American Journal of Clinical Medicine Research, 2020, Vol. 8, No. 1, 1-4
Available online at http://pubs.sciepub.com/ajcmr/8/1/1
Published by Science and Education Publishing
DOI:10.12691/ajcmr-8-1-1

First Report of Missense Mutation at c.1664A>G (p.Y555C) in Krabbe Disease: Genomic Analysis in the Diagnosis of Genetic Disorders

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Received October 07, 2019; Revised November 14, 2019; Accepted December 10, 2019

Abstract **OBJECTIVES:** Genetic disorders contribute to severe morbidity and mortality among neonates and children. Most of these conditions could be attributed to the inheritance of defective gene/chromosome from the parents. Consanguinity, or coming from the same ancestral lineage is considered as a predisposing factor for the development of genetic anomalies. Krabbe disease or globoid cell leukodystrophy is an autosomal recessive disorder due to the mutation of the gene coding for galactosyl ceramidase or galactocerebrosidase (GALC gene). We present a brief report of Krabbe disease attributed to a missense mutation at C.1664A>G (p.Y555C) in exon 14, which was previously not reported in the literature as a pathogenic variant. **METHODS:** A combination of clinical symptoms and laboratory diagnostic methods were used to diagnose the Krabbe disease/globoid cell leukodystrophy. The deoxyribonucleic acid (DNA) sequencing for GALC gene and flanking intronic regions and an enzyme analysis was done to confirm Krabbe disease. Mutational analysis of GALC gene and flanking intronic regions was performed (Sequence analysis of 12 exons (exons-2, 3, 4, 6, 8, 9, 11, 12, 13, 14, 15, 16 & 18). **RESULTS:** A homozygous silent mutation at c.1350C>T (p.5450S) in exon 13, a homozygous missense mutation at c.1664C >G (p.Y555C) in exon 14, homozygous silent mutation at c. 1685T>C (p. I562I) in exon 15, homozygous silent mutation at c. 1698A>T (p. V566V) in exon 15, and a homozygous intronic variant IVS15+5C>G was observed. **CONCLUSION:** A homozygous missense mutation at c.1664C >G (p.Y555C) in exon 14 was observed along with undetectable enzyme activities of β-galactocerebrosidase for the first time in a patient with Krabbe disease.

Keywords: *genetic disorders, India, Krabbe disease, globoid cell leukodystrophy, C.1664A>G (p.Y555C), missense mutation, neurological abnormalities*

Cite This Article: Sabitha Vadakedath, and Venkataramana Kandi, "First Report of Missense Mutation at c.1664A>G (p.Y555C) in Krabbe Disease: Genomic Analysis in the Diagnosis of Genetic Disorders." *American Journal of Clinical Medicine Research*, vol. 8, no. 1 (2020): 1-4. doi: 10.12691/ajcmr-8-1-1.

1. Introduction

Neonates and children suffer from several developmental and genetic disorders. Most of which could be inherited from the parents, and include congenital skeletal disorders (achondroplasia, osteogenesis imperfecta, and skeletal dysplasia), congenital craniofacial anomalies (craniosynostosis syndrome, microcephaly, cleft defect, holoprosencephaly, and other associated syndromes), cytogenetic anomalies (Beckwith-Wiedemann syndrome (over-growth disorder leading to tumours), Turners syndrome (short stature, ovarian insufficiency, and sexual immaturity), and Downs syndrome (dysmorphism, and mental retardation). Microdeletion/sub-microscopic deletions, and microduplication disorders in chromosomes (phenotypic defects which have not yet been sufficiently understood), and dermatological deficiencies (inherited single-gene disorders, malignancies) are also prevalent among neonates and children. Genetic disorders involving glycogen metabolism, and storage disorders have also been observed among children. Other inborn metabolic disorders, genetic disorders presenting as renal diseases, and genetic disorders presenting as neurological deficiencies have also been frequently observed in children [1] as shown in Table 1.

Krabbe disease or globoid cell leukodystrophy is an autosomal recessive disorder due to the mutation of the gene coding for galactosyl ceramidase or galactocerebrosidase (GALC gene) [2]. It is located on the long arm of chromosome 14 and is 57kb in length consisting of 17 exons coding for 669 amino acids. The 5' end of the gene is rich with GC sequence and the promoter activity is seen between -176 to -24 upstream of the initiation codons. GALC gene produces an 80kd protein that splits into 30kd and 50kd subunits which in turn aggregate to produce a high molecular weight complex protein in very small amounts which work efficiently and with stability. A missense mutation at the coding region with 30kd deletion manifests as deficiency of GALC

enzyme. These mutations may be a deletion, transversion ((A-C) found on C.1700, C.1652), or a transition ((T-G) found on C.1796, (G-A) found on C.857, C.809, (C-T) found on C.1586).

2. Methods

We share of experience of observing a 6-year-old boy, who was brought to the paediatric clinic attached to the Chalmeda Ananda Rao Institute of Medical Sciences, Karimnagar, Telangana, India, with complaints of involuntary, jerky movements of the limbs, unable to hold the neck, and urinary incontinence. The patient was bedridden and needed to be attended for doing most of the daily activities. On examination, a positive Babinski sign was noted with the presence of hypertonicity and hyper-reflexes of both upper and lower limbs. There was no visual deficiency, and the patient had the normal intellectual ability on par with his age. Family history revealed that the parents were a third-degree consanguineous couple. The patient here is the first child of the parents, with another younger child (sibling) who is now 4-year-old, normal, and going to school. Parents of the patient revealed that the boy had feeding problems and required bottle-feeding. There was a history of seizures when the boy was a 2-year-old and was then treated symptomatically. Later, slowly and gradually the boy developed tetra-paresis that made him unable to walk, talk and caused difficulty in swallowing [3].

Diagnosis of Krabbe disease was made based on a combination of clinical symptoms, and laboratory diagnostic methods. The brain magnetic resonance imaging (MRI) was performed to assess the neurological abnormalities. Leukocyte enzyme analysis using artificial fluorogenic substrates was performed to detect the activities of β-galactocerebrosidase

The deoxyribonucleic acid (DNA) sequencing for GALC gene and flanking intronic regions was performed to detect mutations in the GALC gene and flanking intronic regions (Sequence analysis of 12 exons (exons- 2,3,4,6,8,9,11,12,13,14,15,16 &18).

Table 1. Various genetic disorders in children

Genetic disorders involving glycogen metabolism/storage disorders	glucose-6-phosphatase deficiency, glycogen branching enzyme deficiency, glycogen debranching deficiency, lactate dehydrogenase deficiency, liver glycogen synthase deficiency, liver phosphorylase deficiency, lysosomal acid alpha-glucosidase deficiency, lysosome-associated membrane protein 2 deficiency, myophosphorylase deficiency, phosphofructokinase deficiency, phosphoglycerate kinase deficiency, phosphorylase b kinase deficiency.
Other inborn metabolic disorders include	congenital creatinine metabolism disorder, tyrosine metabolism disorders, galactosemia, Gaucher's disease (lipid storage disease), Mucopolysaccharidoses, organic acidemias, fatty acid oxidation disorders, maple syrup urine disorders (branched chain keto-aciduria), phenylketonuria, urea metabolism disorders, Wilson disease (defective copper transport-copper accumulation) and other metabolic myopathies related to defective purine metabolism
Genetic disorders presenting as renal diseases	autosomal dominant polycystic kidney disease (cystic dilations in nephrons), autosomal dominant tubulointerstitial kidney disease (late onset, slowly progressive kidney dysfunction), autosomal recessive polycystic kidney disease (cystic dilations, and hepatic fibrosis), congenital, and infantile nephrotic syndrome, cystinosis (lysosomal storage disease presenting as organ/tissue dysfunction), Nail-Patella syndrome or hereditary osteo-onychodysplasia/HOOD syndrome (limb, and pelvic skeletal abnormalities, renal disease), primary hyperoxaluria (excessive oxalate deposition in organs, kidney dysfunction), Williams Beuren syndrome/Williams syndrome (facial anatomical abnormalities).
Genetic disorders presenting as neurological deficiencies	Ataxia-telangiectasia (immune deficiency, cerebellar ataxia, and other neurological defects), Charcot-Marie-tooth disease/ Dejerine-Sottas disease (peripheral neuropathies), Fabry disease (X-linked glycolipid storage disease causing deficiency of the lysosomal enzyme alpha-galactosidase A (GLA gene), fragile X syndrome/Martin-Bell syndrome (intellectual disability, mental retardation, and autism), Friedreich ataxia (Motor dysfunction), hereditary sensory autonomic neuropathies (progressive distal sensory loss, weakness, and muscle wasting), Huntington disease (chorea, motor dysfunction, dystonia, cognitive, and behavioural problems), hereditary haemorrhagic telangiectasia/Osler-Weber-Rendu syndrome (epistaxis, gastrointestinal bleeding, and iron deficiency anaemia), metachromatic leukodystrophy/sulphatide lipidosis (lysosomal storage disease leading to progressive demyelination of peripheral and central nervous system), von Hippel-Lindau disease (benign and malignant tumours), neurofibromatosis/von Recklinghausen disease (macular, and cutaneous neurofibromas), Niemann-Pick disease (defective sphingomyelin storage, and neurological deficiency), Rett syndrome (autism, seizures, gait abnormalities), tuberous sclerosis complex disease (benign hamartomas in various organs), other hereditary ataxias and intellectual disability disorders

Table 2. Interpretation of mutational analysis of exons and flanking intronic regions

Mutational analysis of GALC gene Exon/Intronic	**Point of mutation**	**Type of mutation**	**Pathology**
Exon 13	c.1350C>T (p.5450S)	homozygous silent mutation	Predicted Benign
Exon 14	**c.1664C >G (p. Y555C)**	**homozygous missense mutation**	**Potentially pathogenic as correlated by enzyme deficiency, and not reported in the literature previously**
Exon 15	c. 1698A>T (P. V566V)	homozygous silent mutation	Predicted Benign
Exon 15	c. 1685T>C (p. I562I)	homozygous silent mutation	Predicted Benign
Intronic variant	IVS15+5C>G	homozygous intronic variant	Predicted Benign

3. Results

The MRI study showed altered signals at the posterior peri-ventricular white matter, the body, and splenium of the corpus callosum, posterior internal capsule, and the peri-trigonal regions. Minimal involvement was noted at the sub-cortical fibres of the parietal lobes. Other observations include a normal grey matter, normal bilateral thalamus, and basal ganglia. There was no involvement of the brain stem and cerebellum. Other insignificant findings include no haemorrhage, no mid-line shift, and no extra-axial fluid collection with a normal pituitary gland.

Mutational analysis of GALC gene and flanking intronic regions was performed (Sequence analysis of 12 exons (exons- 2,3,4,6,8,9,11,12,13,14,15,16 &18). Results revealed homozygous silent mutation at c.1350C>T (p.5450S) in exon 13, a homozygous missense mutation at c.1664C >G (p. Y555C) in exon 14, homozygous silent mutation at c. 1685T>C (p. I562I) in exon 15, homozygous silent mutation at c. 1698A>T (P. V566V) in exon 15, and a homozygous intronic variant IVS15+5C>G. Except for the homozygous missense mutation at c.1664C >G (p. Y555C) in exon 14, which was not previously reported in the literature, all others indicate a benign variant as shown in Table 2.

The Leukocyte enzyme analysis using artificial fluorogenic substrates detected no activities of the β-galactocerebrosidase enzyme. The enzyme was not detected in the patient (deficient activities of β-galactocerebrosidase in leukocytes) as against a normal range of 4-40 nmole/17h/mg in healthy individuals.

4. Discussion

A diagnosis of Krabbe disease was confirmed based on the homozygous missense mutation on C.1664A>G (p.Y555C) in exon 14 (which was not reported in the literature previously) and enzyme analysis of β-galactocerebrosidase (no enzyme detected in the leukocytes).

A literature search in PUBMED for reports of mutations leading to Krabbe disease revealed only one publication from India by Singhal BS, who noted that most cases of leukodystrophies were diagnosed by clinical features, MRI studies, enzyme analysis, and genetic studies. They also reported a mutation in exon 2 of MLC 1 gene, which codes for a protein that is secreted in brain, spleen, and leukocytes [4]. There are only a few reports on Krabbe disease from India, and most of the diagnosis was done using clinical, radiological, and enzyme analysis, and not confirmed by genetic or mutational analysis [5-10].

A study by Gulati S et al. form North India who reviewed cases of leukodystrophies had reported that there were five reported cases of Krabbe disease, and most cases were diagnosed based on clinical and radiological grounds, and that confirmatory tests including the genetic analysis was not performed due to financial constraints and the unavailability of such facilities [11].

A recent report from China by Zhao S et al. studied 22 cases by including their clinical presentations, plasma psychosine levels, and β-galactocerebrosidase gene mutations. The results of this study had noted that Plasma psychosine levels were elevated in Krabbe disease and identified 8 novel mutations, including 7 missense mutations, p.H253Y, p.S259L, p.P318L, p.F350V, p.T428A, p.L530P, p.G586D, and 1 splicing mutation, c.1251+1G>A. The same study had observed that p.P154H was the most common mutation in Chinese population and missense mutations predominated as compared to the mutation types reported from Europe and Japan [12].

5. Conclusion

This report is first of its kind from India, where the diagnosis of Krabbe disease was confirmed by utilizing molecular methods and reported the gene, and the mutation responsible for the disease. There is an urgent need of a comprehensive registry of genetic disorders in India. The government should become proactive in the development of programs, where the potential parents are identified, counseling is provided, and genetic analysis for the probable genetic disorders is done to reduce the incidences of genetic disorders. In view of no specific recommended treatment modalities for most genetic diseases, prevention should be considered as better than cure.

6. Recommendations

Therefore, it is important to understand the pathology of leukodystrophies, and regularly update the clinical, neurological, and the genetic profile of the patients, which could help in the development of improved therapeutic strategies and better management of patients.

A comprehensive database of genetic disorders and inborn errors of metabolism could play an important role in encompassing physicians' knowledge, community education, population screening, genetic counseling, carrier identification, and neonatal screening.

7. Limitations of the Study

Large deletion which has been associated with a common polymorphism, p.R184C was not observed in our patient. We could not check for the mutation Y555C, the large deletion of R184C in both the parents. Detection of heterozygous status in both the parents would have confirmed the homozygous status in the child.

Acknowledgements

We would like to thank Dr. Marie T Vanier MD PhD, Emeritus Director of Research at INSERM, Lyon, France for her valuable feedback on the report.

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