in the lipoate synthesis (*LIAS* and *LIPT2*) and iron-sulphur cluster biogenesis (*BOLA3*, *NFU1*, *ISCA2*, *GLRX5* and *IBA57*) affecting the glycine metabolism.[2-8]

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Conflict of interest

Authors declare no conflict of interest.

Glycine cleavage system H protein (*GCSH*) gene encodes for H protein which is involved in GCS as well as lipoate synthesis.[2] Though variants in *GCSH* have been proposed as a possible cause of NKH, till date there are no published reports in the literature.[1,9,10] We herein report three individuals from two unrelated Indian families with variant NKH and a biallelic variant in *GCSH*.

Methods and Results:

Clinical report

Proband 1 (P1) is a three-year-old male, first born to non-consanguineous parents, from family 1 (Figure 1A). He was apparently well till day 32 of life after which he developed excessive crying, lethargy and poor feeding. He had not attained any developmental milestones. He had myoclonic seizures which were controlled using anti-epileptic drugs (AEDs). At three years, his weight was 13kg (−0.6 SD), length was 96cm (0 SD), and head circumference was 45cm (−5 SD). High-performance liquid chromatography at one year of age showed elevated levels of plasma glycine (1,332nmoles/ml; ref: 81-436nmoles/ml), CSF glycine (99nmoles/ml; ref: 2.20-14.20nmoles/ml) and CSF glycine/plasma glycine ratio (0.07; ref: 0.01-0.04). Brain imaging findings at day 45 and one year are depicted in Figure 2A. MR spectroscopy (MRS) at one year obtained from the putamen at TE 30 revealed a small lactate peak.

We ascertained two female siblings, born to non-consanguineous parents, from family 2 (Figure 1B). Proband 2 (P2) became symptomatic after the first 48 hours of life with poor feeding, hypotonia, lethargy and seizures. Blood investigations showed hypernatremia and persistent metabolic acidosis. She continued to have recurrent seizures despite multiple AEDs. Elevated arterial blood lactate levels (9.4mmol/L; ref: 0.5-1mmol/L) and metabolic acidosis were observed at two months of age. Imaging findings of MRI and MRS performed at 3 months are shown in Figure 2B. Until one year, she had not achieved any developmental milestones and was tube fed. Thin layer chromatography of amino acids in urine performed at ten months showed presence of glycine. She succumbed at one year ten months due to resistant seizures.

Proband 3 (P3) had severe respiratory acidosis, seizures, hypotonia and poor respiratory efforts on day 3 of life. Parents decided to discontinue the life support on day 6 of life. She succumbed on day 10. Plasma lactate (day 4) was found to be normal. MRI of the brain on day 4 is depicted in Figure 2C. Detailed clinical and neuroimaging findings are provided in the ‘clinical report’ section of the supplementary material.

Genetic analysis

Informed consent approved by the institutional ethics committee was obtained from the families. Trio exome sequencing (ES), performed in family 1 revealed the presence of start codon variant, c.1A>G, p.(?) in *GCSH* (NM_004483.5) in a homozygous state in P1. This region was poorly covered with a total of seven reads supporting c.1A>G change in P1 (Figure 1D). Sanger validation of the variant c.1A>G, confirmed this variant in homozygous state in P1 (Figure 1C) and in heterozygous state in parents.

Singleton ES was performed in family 2 for P3. A start codon variant, c.1A>G, p.(?) in *GCSH* in the homozygous state was identified in concordance with the observed phenotype in P3 (Supplemental table S1-S2). Segregation analysis and Sanger validation showed the presence of this variant in homozygous state in P2 and in heterozygous state in parents (Figure 1E). Information on allele frequency of this variant and results of *in silico* prediction tools have been provided under 'methods and results section' of supplementary material.

Homozygosity mapping, performed for family 1 and 2 showed a shared 490kb (Chr16:80718879-81213378) region of homozygosity flanking this variant (Supplemental table S4). The estimated pairwise kinship coefficient is 0.0201 indicating that these are unrelated individuals.

Discussion

GCSH gene on chromosome 16, spans 13.5 kb region and is composed of five exons. It encodes for H protein, part of the multiprotein GCS complex. It has a lipoyl-binding domain in exon 1 (between 66-148 amino acids) which covalently binds to LA. GCS consists of P protein, T protein, H protein and L protein encoded by *GLDC, AMT, GCSH* and *DLD* respectively.[11] GCS is involved in catabolic degradation of glycine into CO_2, NH_3 and methylene tetrahydrofolate.[12] H protein shuttles the methylamine group of glycine from the P protein to the T protein.[13] It also participates in the synthesis and transfer of LA from H protein to the E2 subunits of pyruvate dehydrogenase complex, alpha-ketoglutarate dehydrogenase and branched chain ketoacid dehydrogenase, as well as the E3-binding protein of pyruvate dehydrogenase complex.[2,14] Hence, defects in H protein are likely to cause mitochondrial dysfunction.

GCSH was earlier proposed as a candidate gene for NKH and variant NKH owing to its role in GCS and lipoate synthesis.[2,13] Hiraga et al (1981) showed that the activity of the H protein was significantly reduced as compared to the P protein in the liver and brain in an individual with NKH, leading to deficient GCS activity.[13] In two individuals with NKH, a deletion of 5 kb SacI fragment of the H protein cDNA probe was identified by southern blot analysis.[15] Kure et al (2002) reported a heterozygous acceptor splice site variant, c.425-1G>T in *GCSH* in a Japanese family causing transient neonatal hyperglycinemia.[9] A recent study has shown that homozygous *Gcsh* null mice undergo early embryonic lethality, prior to mid-gestation, suggesting the role of this gene beyond GCS.[16] Although *GCSH* has been sequenced previously in several families with NKH, no pathogenic variants have been documented in this gene till date.[1] This could be attributed to either extreme rarity of the condition, ethnic disparity of carriers for *GCSH* variants across different populations, and/or inadequate coverage of exon 1 in individuals tested by ES.

The variant, c.1A>G in *GCSH* occurs at the initiation site and thus likely to affect the translation of the GCSH protein. There are no other protein coding transcripts for this gene. In the absence of any other in-frame 'ATG' except in the last exon of *GCSH*, it is predicted that the translation machinery may recognize the out of frame 'ATG' present in the exon 2, which is likely to cause a shift in the reading frame and formation of a shortened protein with an altered amino acid sequence. The absence of consanguinity and a small size of

the shared region suggested the possibility of a founder effect rather than inbreeding. No pathogenic variants were identified in any other gene known to cause NKH in both families (Supplementary table S3).

Classic forms of NKH affect the GCS alone and most of the clinical manifestations result from accumulation of glycine. Individuals with severe forms of NKH have an early onset intractable epilepsy, hypotonia and developmental arrest.[17] Variant forms of NKH have an accumulation of glycine as well as lactic acidosis consequent to mitochondrial dysfunction.[2,5] Hence, the clinical presentation of these disorders is a combined phenotype of severe NKH and mitochondriopathies.[5-8] All probands presented in neonatal and early infantile periods with excessive crying, lethargy, poor sucking, poor respiratory efforts requiring ventilation, and intractable seizures. Most of the clinical manifestations in the present probands overlap with severe forms of classic/variant NKH.

In classic NKH, the elevation of CSF glycine, plasma glycine and CSF/plasma glycine ratio ranges from 40-510nmoles/ml, 342-2362nmoles/ml and 0.09-0.45, respectively.[18] However, individuals with variant NKH show milder elevation of glycine as compared to classic NKH with levels of CSF glycine, plasma glycine and CSF/plasma glycine ratio ranging from 15-90nmoles/ml, 400-1000nmoles/ml, and 0.02-0.09, respectively.[5] The elevated glycine levels observed in P1 are similar to those observed in classic forms of NKH. However, quantitative levels of glycine were not available in P2 and P3. Lactic acidosis along with presence of hyperglycemia as observed in individuals with variant NKH was seen in P1 and P2.[2,5]

The MRI findings seen in classic forms of NKH in the acute-phase include diffusion restriction in the posterior limb of internal capsule, lateral thalami, and in the dorsal aspect of midbrain and pons.[19] Few of the findings of classic NKH were observed in P3. The imaging features in variant NKH include Leigh-like lesions, leukodystrophy, secondary neurodegeneration, and increased lactate on MRS.[3-5] Leigh-like lesions and increased lactate on MRS were observed in P1 and P2.

Hence, the clinical, biochemical and neuroimaging findings observed in the individuals in our study are suggestive of variant NKH. The presence of this predicted pathogenic variant in three individuals from two unrelated families provides evidence for the association of *GCSH* with variant NKH. Functional experiments for the evidence of pathogenicity of the observed variant could not be carried out due to unavailability of further samples from the probands. Reporting of further families will help us to validate this novel disease-gene association.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Data availability

The data can be shared upon a reasonable request to the corresponding author.

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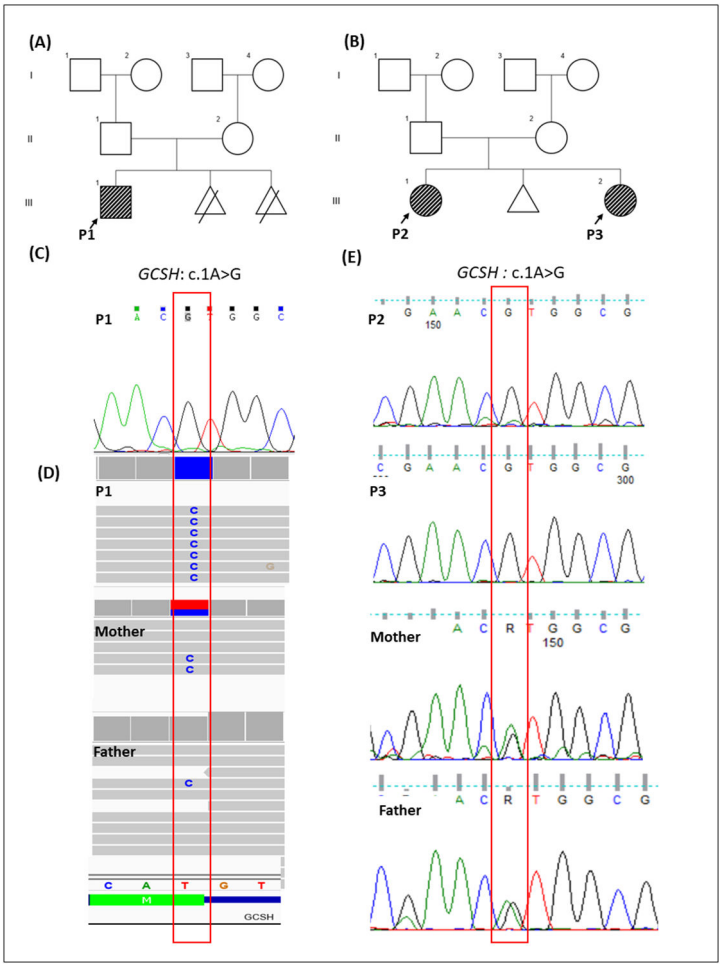

Figure 1:

(A) Pedigree of the family 1 **(B)** Pedigree of the family 2 **(C)** Sanger validation and bi-allelic segregation analysis of the variant c.1A>G in *GCSH* in P1 **(D)** Integrative Genomic Viewer (IGV) snapshots of family 1 showing the variant c.1A>G in homozygous state in proband, heterozygous state in mother, and one read supporting mutant allele in the father **(E)** Sanger validation and bi-allelic segregation analysis of the variant c.1A>G in *GCSH* in family 2.

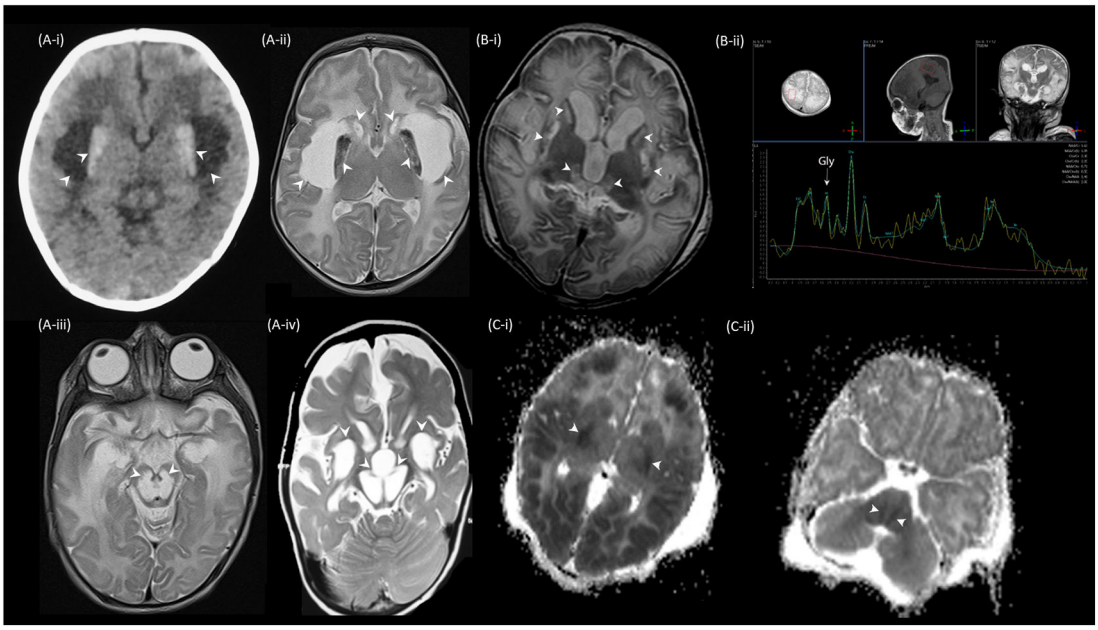

Figure 2:

(A) Neuroimaging findings of P1 **(i)** CT scan at day 45 shows symmetric hyperdensity in bilateral basal ganglia and hypodensity in the adjacent white matter **(ii)** MRI findings performed on day 45 showing bilateral symmetrical T2 hypointensity in putamen, T2 hyperintensity in the caudate nucleus and white matter adjacent to basal ganglia **(iii)** T2 hyperintensity is also noted in the white matter tracts of the brain stem **(iv)** MRI of P1 performed at 1 year shows atrophy with cystic encephalomalacia in the previously affected basal ganglia and brainstem **(B)** MRI of P2 at 3 months shows **(i)** T2 hyperintense foci in bilateral lentiform nucleus and head of caudate nucleus and posteromedial thalamus **(ii)** MRS from cerebral white matter shows elevated glycine peak at 3.5 ppm and increased lactate **(C)** MRI of P3 at day 4 of life shows **(i)** a focal area of diffusion restriction with a reduced ADC values in the posterior limb of internal capsule, **(ii)** corona radiata, parieto-occipital gray matter and in the posterior tegmental tracts of brainstem.