Disorders of Tetrahydrobiopterin Metabolism: Experience from South India

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Abstract

Background: Disorders of tetrahydrobiopterin metabolism represent a rare group of inherited neurotransmitter disorders which manifest mainly in infancy or childhood with developmental delay, neuroregression, epilepsy, movement disorders and autonomic symptoms.

Methodology: A retrospective review of genetically confirmed cases of disorders of tetrahydrobiopterin metabolism over a period of three years (Jan 2018 to Jan 2021) was performed across two paediatric neurology centres from South India.

Results: A total of nine patients(M:F=4:5) fulfilled the eligibility criteria. The genetic variants detected include homozygous mutations in the *QDPR*(n=6), *GCH1*(n=2) and *PTS*(n=1) genes. The median age at onset of symptoms was 6-months(range 3-78 months), while that at diagnosis was 15-months (8-120 months) resulting in a median delay in diagnosis of 9-months. The main clinical manifestations included neuroregression (89%), developmental delay(78%), dystonia(78%) and seizures(55%). Management strategies included phenylalanine restricted diet, levodopa/carbidopa, 5-Hydroxytryphtophan, and folinic acid. Only, Patient-2 afforded and received BH4 supplementation at a sub-optimal dose later in the disease course. We had a median duration of follow up of 15 months (range 2-48 months). Though biochemical response has been marked, except for patients with GTPCH deficiency, only mild clinical improvement was noted with regards to developmental milestones, seizures or dystonia in others.

Conclusion: Tetrahydrobiopterin deficiencies represent an rare yet potentially treatable cause for non-phenylketonuria hyperphenylalaninemia when diagnosed and treated early in life. Screening for disorders of biopterin metabolism in patients with hyperphenylalaninemia prevents delayed diagnosis. This study expands the genotype phenotype spectrum of patients with disorders of tetrahydrobiopterin metabolism from South India.

Introduction

Disorders of tetrahydrobiopterin (BH4) metabolism were initially described in the late 19th century as 'atypical' or 'malignant Phenylketonuria' (PKU) in a subset of patients diagnosed to have hyperphenylalaninemia (HPA) who were not responding to dietary intervention [1,2]. BH4 is an essential cofactor for the enzymatic hydroxylation of three aromatic amino acids, namely phenylalanine, tryptophan and tyrosine; three isoforms of nitric-oxide (NO) synthase and alkylglycerol mono oxygenase [3,4]. In addition to phenylalanine, enzymatic hydroxylation of aromatic amino acids results in synthesis of monoamine neurotransmitters particularly serotonin, dopamine and its metabolites[3,4]. Biosynthesis of BH4 occurs from guanosine triphosphate (GTP) through a three-step reaction catalysed by the enzymes guanosine triphosphate cyclohydrolase (GTPCH), 6- pyruvoyl tetrahydropterin synthase (PTPS), and sepiapterin reductase (SR). BH4 reacts with aromatic amino acid hydroxylase as an active cofactor and is converted into pterin-4α-carbinolamine. The regeneration of BH4 from pterin 4α carbinolamine is mediated by the enzymes pterin 4α carbinolamine dehydratase (PCD) and dihydropteridine reductase (DHPR) [3,4].

Disorders of BH4 metabolism are rare heterogenous group of inherited neurotransmitter disorders occurring due to disruption in the biosynthesis or regeneration of BH4 [3,4]. Pathophysiology is twofold due to – (i) Elevated phenylalanine levels leading to accumulation of toxic metabolites such as phenyl acetic acid, phenyl pyruvic acid and phenyl lactic acid resulting in neurological deterioration, and (ii) impaired monoamine neurotransmitter synthesis leading to deficiency of 5-hydroxytryptophan (5-HT), dopamine, nor-epinephrine and epinephrine [3,4]. BH4 deficiency can present in infancy or childhood with varied spectrum of manifestations comprising of developmental delay, cognitive impairment, epilepsy and extrapyramidal symptoms. Diagnosis is primarily by biochemical evaluation of blood phenylalanine levels, cerebrospinal fluid neurotransmitters levels, urinary pterins levels, erythrocyte DHPR activity and genetic confirmation of the pathogenic variants. Treatment includes management of HPA by dietary restriction, BH4 supplementation and replacement of monoamine neurotransmitters [3-7].

BH4 synthesis defects was first described by Smith et al., in 1975 [2]. The incidence of BH4 defects varies geographically between 2 to 10% in patients detected to have HPA [8,9]. There is paucity of literature about BH4 synthesis defects from India. Opladen et al., 2012 reported 626 patients with BH4 deficiencies worldwide of which 2.1 % of patients were from India [5]. Vykuntaraju et al., 2018 reported first case of DHPR deficiency from India [10] The aim of this study was to describe in detail the clinical characteristics, laboratory profile, radiological profile, treatment profile and outcome in genetically confirmed patients with BH4 deficiency from two paediatric neurology centres at south India.

Subjects And Methods

Study design

This was a retrospective descriptive study done from two paediatric neurology centres; a quaternary care center for neurological disorders and a tertiary level institute for child health from south India. The case records of the patients who underwent appropriate genetic counselling and genetically confirmed to have disorders of biopterin metabolism and under the care of the authors from January 2018 and January 2021 were included. A prior informed consent was obtained for all the patients who had underwent genetic testing and were enrolled in the study. Patients were excluded from the analysis if genetic confirmation was unavailable.

Data collection

Data about the clinical features, consanguinity, perinatal events, clinical examination, blood phenylalanine levels, electroencephalography (EEG), neuroimaging findings, genetic analysis, treatment and outcome were collected and the pertinent data was tabulated in a Microsoft excel worksheet. Blood phenylalanine levels were measured using electrospray ionization tandem mass spectrometry. Sequential blood phenylalanine levels from baseline while the patient was on treatment was also recorded. EEG was performed in the Galileo system by using the standard 10-20 system of electrode placement. Both

awake and sleep records were captured. Magnetic Resonance Imaging (MRI) studies of the brain were analysed by an independent neuroradiologist blinded to patient's clinical details. The sequences included T1 (axial), T2 (axial, coronal, sagittal), fluid-attenuated inversion recovery (FLAIR axial), susceptibility weighted imaging (SWI) and diffusion weighted imaging (DWI). Targeted exome sequencing was done to detect genetic mutations. In house bioinformatic pipeline was employed for analysis of raw data, variant calling and annotation.

Results

A total of nine patients (4 males and 5 females) were genetically confirmed to have disorders of biopterin metabolism. Six patients had deficits in *QDPR* gene (DHPR deficiency), two had mutations in *GCH1* gene (GTPCH deficiency) and Patient-9 had mutation in *PTS* gene (PTPS deficiency). The clinical features have been described in detail in Table 1. The biochemical profile, EEG and neuroimaging findings are summarized in Table 2.

Clinical features (n=9)

The mean age at onset of symptoms in our cohort was 17.3 ± 24.3 months (range - 3 to 78 months). The mean age at diagnosis was 37 ± 42.4 months (range - 8 to 120 months). There was a median delay of 9 months in the diagnosis.

QDPR gene mutation (DHPR deficiency) (n=6)

Six patients (2 males and 4 Females) had mutations in *QDPR* gene (DHPR deficiency) with the mean age at onset of symptoms being 11.8 ± 9.8 months (range 3 to 24 months). Clinical features in these patients included developmental delay (5/6), regression of milestones (6/6), seizures (3/6), dystonia (4/6) and ataxia (1/6). Consanguinity was present in (5/6) patients. On examination, microcephaly was seen in (2/6) patients, generalized dystonia in (4/6) patients, generalized hypotonia in two patients and oculogyric crisis in one patient. Patient-3 had multiple café-au-lait spots in the body. Patient 4 had history of recurrent upper respiratory tract infections.

GCH1 gene mutation (GTPCH deficiency) (n=2)

Patients-7 and 8 had mutations in *GCH1* gene. Patient-7 was an eight-month-old girl presented with developmental delay, oculogyric crisis, intermittent dystonia and hypotonia from 4-months of age. On examination, she had microcephaly, intermittent oculogyric crisis and dystonia. Patient-8 was an eight-year-old boy who had presented with toe walking and abnormal posturing of both feet while walking since 6.5 years of age, tremulousness and posturing of both upper limbs since 7.5 years of age with diurnal variation. On examination, he predominantly had foot dystonia with preserved reflexes.

PTS gene mutation (PTPS deficiency) (n=1)

Patient-9 was incidentally detected to have increased phenylalanine levels on new-born screening. He was observed to have delayed developmental milestones at around 3-months of age. Later developed dystonia and had myoclonic epilepsy at 6-months. On examination, he had hypopigmented skin and hair, hypotonia and generalised dystonia. Parents were unable to start on phenylalanine restricted diet due to financial constraints.

Biochemical findings (n=9)

Prior to the treatment, in patients with DHPR deficiency, the mean and the median levels of blood phenylalanine was 394.33 ± 191.5 µmol/L and 395 µmol/L respectively (range: 287 - 676 µmol/L). Phenylalanine tyrosine ratio (Phe/Tyr ratio) was increased in patients with DHPR deficiency with a mean and median of 10.45 ± 6.45 and 8.36 respectively (range 3.6 to 21.26). Patient-6 with DHPR deficiency had normal phenylalanine levels when evaluated here at the age of 10 years. However, Phe/Tyr ratio was elevated to 3.5 (cut-off being 1.5) [11] that gave rose to a suspicion of HPA. In patients with GTPCH deficiency (Patients-7,8) and PTPS deficiency (Patient-9) serum phenylalanine levels were 300, 69 and 640 µmol/L and P/T ratio was 18.97, 1.1 and 18.97 respectively. Oral BH4 loading test, urinary pterins, CSF neurotransmitter analysis, CSF 5-methylenetetrahydrofolate analysis and blood erythrocyte DHPR activity was not performed due to financial constraints, transportation difficulties and non-availability in India.

Electroencephalography (EEG) findings(n=8)

Eight patients in the study, six with DHPR deficiency, one with PTPS deficiency and one with GTPCH deficiency had underwent EEG to detect clinical and sub-clinical seizures. EEG was normal in 5/8 patients. EEG was abnormal in patient-2, 3 and 5. Patient-2 had multifocal inter-ictal epileptiform discharges (IEDs), patient-3 had classical hypsarrhythmia and patient-5 had sleep accentuated, near-continuous, generalized (fronto-central dominant) spike/polyspike wave discharges of 1-2 Hz with marked reduction in IEDs on awakening consistent with electrical status epilepticus during slow wave sleep (ESES) (Figure 1A-C).

Neuroimaging findings (n=9)

MRI brain was normal in 4/9 patients, two patients each with DHPR deficiency (P-1,2) and GTPCH deficiency (P-7,8). MRI findings were consistent with delayed myelination in two patients, one each of DHPR deficiency (P- 3) and PTPS deficiency (P-9). Patient-4 with DHPR deficiency had T2 hyperintensity of bilateral central tegmental tracts with diffusion restriction (Fig 2A,B&C). Patient-5 with DHPR deficiency had T2 hyperintensities in the subcortical white matter of both frontal, parietal and occipital lobes with partial inversion on FLAIR (Fig 2D,E&F). Patient-6 with DHPR deficiency had bilateral symmetrical calcification in the lentiform nuclei, anterolateral thalami and subcortical white matter of all the lobes with occipital preponderance(Fig 2G, H, I). Calcification was also noted in the white matter of both cerebellar hemispheres.

Mutational analysis (n=9)

We detected nine different variations in the three genes related to BH4 deficiency among the cases studied (Table 3). Pathogenic/likely pathogenic (P/LP) variants were identified in all the 9 cases according to the American College of Medical Genetics and Genomics (ACMG) criteria [12]. All the variations identified were in homozygous state among which seven were missense and two were splice site variations. QDPR gene variations were seen in six cases - six of these predicted to be P/LP (c.68G>A; p.Gly23Asp, c.296-1G>T, c.488G>T; p.Ser163Ile, c.635T>C; p.Phe212Ser, c.680T>C; p.Leu227Pro and c.295+5G>T). Three of these variants were novel (c.296-1G>T, c.295+5G>T and c.680T>C) and remaining three were known. Among the three known variants, c.68G>A is reported in Clinvar (RCV000000519.2) and Human Gene Mutation Database(HGMD) (CM930632) database, while c.457C>T and c.488G>T have been reported in HGMD. Two different GCH1 gene variations (c.703C>G; p.Arg235Gly and c.457C>T; p.His153Tyr) were observed in 2 cases, both were likely pathogenic and known. Further, both these variants were localized in the GTP cyclohydrolase I domain of the GCH1 protein. PTS gene variation (c.65C>G; p.Ala22Gly) was observed in one case which was classified as likely pathogenic. The observed variation lies in the 6-pyruvoyl tetrahydropterin synthase domain of the PTS protein and has been reported in HGMD database (CM064184). A schematic diagram of the genes - *QDPR*, *GCH1* and *PTS*, and the mutations found in this study is shown in Figure 3. Patient-3 with multiple café-au-lait spots had an additional heterozygous LP missense variant c.2288T>C on exon 19 of the NF1 gene (chr17:g.31227254T>C; Depth: 214x) that results in the amino acid substitution of Proline for Leucine at codon 763 (p.Leu763Pro; ENST00000358273.9). Patient-4 with recurrent respiratory tract infections had an additional pathogenic homozygous 5' splice site variant in intron 11 of the *ZMYND10* gene (chr3:g.50379003A>C; Depth: 84x) that affects the invariant GT donor splice site downstream of exon 11 (c.1247+2T>G; ENST00000231749.3).

Treatment and outcome (n=9)

The treatment and follow up details have been summarized in Table 4. All the patients in the study were treated with levodopa/carbidopa, folinic acid and low phenylalanine diet. Patient-6 and Patient-8 with normal levels of PHA didn't receive low phenylalanine diet. Five patients (4 with DHPR deficiency and Patient-7 with GTPCH deficiency) received 5-hydroxytryptophan (5- HTP) at a median dose of 3mg/kg/day. Patient-2 with DHPR deficiency could access BH4 and received it at 2mg/kg/day at a sub-optimal dose later in the disease course. For seizures, patients were treated with levetiracetam, clobazam, valproate, and topiramate. Patient-5 with epileptic encephalopathy showed moderate clinical response to a course of pulse intravenous methylprednisolone with complete electrographical resolution (Figure 1D). Patients additionally received trihexyphenidyl and tetrabenazine for dystonia. The mean and median duration of follow up of the study cohort was 19.8 ± 15.5 months and 15-months (range 2-months to 4-years). There was variable clinical response in terms of improvement in developmental milestones, reduction in dystonia and seizures as described in Table 4. Significant improvement in milestones and complete cessation of seizures was noted in a Patient-4 with DHPR deficiency. In Patient-7, administration of levodopa at 1 mg/kg/day resulted in dyskinesias that disappeared with reduction of dose to 0.5 mg/kg/day. This child showed significant motor improvement with low dose levodopa as well as folinic acid. Folinic acid is given to replete CSF 5-methyltetrahydrofolate levels that might become low as a result of levodopa/carbidopa supplementation [5, 13]. Patient-8 with GTPCH deficiency had significant alleviation of dystonia with a small dose of levodopa, thus conforming the criteria of dopa-responsive dystonia. Biochemically, in patients with DHPR deficiency, there was reduction in the phenylalanine levels with mean and median blood levels of 120.6 ± 96.4 µmol/L and 103.3 µmol/L (range 20 to 269.7 µmol/l) respectively at a median follow up of 13.5 months. Patient-2 with DHPR deficiency who was treated with BH4 supplementation had lower blood phenylalanine levels 20 µmol/l at a follow up duration of 27 months. In Patient-7 with GTPCH deficiency and Patient-9 with PTPS deficiency the blood phenylalanine levels were 350 and 200 µmol/L at a follow up duration of 40 and 16-months respectively. As blood phenylalanine levels were normal at baseline in Patient-6 and 8 repeat analysis wasn't done. The mean and median development quotient of patients in our study at the last follow up was 44.8 ± 28.9 and 30 (range 10 to 100). Repeat neuroimaging to look for any radiological response was not done in any of our patients.

Discussion

In the present study, we have described the clinical features, investigations, treatment and outcome of 9 genetically confirmed patients with BH4 deficiency from South India. The disorders of BH4 metabolism are broadly classified into six types based on the specific enzyme defects into autosomal recessive (AR) or autosomal dominant (AD) GTPCH deficiency, PTPS deficiency, DHPR deficiency, SR deficiency, and PCD deficiency. PTPS deficiency is the commonest cause of BH4 deficiency (56.7%), followed by DHPR deficiency (34.7%), GTPCH deficiency (4.9%) and PCD deficiency (3.7%) [5, 14]. SR and PCD deficiencies are mild and rarely described.

Most of the reports on disorders of BH4 metabolism are from western literature, Japan, and China where universal new-born screening for PKU is practised [5-9]. Opladen et al., 2012 has described the largest cohort of 626 patients with BH4 deficiency from the BIODEF database [5]. Unlike previous studies most of the patients in our cohort belonged to DHPR deficiency(66.6%), followed by AR GTPCH deficiency (22.2%) and PTPS deficiency (11.1%). This discrepancy could be because of the referral bias as ours was tertiary/quaternary health care centre. All our patients were diagnosed when symptomatic except one who was detected on new-born screening. Our patients had an median age at onset of symptoms of 6 months and were diagnosed around 15 months (8 months – 120 months) which is delayed as compared to previous studies [6]. Though there is glaring biochemical difference among the various BH4 deficiencies clinical

features overlap in most and can mimic cerebral palsy, extrapyramidal disorder or genetic epilepsies. The cardinal clinical features described include hypotonia with developmental delay, cognitive impairment, seizures, autonomic disturbances and movement disorders mainly dystonia, oculogyric crisis, dyskinesias and early onset parkinsonism [3-5]. Other less-described symptoms are swallowing difficulties, hypersalivation, sleep disturbances, psychological issues, prematurity, low birth weight and central hypothyroidism [15]. Hypopigmented skin and hair, characteristic musty body odour, and eczema are few systemic findings [16]. In our cohort neuroregression (89%) was the most common presentation followed by developmental delay (77.7%), dystonia (77.7%), seizures (55.5%) and behavioural problems (22.2%). Extrapyramidal symptoms dominated in patients with GTPCH deficiency, seizures in DHPR and PTPS deficiency, whereas regression and developmental delay was present across the cohort. Ataxia was observed in Patient-1. Consanguinity was high and noted in 89%. Microcephaly (33%), oculogyric crisis (22%), hypotonia (22%) and dystonia (77.7%) were remarkable examination findings. Microcephaly is commonly reported in patients with PTPS and DHPR deficiency, while it is uncommon in GTPCH deficiency[17]. In our study, the patients with PTPS and GTPCH deficiency as well as one patient with DHPR deficiency reportedly had microcephaly. Normally in patients with PKU or BH4 deficiencies there will be decreased skin and hair pigmentation due to reduced levels of tyrosine and competitive inhibition of tyrosine uptake by phenylalanine [18]. But skin and hair changes were not that prominent in our cohort (11%) probably as blood phenylalanine levels were not markedly elevated. Prematurity, low birth-weight, musty odour and autonomic nervous system symptoms was not present in any of the patients in our cohort.

Hyperphenylalaninemia (HPA) is classically defined as plasma phenylalanine levels greater than 120 µmol/l (2mg/dl) [19]. HPA can result either from deficiency of the enzyme phenylalanine hydroxylase or its cofactor BH4. Disorders of BH4 metabolism are rare and contribute to around 2% of the cases detected to have HPA [19]. HPA is seen in almost all the subtypes of BH4 deficiency except in AD-GTPCH and SR deficiency. Only mild HPA was detected in our cohort with highest blood phenylalanine levels pre-treatment being 676 µmol/l. Patient-6 with DHPR deficiency had an increased Phe/tyr of 3.5 with a borderline baseline phenylalanine levels suggesting HPA. Surprisingly, Patient-8 with AR-GTPCH deficiency didn't have HPA [20]. Ideal investigation after detecting HPA is to assess blood or urinary pterins (biopterin, neopterin, sepiapterin), CSF analysis of neurotransmitters [5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA)], pterins and 5-MTHFR and erythrocyte DHPR activity which will help in diagnostic confirmation and also in differentiating among the various subtypes of BH4 deficiencies [14]. The presence of HPA with normal BH4 cofactor metabolites suggests PKU and excludes BH4 disorders. Unfortunately as ours is a resource limited setting the above mentioned biochemical tests could not be performed in our study.

EEG changes previously described in the literature are non-specific [5,6]. Three patients in our cohort with DHPR deficiency had abnormal EEG findings, Patient-2 had multifocal epileptiform discharges, Patient-3 had classical hypsarrhythmia and Patient-5 had an EEG consistent with ESES. Drug refractory epilepsy was observed in all the three cases with abnormal EEG. Neuroimaging findings in BH4 deficiencies are indeterminate and not routinely recommended for diagnosis [14]. In the cohort by Ye et al., 2012 cerebral MRI abnormalities were seen nearly in 43% and mainly showed cerebral white matter changes[6]. Imaging changes are commonly described in patients with DHPR and PTPS deficiency. Similar to our cohort, the imaging findings described include delayed myelination, signal changes in parieto-occipital white matter, cerebral atrophy and central tegmental tract (CTT) hyperintensities [21, 22] . Intracranial calcifications like in our Patient-6 with DHPR deficiency, mainly in basal ganglia and subcortical regions have been previously described in patients with DHPR deficiency and in only one patient with PTPS deficiency [23]. Intracranial calcifications have been hypothesized to be secondary to cerebral folate depletion in DHPR deficiency. Other imaging findings in BH4 deficiency include subcortical cyst like lesions on T1 weighted images [22]. However, no correlation has been observed with the imaging features and degree of neurological impairment [23]. A total of 11 genetic variants were identified in our cohort with 9 P/LP variants related to BH4 disorders: 6 in the *QDPR* gene, 2 in the *GCH* gene and 1 in the *PTPS* gene. Most of the variants have been previously reported in ClinVar or HGMD except three novel variants of *QDPR* gene c.296-1G>T, c.295+5G>T and c.680T>C.

Earlier the initiation of treatment in BH4 disorders better is the neurodevelopmental outcome [5,6]. The main target of treatment is to lower the plasma phenylalanine levels by low phenylalanine diet in conjunction with BH4 supplementation, and replacement of monoamine neurotransmitters namely L-Dopamine and 5-HT [14]. Sapropterin hydrochloride, synthetic analogue of BH4 was first approved by FDA in 2007 for the treatment of BH4 responsive HPA [7]. Oral BH4 at therapeutic dose doesn't cross blood brain barrier and is mainly involved in lowering peripheral blood phenylalanine levels. However oral BH4 is expensive and most of our patients didn't receive it due to non-affordability and non-availability in India. Only one of our patient was able to access BH4 and received it at a suboptimal dose of 2mg/kg/day later in the disease course. The actual recommended dose of BH4 is around 5-10mg/kg/day. Sometimes a higher dose of BH4 (upto 20 mg/kg/day) is required to maintain optimal CSF BH4 levels [24]. Among patients with DHPR deficiency, traditionally treatment with only low phenylalanine diet and neurotransmitter replacement has been advocated with controversial role of BH4 as the defect is mainly in BH4 recycling. This is because, a very high dose of BH4 (>20mg/kg) will be required if BH4 is solely used for treatment of HPA in DHPR deficiency. This leads to an excess of BH2 which in turn leads to inhibition of aromatic acid hydroxylases, NO uncoupling resulting in oxidative stress and neurotoxicity [25]. Nonetheless Coughlin et al., 2013 has reported clinical improvement in a case of DHPR deficiency with oral supplementation of BH4 (upto 40mg/kg) along with levodopa and 5-HT [26]. Patient-2 with DHPR deficiency who received BH4 at 2mg/kg/day had no adverse effects and the dose was inadequate for any significant clinical improvement.

Levodopa in combination with peripheral decarboxylase inhibitor (carbidopa/benserazide) is recommended at a dose of 3-7 mg/kg/day (GTPCH deficiency) and upto 10 mg/kg/day in rest of the BH4 disorders starting at a low dose of 0.5-1mg/kg/day [14]. All the patients in our cohort received levodopa/carbidopa at a median dose of 3mg/kg/day (range 1-10mg/kg). 5-HT starting at a dose of 1-2 mg/kg/day (target dose 5mg/kg/day) is recommended in all BH4 disorders except AD-GTPCH and PCD deficiency [14]. Only five patients in our cohort received it at a median dose of 3mg/kg/day (range 1-5mg/kg) due to affordability issues. Folinic acid (10-20mg/day) is beneficial in patients with DHPR deficiency as it leads to secondary cerebral folate depletion and also in cases with low CSF 5-MTHFR [14]. Owing to lack of facility for monitoring CSF 5-MTHFR, all the patients in our study received folinic acid at a dose of 10-15mg/day. Additionally children received symptomatic management for seizures and dystonia. Medication related adverse events was not observed in our patients, except P-7 with GTPCH had mild dyskinesias with L-Dopa. Ideally monitoring of treatment by blood/plasma phenylalanine levels, CSF HVA, 5-HIAA and 5-MTHFR and titration of dosage of BH4, levodopa and 5-HT is essential to ensure optimal level of neurotransmitters required for brain development [14]. Prolactin is another indirect marker used for assessing effectiveness of levodopa in order to avoid invasive lumbar puncture [27]. Selective monoamine

oxidase inhibitors, dopamine agonists, catechol-o-methyltransferase inhibitors, selective serotonin reuptake inhibitors are the next line agents suggested for treatment of BH4 disorders [14].

We had a median duration of follow up of 15 months in our study, with a median reduction of blood phenylalanine levels by 190 µmol/l. Though biochemical response has been marked, except for patients with GTPCH deficiency, only mild clinical improvement was noted with regards to developmental milestones, seizures or dystonia. Developmental quotient at follow up in 8/9 patients was < 70 suggesting severe developmental delay. This finding is in line with other studies wherein plasma phenylalanine levels had no correlation with the developmental quotient. Rather, developmental quotient inversely correlated with the age of initiation of treatment [6, 23]. Irreversible neuronal damage (basal ganglia calcification, neuronal loss) in DHPR deficiency occurs at a very early age that fails to amend with therapy. Hence, detection of the disorder within first month of life will ensure good neurological prognosis [28]. Patients who were initiated on treatment at early age, had higher incidence of better neurological outcome with lesser children having mental retardation, hypotonia or seizures [5,28]. However, few patients develop severe phenotype despite initiation of treatment at an early age [29]. Children with later age of onset of treatment displayed mental retardation, aggressive behaviour, irritability and autistic features, despite improvement in IQ [23, 30]. Moreover, patients with earlier age of onset of treatment had achieved normal IQ. Although our study comprised of only 9 patients, the mean development quotient of 44.8 ± 28.9 must be interpreted with caution. Yet, it is much lower than the development quotient observed after 3 years of treatment in another study (78±15) [23].

Our data indicates that patients in our follow up have much worse neurological outcome than reported in previous studies [30].There are two factors leading to this wide discrepancy. Firstly, these studies were done in countries where new-born screening for these disorders has been implemented with good coverage, resulting in detection in the neonatal period, leading to early initiation of treatment. Secondly, most of the patients were treated with BH4 supplements unlike our patients, hence affirming the fact that BH4 is indispensable in the treatment for BH4 deficiency. However, these factors may not hold true for all patients. Patient-1 diagnosed and started on treatment at the age of 4 years improved tremendously, while Patient-2 and Patient-5 diagnosed and started on earlier treatment has poor developmental outcome, with drug refractory seizures [28]. The overall outcome of these patients with BH4 deficiency is tremendously conditioned by the age of initiation of treatment and the adequacy of the dose of the medication. Folinic acid administration, particularly plays a very significant role in the developmental outcome [29]. Patients who have developed severe developmental delay and low CSF HVA and HIAA values before starting treatment are unlikely to make complete recovery even with the best medical management [28]. The lack of wide accessibility to pterin and CSF neurotransmitter analysis makes monitoring of treatment highly dependent on clinical assessment, that may not give sufficient evidence for dosage optimization, thereby leading to subtle progressive brain damage [16, 23]. In addition to serum phenylalanine, regular monitoring of serum prolactin may remove impediments to a certain extent in this regard.

Management of BH4 deficiency in India poses a major challenge. The non-availability of diagnostics and lack of national neonatal screening programmes for detection of these disorders is compounded by the lack of BH4 supplements for the diagnosed patients [31]. Universal new-born screening for HPA in conjunction with BH4 cofactor metabolites is essential since 25% of children with PTPS and GTPCH deficiency and 40% of children with DHPR deficiency are asymptomatic in the new born period and remain asymptomatic until 4 months of age [5, 32]. The new born screening may infact be normal on day one of life and hence a repeat testing is essential later after day-3 of life [30]. The first step towards inclusion into the new-born screening programme in India as well as availability of BH4 supplements will pave way for earlier identification and better neurodevelopmental outcome for Indian children with this rare yet treatable neurotransmitter disorder.

To the best of our knowledge, this is the first Indian study described on a cohort of genetically confirmed patients with BH4 deficiency. Strengths of the study includes strict inclusion of only genetically confirmed cases of disorders of biopterin metabolism, elaborate description of clinical features, investigations, treatment, and outcome. Limitations include the retrospective nature with non-uniform data and follow up, lack of facility to perform BH4 cofactor analysis, segregation analysis, parental analysis, and functional studies. Although the study suffers from its inherent limitations, this is a novel endeavour to highlight the challenges faced during management of patients with BH4 deficiency in India. This study also highlights the need for initiation of nationwide new-born screening which is an unmet need of the hour not only for HPA but also for various other inborn errors of metabolism which have a better outcome when detected and treated early.

Conclusion

Tetrahydrobiopterin deficiencies represent an rare yet potentially treatable cause for non-phenylketonuria hyperphenylalaninemia when diagnosed and treated early in life. Developing a policy of new-born screening and implementing at national level can help prevent devastating neurological deterioration. This study expands the genotype phenotype spectrum of patients with disorders of tetrahydrobiopterin metabolism from South India.

Declarations

Funding :

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Conflicts of Interest :

None

Availability of data and material :

Everything has been presented in the manuscript. Any additional data required shall be provided

Code availability:

Not applicable

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Yes

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Tables

Table 1

Clinical features of patients with disorders of tetrahydrobiopterin metabolism

Details	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	P
Age at onset of symptoms	2 years	3 months	3 months	6 months	11 months	2 years	4 months	6 m
Age at diagnosis	4 years	9 months	18 months	15 months	12 months	10 years	8 months	8
Gender	Female	Female	Male	Male	Female	Female	Female	M
Initial Manifestations	Ataxia	Delayed milestones	Neuroregression with dystonia and seizures	Neuroregression with seizures	Neuroregression with seizures	Neuroregression with dystonia	Delayed milestones	A tw pe of lo lir
Clinical features	Developmental delay, dystonia and ataxia since 2 years of age	Developmental delay, regression of milestones, seizures, dystonia from 9-months of age, epileptic spasms	Developmental delay, dystonia at 9-months, epileptic spasms followed by regression	Regression of milestones at 6 months of age, seizures, recurrent respiratory tract infections	Regression of milestones with seizures at 11-months of age , irritability and constipation	Regression of milestones on the background of developmental delay, dystonia since 2-years of age	Developmental delay, irritability, oculogyric crisis, dystonia and choreoathetoid movements since 4-months	D of lir si ye up lir si ye ag
Developmental delay	+	+	+	+	-	+	+	-
Regression of milestones	+	+	+	+	+	+	+	-
Seizures	-	+	+	+	+	-	-	-
Behavioural problems	-	-	-	-	+	-	+	-
Oculogyric crisis	-	-	-	-	+	-	+	-
Dystonia	+	+	+	-	-	+	+	+
Ataxia	+	-	-	-	-	-	-	-
Consanguineous parents	Yes	Yes	Yes	Yes	No	Yes	Yes	Y
Examination								
Microcephaly	No	No	Yes	No	No	No	Yes	N
Skin and hair findings	-	-	Café – au – lait spots	-	-	-	-	-
Ocular findings	-	-	-	-	Oculogyric crisis	-	Oculogyric crisis	-
Tone	Variable	Variable	Variable	Hypotonia	Hypotonia	Variable	Variable	V
Extrapyramidal features	Dystonia	Dystonia	Dystonia	-	-	Dystonia	Dystonia	D

Table 2

Biochemical and radiological pattern in patients with disorders of tetrahydrobiopterin metabolism

Details	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Blood Phenylalanine levels before starting treatment (20-150 µmol/l)	287.2	340	450	676	495	118	300	69.2
Blood Phenylalanine tyrosine ratio (cut off 1.5)	21.26	9.70	7.02	6.56	14.6	3.6	18.97	1.1
EEG	Normal	Multifocal inter-ictal epileptiform discharges	Hypsarrhythmia	Normal	Sleep activated near continuous spike-wave discharges	Normal	Normal	Not done
MRI brain	Normal	Normal	Delayed myelination	T2 hyperintensity and diffusion restriction of central tegmental tracts	Subcortical T2 hyperintensities with cerebral and cerebellar atrophy	Calcification in bilateral lentiform nuclei, thalami, cerebellar hemispheres and frontal, parietal, temporal and occipital white matter	Normal	Normal
Mutational analysis	QDPR gene Exon 5 c.488G > T (p.Ser163Ile)	QDPR gene Exon 1 c.68G>A (P.Gly23Asp)	QDPR gene Intron 3 c.295+5G>T **(5' splice site)**	QPDR gene Exon 7 c.680T>C (p.Leu227Pro)	QPDR gene Intron 3 (c.296-1G>T) (3' splice site)	QPDR gene Exon 7 c.635T>C (p.Phe212Ser)	GCH1 gene Exon 6 c.703C>G (p.Arg235Gly)	GCH1 gene Exon 3 c.457C>T(p
Additional variants	-	-	NF1 gene Exon 19 c.2288T>C (p.Leu763Pro)	ZMYND10 gene Intron 11 c.1247+2T>G (5' splice site)	-	-	-	-
Diagnosis	DHPR deficiency	DHPR deficiency	DHPR deficiency with Neurofibromatosis-1	DHPR deficiency with Primary ciliary dyskinesia-22	DHPR deficiency	DHPR deficiency	GTPCH deficiency	GTPCH defi

EEG- electroencephalography, MRI- Magnetic Resonance Imaging, DHPR- dihydropteridine reductase, GTPCH - GTP cyclohydrolase I, PTPS- 6-pyruvoyl-tetrahy

Table 3

Detailed description of mutational analysis of patients with disorders of tetrahydrobiopterin metabolism

Proband	Gene (Location)	Genomic coordinate	Variant	Zygosity	Constraint Scores	Population Frequency (gnomAD, 1kG, 100kGenomeAsia)	Functional prediction	Splicing prediction
Patient 1	QDPR (Exon 5)	chr4:17493912C>A AA residue reported in HGMD	c.488G>T (p.Ser163Ile)	Homozygous	pLI=0 Z=0.28	Absent	11 deleterious (BayesDel_addAF, DANN, DEOGEN2, EIGEN, FATHMM-MKL, LIST-S2, M-CAP, MVP, MutationAssessor, MutationTaster and SIFT)	No impact
Patient 2	QDPR (Exon 1)	chr4:17513610C>T ClinVar reported HGMD reported	c.68G>A (p.Gly23Asp)	Homozygous	pLI=0 Z=0.28	Absent	12 deleterious (BayesDel_addAF, DANN, DEOGEN2, EIGEN, FATHMM-MKL, LIST-S2, M-CAP, MVP, MutationAssessor, MutationTaster, PrimateAI and SIFT)	No impact
Patient 3	QDPR (Intron 3)	chr4:17505997C>A	c.295+5G>T (5' splice site)	Homozygous	pLI=0 Z=0.28	Absent		Predicted Splice Donor loss (GeneSplicer, MaxEntScan, NNSplice, PWM) varSEAK: Loss of function for authentic Splice Site. Exon Skipping
Patient 4	QDPR (Exon 7)	chr4:17488809A>G	c.680T>C (p.Leu227Pro)	Homozygous	pLI=0 Z=0.28	Absent	9 deleterious (BayesDel_addAF, DEOGEN2, EIGEN, FATHMM-MKL, LIST-S2, MVP, MutationAssessor, MutationTaster and SIFT) vs 2 benign (DANN and PrimateAI)	No impact
Patient 5	QDPR (Intron 3)	chr4:17503483C>A	c.296-1G>T (3' splice site)	Homozygous	pLI=0 Z=0.28	Absent		Predicted splice acceptor loss, causes frameshift (GeneSplicer, MaxEntScan) VarSEAK: Loss of function for authentic Splice Site. Use of cryptic site 11 nt downstream of 3' ss
Patient 6	QDPR (Exon 7)	chr4:17488854A>G AA residue reported in HGMD	c.635T>C (p.Phe212Ser)	Homozygous	pLI=0 Z=0.28	Absent	10 deleterious (BayesDel_addAF, DANN, DEOGEN2, EIGEN, FATHMM-MKL, M-CAP, MVP, MutationAssessor, MutationTaster) and SIFT) vs 2 benign (LIST-S2 and PrimateAI)	No impact
Patient 7	GCH1 (Exon 6)	chr14:55310785G>C AA residue reported in HGMD	c.703C>G p.Arg235Gly	Homozygous	pLI=0.9 Z=1.52	Absent	11 deleterious (BayesDel_addAF, DANN, DEOGEN2, EIGEN, FATHMM-MKL, LIST-S2, M-	No impact

							CAP, MVP, MutationAssessor, MutationTaster and SIFT) vs 1 benign (PrimateAI)	
Patient 8	GCH1 (Exon 3)	chr14:55326451G>A AA residue reported in HGMD	c.457C>T (p.His153Tyr)	Homozygous	pLI=0.9 Z=1.52	Absent	12 deleterious (BayesDel_addAF, DANN, DEOGEN2, EIGEN, FATHMM-MKL, LIST-S2, M-CAP, MVP, MutationAssessor, MutationTaster, PrimateAI and SIFT)	No impact
Patient 9	PTS (Exon 1)	chr11:112097231C>G HGMD reported	c.65C>G (p.Ala22Gly)	Homozygous	pLI= 0 Z= 0.13	Absent	11 deleterious (BayesDel_addAF, DANN, DEOGEN2, EIGEN, FATHMM-MKL, LIST-S2, M-CAP, MVP, MutationAssessor, MutationTaster, SIFT) vs 1 benign (PrimateAI)	No impact

HGMD- Human Gene Mutation Database

Table 4

Treatment and follow up of patients with disorders of tetrahydrobiopterin metabolism

Details	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Treatment									
Low phenylalanine diet	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes
BH4 (mg/kg/day)	No	Yes, 2 mg/kg/day	No	No	No	No	No	No	No
Levodopa-carbidopa (mg/kg/day)	3 mg/kg	3 mg/kg	3 mg/kg	5 mg/kg	3 mg/kg	10 mg/kg	1 mg/kg	6 mg/kg	3 mg/kg
5 -HTP (mg/kg/day)	No	3mg/kg	No	5 mg/kg	2 mg/kg	1 mg/kg	3 mg/kg	No	No
Folinic acid (mg/day)	15mg	15mg	15mg	10 mg	10 mg	10 mg	15mg	10mg	15mg
Follow up									
Follow up duration in months	48	27	6	2	15	12	40	12	16
Improvement in milestones	Complete	Minimal	Minimal	Significant	Minimal	No	Significant	Baseline milestones were normal	Minimal
Improvement in dystonia	Yes, mild improvement	Yes, mild improvement	Yes, mild improvement	N/A	N/A	Yes, mild improvement	Yes, significant improvement	Yes, significant improvement	Yes, mild improvem
Improvement in seizures	N/A	No, seizures remained drug refractory	No, seizures remained drug refractory	Yes, complete cessation of seizures	No, seizures remained drug refractory	N/A	N/A	N/A	Yes, mild reduction seizure frequency
Serum phenylalanine levels in last follow up (µmol/l)	150	20	60	269.7	103.3	Not repeated as baseline was normal	350	Not repeated as baseline was normal	200
Development quotient (DQ) at last follow up	70	30	30	41.1	22.2	10	70	100	30
BH4- tetrahydrobiopterin , 5-HTP- 5 hydroxytryptophan, N/A- Not applicable									

Figures

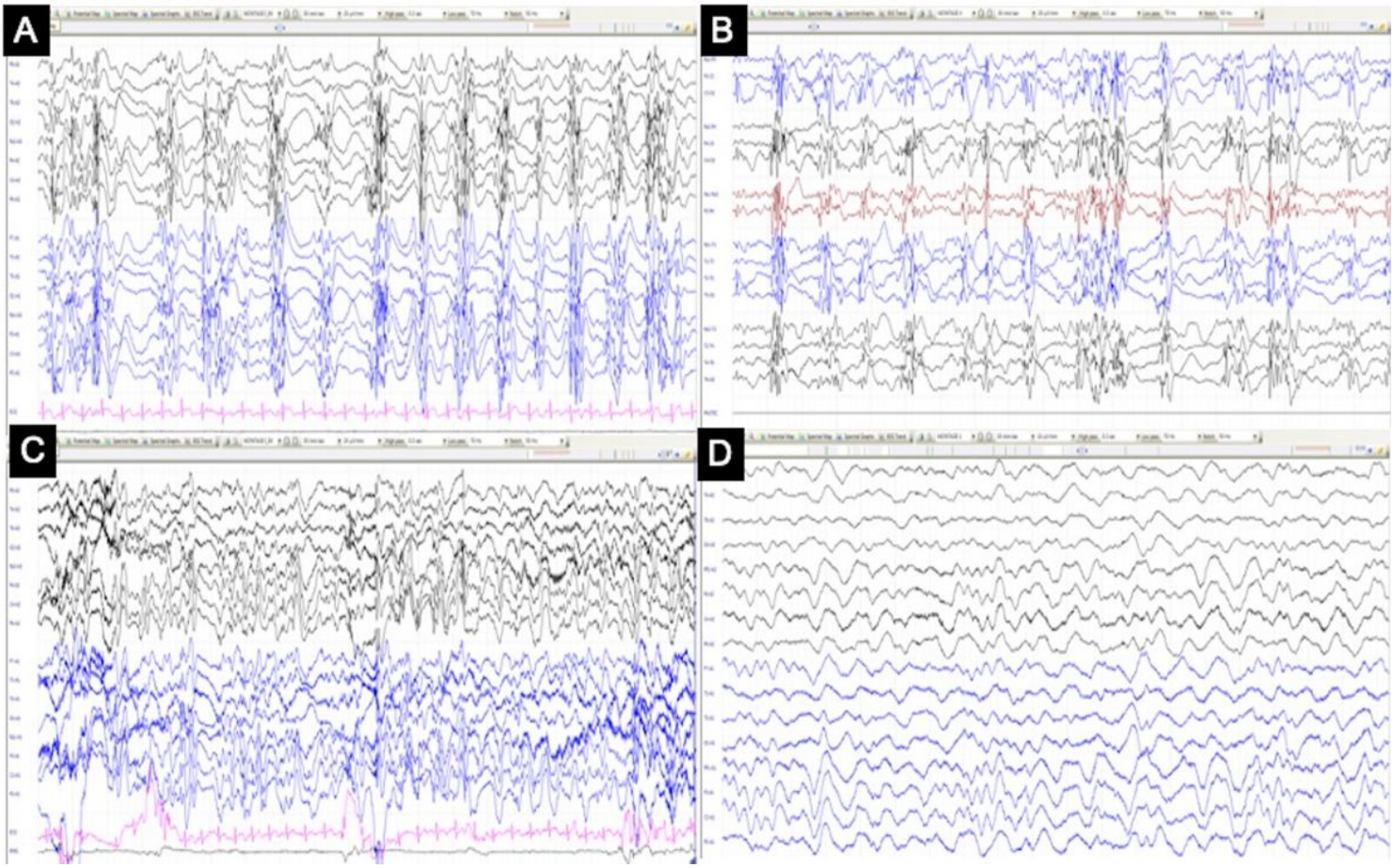

Figure 1

Eight patients in the study, six with DHPR deficiency, one with PTPS deficiency and one with GTPCH deficiency had underwent EEG to detect clinical and sub-clinical seizures. EEG was normal in 5/8 patients. EEG was abnormal in patient-2, 3 and 5. Patient-2 had multifocal inter-ictal epileptiform discharges (IEDs), patient-3 had classical hypsarrhythmia and patient-5 had sleep accentuated, near-continuous, generalized (fronto-central dominant) spike/polyspike wave discharges of 1-2 Hz with marked reduction in IEDs on awakening consistent with electrical status epilepticus during slow wave sleep (ESES) (Figure 1A-C).

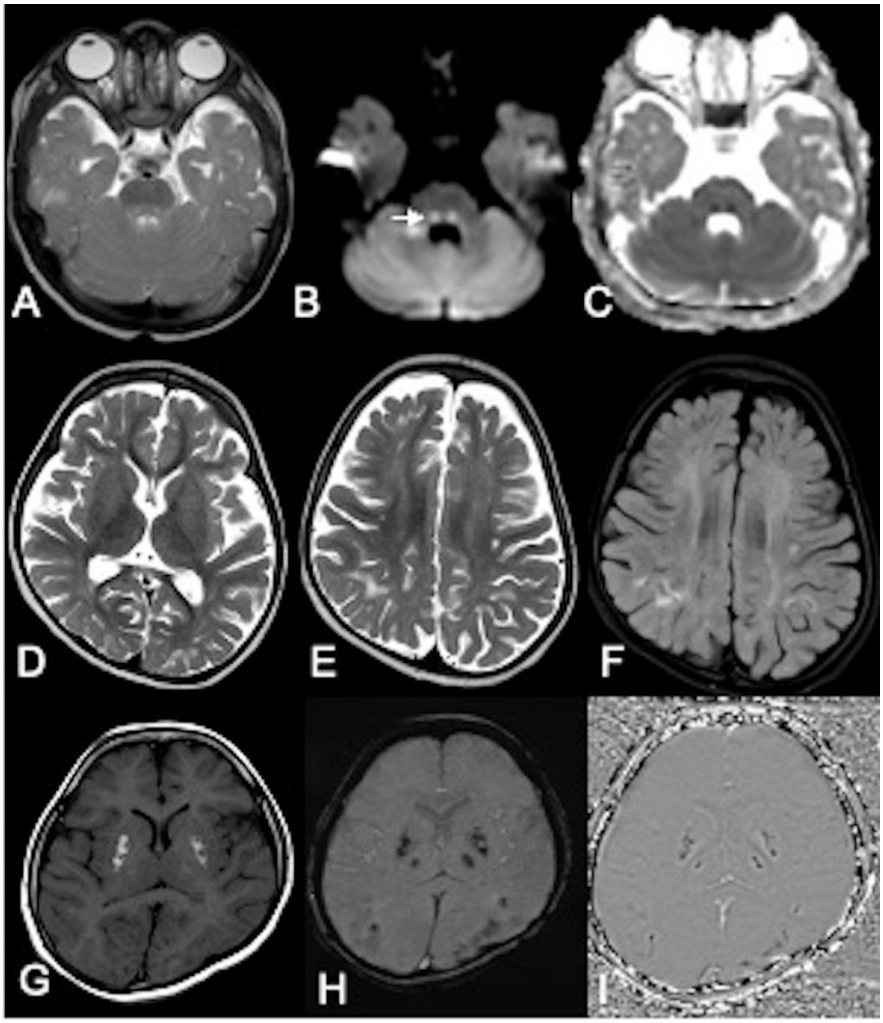

Figure 2

MRI brain was normal in 4/9 patients, two patients each with DHPR deficiency (P-1,2) and GTPCH deficiency (P-7,8). MRI findings were consistent with delayed myelination in two patients, one each of DHPR deficiency (P- 3) and PTPS deficiency (P-9). Patient-4 with DHPR deficiency had T2 hyperintensity of bilateral central tegmental tracts with diffusion restriction (Fig 2A,B&C). Patient-5 with DHPR deficiency had T2 hyperintensities in the subcortical white matter of both frontal, parietal and occipital lobes with partial inversion on FLAIR (Fig 2D,E&F). Patient-6 with DHPR deficiency had bilateral symmetrical calcification in the lentiform nuclei, anterolateral thalami and subcortical white matter of all the lobes with occipital preponderance(Fig 2G, H, I). Calcification was also noted in the white matter of both cerebellar hemispheres.

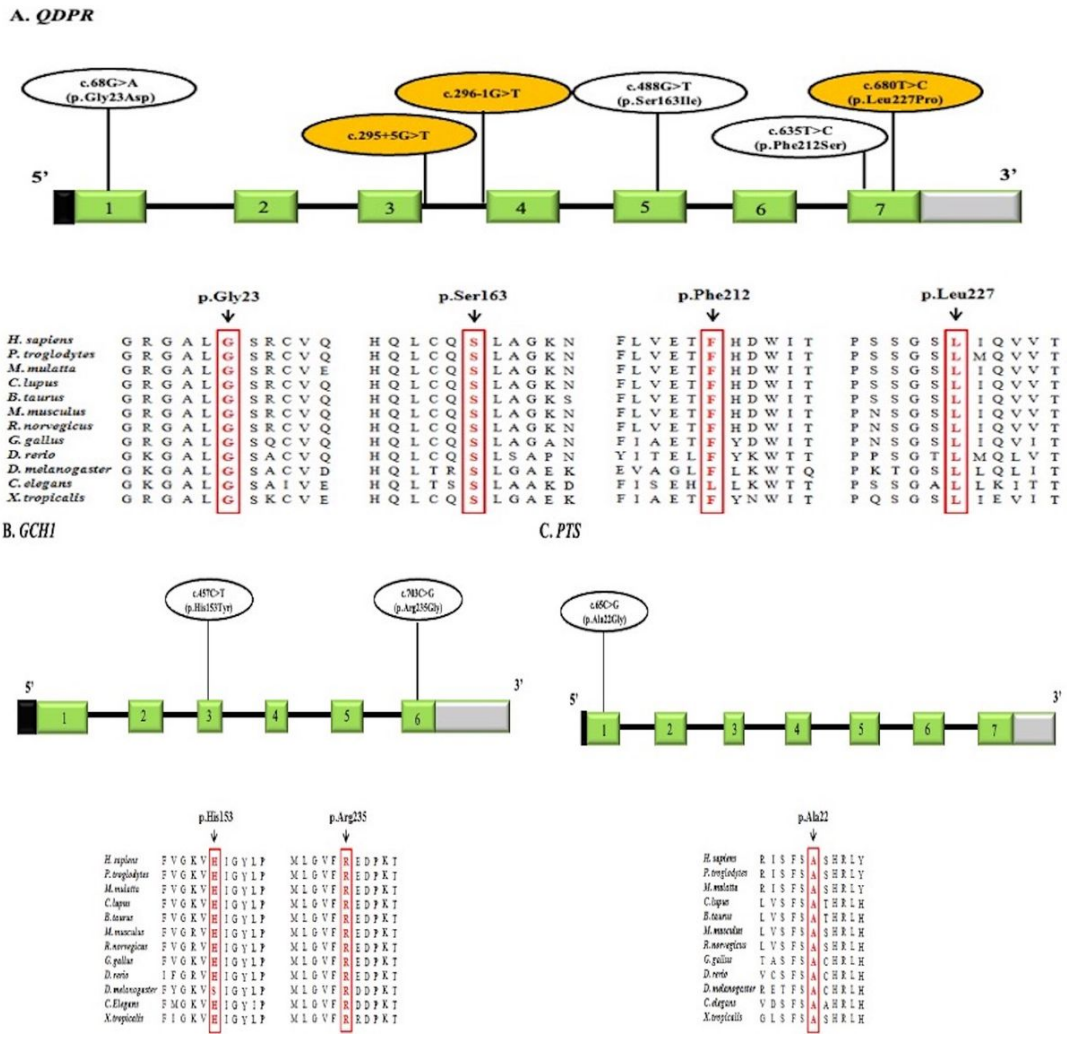

Figure 3

The observed variation lies in the 6-pyruvoyl tetrahydropterin synthase domain of the PTS protein and has been reported in HGMD database (CM064184). A schematic diagram of the genes - QDPR, GCH1 and PTS, and the mutations found in this study is shown in Figure 3.