

Leigh's Syndrome: A Case Report

H Raghavendra Prasad, K Neelima, M Shalini, G Chandak

Citation

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Abstract

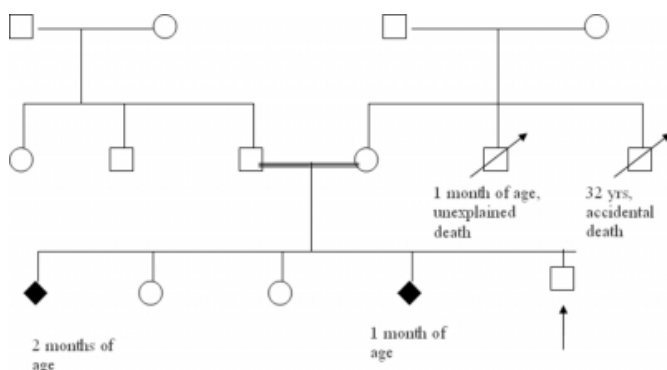
Leigh Syndrome (LS) is a rare, progressive neurodegenerative disorder and the case reports in Indian literature are scanty. Nevertheless, it is one of the few neurometabolic disorders where supportive treatment is found to ameliorate clinical symptoms, at least partially. Apt use of neuro-imaging and genetic analysis needs to be emphasized if the disease is to be diagnosed at the earliest presentation. Genetic analysis for mutation detection has significant diagnostic and prognostic implications. We report a two and a half year old Indian child with clinical and laboratory features of Leigh Syndrome suggested by neuro-imaging and genetic analysis.

CASE REPORT

A two and a half year old boy, born full term, out of second degree consanguineous marriage to a fifth gravida mother presented with cough, cold, fever and increased work of breathing for 2 weeks prior admission. He was mechanically ventilated for 9 days for acute bronchopneumonia with respiratory failure at a local hospital before admission into our hospital. The mother has a history of two abortions prior to the birth of this child and unexplained neonatal death on the matriarchal lineage. His birth weight was 3 kg and there was no history suggestive of perinatal asphyxia. He had two sisters who were healthy.

Figure 1

Figure 1: Pedigree Chart



The child was asymptomatic till 9 months of age when he developed fever and seizures and was administered symptomatic treatment. He had attained standing with support, sitting without support and bisyllables by that age. One and a half months later, he was noticed to have

difficulty in speaking and subsequent arrest in gaining further developmental milestones in all domains. There has also been a loss of personal-social and language milestones since then and he has also failed to thrive.

On admission into our hospital, the child was febrile and chest auscultation revealed bilateral crepitations and rhonchi. On neurological examination, he was conscious but not oriented to the surroundings. His right pupil was normal in size and reacting to light but his left pupil was dilated and not reacting (pharmacological mydriasis). There were no other cranial nerve palsies. Motor system examination showed hypertonia (lower limbs more than upper limbs and left side more than right side) and involuntary movements-bilateral choreoathetoid movements and myoclonic jerks. His deep tendon reflexes were exaggerated and plantar reflexes were equivocal. He did not fix his eyes or follow light. Fundus examination and visual evoked potentials were normal. He also had a palpable liver of 2 cm below the costal margin.

In view of the above a clinical possibility of a mitochondrial disorder was considered and he was investigated accordingly.

MR Imaging of the brain revealed diffuse cerebral and brainstem atrophy. There were bilateral symmetric T2 hyperintense lesions involving the pulvinar and dorsal aspects of the thalami and in the parietal, occipital and posterior temporal regions. The involved regions showed restriction of diffusion on diffusion-weighted imaging. The imaging findings were consistent with a progressive

neurodegenerative disorder and suggested the diagnosis of a mitochondrial encephalopathy.

Figure 2

Figure 2: Axial Diffusion weighted images reveal restriction of diffusion in the posterior parts of the thalami as well as the cortex of the posterior temporal and occipital lobes on either side.

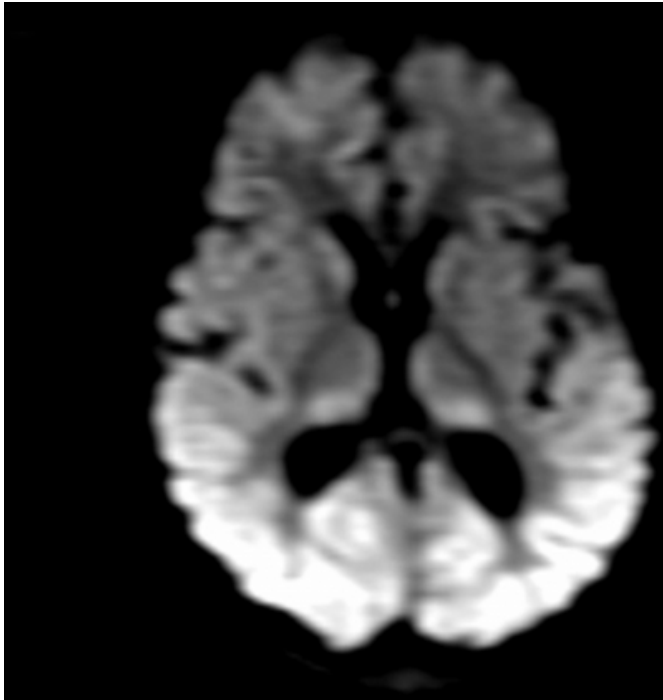


Figure 3

Figure 3: Axial FLAIR images reveal hyperintense lesions involving the thalami on both sides. The grey matter lesions are apparent only as effacement of sulcal spaces in the temporal and occipital regions

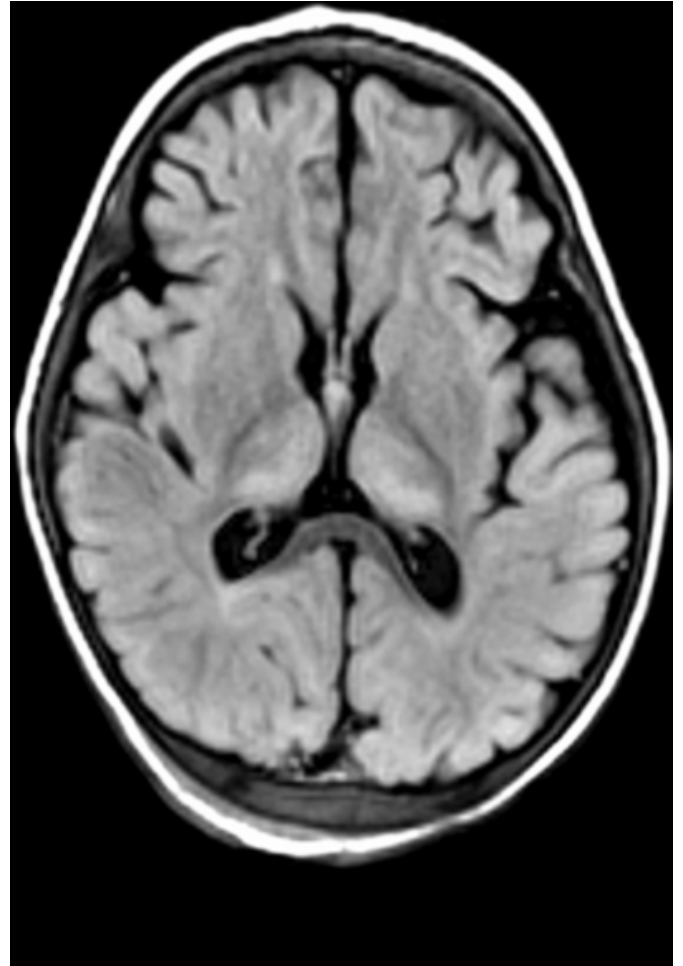
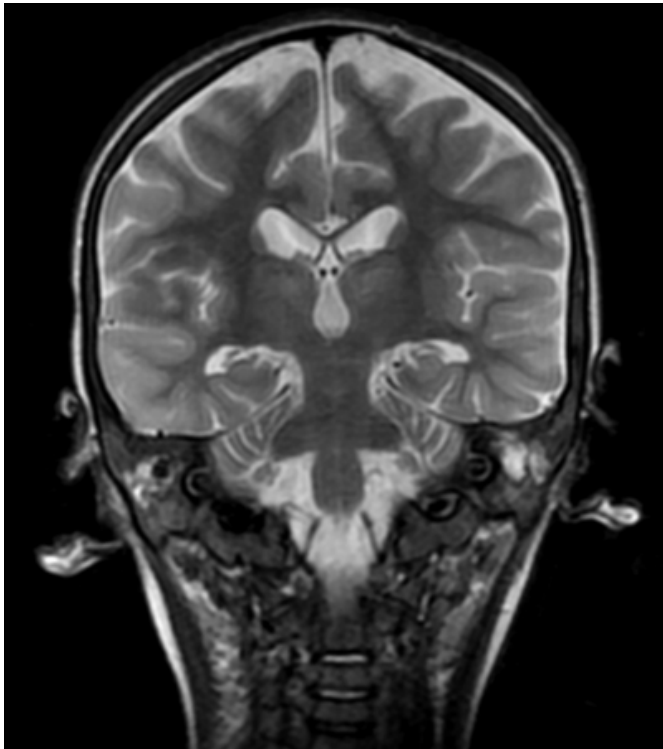


Figure 4

Figure 4: Coronal T2 weighted images reveal T2 hyperintense lesions in the thalami, posterior parietal and temporal lobes on either side



There was no metabolic acidosis and his arterial lactate estimation was normal. Serum ammonia was not elevated. CSF analysis was normal except for a slightly elevated lactate. 2, 4 DNP (dinitro-phenylhydrazine) Test was positive on metabolic screening. This observation in the absence of neuro-infection was suggestive of a mitochondrial disorder, - Leigh Syndrome. To investigate the genetic basis, we screened complete ND5 and ATPase6 genes of the child and his mother for known and novel mutations. Genomic DNA was isolated from the leucocytes and both genes were PCR-amplified using specific primers and the purified products were sequenced using Big dye terminator cycle sequencing ready kit (Ver 3.1, Applied Biosystems, Foster City, CA) on ABI 3730 Genetic Analyzer (Applied Biosystems). The sequences were analyzed using Seqscape software by comparing them with Cambridge reference sequence for mitochondrial genome. ATPase-6 gene harboured two mutations C9094T and A8860G, which were homoplasmic and found in his mother too. While A8860G has been identified in normal individuals, the nature of change of amino acid (leucine to phenylalanine) associated with C9094T mutation may be suggestive of its causal nature. Few homoplasmic polymorphisms such as G12372A and G13194A were

identified in ND5 gene in both the child and his mother, but were likely to be of no significance as per the literature.

Respiratory infection was managed with intravenous antibiotics (piperacillin-tazobactam and tobramycin), salbutamol and budesonide nebulisations and supplemental oxygen. He was also commenced on antiepileptics (phenytoin and valproate). Phenytoin was gradually tapered off and stopped. Specific supportive therapy for the suspected mitochondrial disorder was begun with intravenous thiamine infusions, oral haloperidol, carnitine and alkali supplementation and oral coenzyme Q (ubiquinone). Enteral feeds were commenced and the feed volumes were gradually increased. In view of the persistent fever and the ear discharge showing gram negative bacilli on Gram stain, the antibiotics were upgraded to ceftazidime and amikacin on the 5th day of admission. Over the next 4 days, his fever defervesced and the choreoathetoid movements showed a significant improvement. Supplemental Oxygen was gradually weaned and stopped. He was discharged home after 12 days. He was also started on oral riboflavin on follow up. Later on he was hospitalized twice for acute bronchopneumonia. Subsequent therapy with intramuscular benzathine penicillin once in every 3 weeks reduced the incidence of respiratory infections significantly.

Developmental assessment after 6 months (at the age of 3 years) revealed a developmental quotient of 46, indicating a moderate developmental delay. Though he was dependent in self-help skills, he was able to comprehend simple 1-2 word phrase instructions and express through differential cry and vocalization. (2-3 word vocabulary) Hypertonia improved and there were no involuntary movements. Attention could be aroused but he got distracted easily. He had a concept of household items. Eye contact was present.

DISCUSSION

Leigh Syndrome₁ is a rare, inherited neurodegenerative disorder with characteristic pathological features usually presenting in infancy or early childhood.

It was first reported by Denis Leigh₂ in 1951 in a 7 month old infant. The estimated prevalence of Leigh Syndrome was 2.05 cases per 1,00,000₃. The preschool incidence of Leigh syndrome was 1 out of 32,000₄. In India, Bhavsar VM, Kumta NB₅ described the role of CT Scan of the brain in the diagnosis of Leigh Syndrome in 1991. Ghosh and Pradhan₆ reported two children with Leigh Syndrome suspected clinically and confirmed by MRI in 1996. The children showed partial response to parenteral thiamine and MRI

lesions showed partial improvement with time over one year of follow up. In one of their patients, medulla and spinal cord were involved which is extremely rare. In 2004, Mannan and Sharma et al⁷ reported autopsy proven Leigh Syndrome in a 15-month-old girl admitted with cough and hyperventilation. In 2005, Hombal and Narvekar⁸ reported Leigh Syndrome in a 3-year-old child with regression of milestones and involuntary movements. The diagnosis in their case was based on neuroimaging.

There are at least four genetically determined causes of Leigh Syndrome¹: Pyruvate dehydrogenase complex deficiency, complex I deficiency, complex IV deficiency (COX), and complex V (ATPase deficiency). These enzymes, when defective, are known to disturb oxidative phosphorylation and lead to failure of organs with high oxidative metabolic demand such as neuro-muscular system. All the five complexes except complex II are heterogeneous in their origin i.e. components of all the four complexes are encoded by both nuclear and mitochondrial genomes. ND5 and ATPase6, which are mitochondrial component of complex I and complex V respectively, are one of the frequent genetic causes for occurrence of Leigh Syndrome. The activity of these complexes can be detected by histochemical studies of fresh muscle tissues or cultured skin fibroblasts.

The mutations can arise sporadically or be inherited by autosomal recessive transmission (COX deficiency) or X linked transmission (PDHE1 deficiency) or by maternal transmission (complex V deficiency ATPase6 nt 8993 mutation). Maternal inheritance accounts for 20 % of the cases⁹. Mutations in other mitochondrial genes ND4 and ND6 and nuclear genes such as SURF-1 are also reported to be associated with Leigh Syndrome^{10, 11}. Yang and Sun et al reported A8344G, T8993G, T8993C, A3243G, G604C and SURF-1 mutations in a retrospective study of 65 patients with Leigh Syndrome¹⁰. NARP (neurogenic weakness, ataxia, and retinitis pigmentosa) and Leigh syndromes are associated with a T8993G mutation when the percentage of mutant mitochondrial DNA is low (60- 90%) or high (>90%), respectively. Leigh syndrome is also caused by a second mutation in the same position T8993C¹². T8993C mutation was found to be associated with a slower clinical progression and more frequent sensory neuronal involvement¹³. Thus mutation analysis may be useful for prognostication also. Moslemi, Darin et al found two new mutations T9185C and T9191C in MTATP6¹⁴. Taylor, Morris et al described association of the syndrome with

T12706C ND5 mutation¹⁵. Akagi, Inui et al detected a T-to-G transition at nucleotide 9176 (T9176G) in the mitochondrial adenosine triphosphate 6 gene (MTATP6) in two siblings with Leigh syndrome¹⁶. Preliminary studies have proven the utility of mutation analysis in prenatal diagnosis of Leigh Syndrome^{17, 18}.

Affected children usually become symptomatic within the first year of life with feeding difficulties, vomiting and failure to thrive¹. Motor and language milestones may be delayed. They can have seizures, hypotonia, ataxia, tremor, pyramidal signs, nystagmus, external ophthalmoplegia, ptosis, optic atrophy and decreased visual acuity. Intermittent sighing respirations may be found secondary to brainstem dysfunction. Rarely can it present in the childhood (juvenile form). Respiratory failure is usually responsible for mortality. Biochemical abnormalities include elevated blood and CSF lactate and pyruvate¹⁹.

Neuroimaging^{5, 6, 8, 19, 20} plays an important role in diagnosis as well as follow up of patients with Leigh Syndrome. CT scans may reveal bilateral symmetric areas of low attenuation involving the basal ganglia. On MR imaging, signal changes characterized by low signal on T1 weighted images and high signal on T2 weighted images, have been most commonly reported in the basal ganglia, thalamus and brainstem. Variable involvement of the cerebral and cerebellar cortex, cerebral and cerebellar white matter and the spinal cord has also been reported. The affected regions show restriction of diffusion in the acute phase. MR spectroscopy of the basal ganglia typically demonstrates high lactate levels, decrease in NAA/creatine levels and elevation of choline/creatine ratio. These metabolites are useful indicators of prognosis and response to therapy²¹.

Autopsy findings^{7,9,19} include focal symmetric areas of necrosis in the thalamus, basal ganglia, tegmental gray matter, periventricular and periaqueductal regions of the brainstem and posterior columns of the spinal cord. These lesions show cystic cavitation with neuronal loss, demyelination and vascular proliferation. Demyelination with relative preservation of neurons and axon differentiates these lesions from those of hypoxic or ischemic origin⁷.

Specific therapy for mitochondrial disorders in children is not available. The results and prognosis are variable. The aim of symptomatic treatment is to improve the ATP production and to lower the lactate levels. High concentrations of lactate can inhibit glycolysis, increase triglyceride turnover or modulate the function of ATP-

sensitive K⁺ channels. The osmotic effect of accumulated intracellular lactate can cause cell swelling²². Thiamine, a cofactor of pyruvate dehydrogenase complex has been reported to improve the neurological status in some patients²³. Dichloroacetate has been reported to alter the clinical course of acute deterioration in adult patients with T8993C mutation²⁴. It is delivered to mitochondria via the transport system for pyruvate; and stimulates the overall activity of the PDH complex in most tissues except for intestine and testes. Dichloroacetate inhibits pyruvate dehydrogenase kinase and thus maintains PDH in a nonphosphorylated, catalytically active form²². In children, it was shown to produce clinical and biochemical improvement possibly by reducing toxic accumulation of lactate, but could not prevent the natural progression of the syndrome²⁵. Marked improvement was observed with riboflavin, which nearly normalized the adenosine triphosphate production^{26, 27}. Rapid clinical and biochemical improvement was observed in patients with acute central respiratory failure with the use of intravenous soya bean oil (ketogenic emulsion)²⁸. Ketogenic diet has been found to improve the outcome in those with a deficiency of pyruvate dehydrogenase²⁹. Coenzyme Q and carnitine³⁰ have also been found to be effective. Leung TF, Hui J et al described significant relief of dystonia with intramuscular botulinum toxin³¹. Nucleus transplantation into enucleated oocyte is emerging as a new option for prevention of mitochondrial disorders³².

This child presented to us with regression of developmental milestones, seizures and acute exacerbation caused by a trivial respiratory illness. These symptoms pointed towards a neurodegenerative disorder. There was a maternal history of recurrent abortions and unexplained neonatal death on the matriarchal lineage. Earlier studies have shown that mutations causing Leigh Syndrome can be associated with multiple neonatal deaths³³. Examination revealed delayed development, hypertonia, disorientation and bilateral choreoathetoid movements all of which are recognized features of Leigh Syndrome¹. CSF lactate was slightly elevated, but arterial lactate was normal. Though Leigh Syndrome is conventionally associated with elevated serum lactate, earlier studies have shown that serum lactate can be well within normal limits in spite of definite neuroradiological features and spectroscopic evidence of elevated brain lactate³⁴. MR Imaging of the brain revealed diffuse cerebral and brainstem atrophy. There were bilateral symmetric T2 hyperintense lesions involving the pulvinar

and dorsal aspects of the thalami and in the parietal, occipital and posterior temporal regions. The involved regions showed restriction of diffusion on diffusion-weighted imaging. The imaging findings suggested a progressive Neurodegenerative disorder with the possibility of a mitochondrial encephalopathy. This is consistent with neuroradiologic findings in previous reports of Leigh Syndrome^{5, 6, 8, 19, 20}.

In mutation analysis, we did not find any of the reported mutations such as G13513A, A13084T and T12706C in the ND5 gene. Although few synonymous mutations were observed, they cannot explain the pathogenic role of ND5 gene in this patient. However, two non-synonymous mutations, A8860G replacing threonine with alanine at 112 position (T112A) and C9094T substituting leucine with phenylalanine at 190th codon (L190F) were identified in the ATPase6 gene. A8860G is a well-known polymorphism reported in several mitochondrial disorders and part of a haplogroup H in Northeast German and other populations but C9094T is a novel mutation, never reported in any patient with Leigh Syndrome. It was identified in only two normal, healthy individuals (Indians). At 9094 position in the mitochondrial genome, which is a part of ATPase-6 gene, cytosine (C) is present and as a result of transition cytosine is being replaced by thymine (T). This transition results in substitution of leucine (L) by phenylalanine (F), which may affect the function of ATPase6 protein. C9094T could have some functional consequences in view of the nature of change of amino acid and the residue being conserved across the chimpanzee and gorilla^{35, 36, 37, 38}. The mutation cannot be classified as a pathogenic or even probably pathogenic according to the scoring system criteria³⁹, as functional studies (such as PCR) and biopsy of affected tissues have not been performed. Mutation analysis has not been repeated in another laboratory owing to the lack of facilities. Further studies are essential to determine the exact structural effects of the mutation. The positive 2, 4 dinitro phenyl hydrazine test could possibly be attributed to concurrent valproate therapy⁴⁰.

Thus the child has three minor clinical criteria and one minor biochemical criterion according to the modified Walker's criteria⁴¹ for pediatric population namely Symptoms compatible with an RC defect, Metabolic indicator of an RC defect (elevated CSF Lactate), Previous maternal history of recurrent abortions and unexplained neonatal death on the matriarchal lineage and a Movement disorder. These suggest a probable mitochondrial disorder. There was radiological

evidence of a mitochondrial disorder and earlier researchers have reported patients with Leigh Syndrome whose diagnosis was based on neuroimaging alone^{5,6,8}. Enzymology, histology and functional fibroblast ATP synthesis rate were not performed due to the paucity of facilities. Molecular studies revealed a novel mutation but further research is warranted before the pathogenicity of the mutation is established. There was an appreciable improvement with thiamine, carnitine, coenzyme Q and riboflavin. Repeat neuroimaging was not performed as there were financial constraints and the studies done previously to document radiological improvement with supportive therapy were inconclusive. The frequency of respiratory illnesses which can worsen the neurological picture came down noticeably with intramuscular benzathine penicillin prophylaxis. The child is on regular follow up now for the past 2 years and is able to comprehend simple 1-2 word phrase instructions. Hypertonia and involuntary movements improved. He has a concept of household items. Motor development is normal for age. There are no further episodes of stress related neurological deterioration.

CONCLUSION

Mitochondrial disease cannot be cured completely. Efforts for prevention and prenatal diagnosis are still in the nascent stage. With appropriate investigations, accurate diagnosis and prompt institution of adequate supportive therapy, symptomatic amelioration can be achieved, thereby adding life to the limited years of survival of these children. Further research aimed at prenatal identification of the responsible mutations and prevention of the disease is warranted.

Figure 5

Table 1: Relevant Investigations

Total leucocyte count	13,700 / mm ³
C Reactive Protein (CRP)	8.36 mg / dl
Serum Sodium	129.7 mEq / l
Serum Calcium	7.3mg/dl
Serum Lactate	10.4mg/dl (Normal: 4.5 – 20mg/dl)
Arterial Blood Gas analysis	pH:7.507, PCO2:43.6, PO2:33.2, HCO3:25.7, BE:2.7
Prothrombin Time	16.6 seconds
Control	12.9 seconds
Partial Thromboplastin Time	146.9 seconds
Control	35.2 seconds
Fibrin degradation products	>1600ng/ml
Blood for bacterial antigen detection	Positive for H. influenzae B and N. meningitidis AcyW135
Blood for Culture and sensitivity	Sterile
Serum Bilirubin Total	12.92 mg / dl
Indirect	11.44 mg / dl
Blood for metabolic screening	2,4 DNFH test positive
Urine analysis	8-10pus cells/hpf, Protein +, Ketone bodies+
CSF Cell Count	Nil
Biochemical Analysis	Normal
Gram Stain/AFB Stain	No organisms seen
CSF Bacterial Antigen Detection/Culture	Negative
CSF Lactate	16.8 mg/dl (Normal: < 12 mg/dl)
Ear discharge for Gram stain	Few pus cells, occasional Gram negative bacilli seen

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NAME OF THE DEPARTMENT AND HOSPITAL WHERE THE WORK WAS DONE

CLINICAL WORK

Children's Medical Center KRISHNA INSTITUTE OF MEDICAL SCIENCES 1-8-31/1, Minister Road, Secunderabad, A.P. State INDIA - 500 003 Tel: (0091-40) 2781 4499, Fax: (0091-40) 2784 0980 E-mail: cmc_kims@yahoo.co.in, info@kims.co.in Website: <http://www.kims.co.in>

GENETIC ANALYSIS

Genome Research Group
Centre for Cellular and Molecular Biology (CCMB)
Uppal road, Hyderabad 500 007. INDIA
Tel: (0091-40) 2716 0222
Fax: (0091-40) 2716 0591, 2716 0311

CORRESPONDENCE TO

H.V. Raghavendra Prasad MD Peds, DNB Peds, MNAMS (India), FRCPCH (UK) Consultant Paediatrician Dept. of Paediatrics East Surrey Hospital Canada Avenue Redhill, RH1 5RH, UK Tel: (0044) 1737 768 511 x6769 (Sec) Mobile: (0044) 7756 440 546 E-mail: raghuhv@yahoo.com

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Author Information

H.V. Raghavendra Prasad, Peds, DNB Peds, MNAMS (India), FRCPCH (UK)

Consultant Paediatrician & Intensivist, Children's Medical Center, Krishna Institute Of Medical Sciences

Kharidehal Neelima, MD Peds

Paediatric Registrar, Children's Medical Center, Krishna Institute Of Medical Sciences

Manu Shalini, MSc

Senior Research Fellow, Centre for Cellular and Molecular Biology (CCMB)

GR Chandak, MD, DNB

Scientist & Medical Geneticist, Centre for Cellular and Molecular Biology (CCMB)