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Recurrent Recessive Mutation in *DGUOK* Causes Idiopathic Non-Cirrhotic Portal Hypertension

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Abstract

Despite advances in the diagnosis and management of idiopathic non-cirrhotic portal hypertension (INCPH), its pathogenesis remains elusive. Insight may be gained from study of early onset familial INCPH, in which Mendelian mutations may account for disease. We performed exome sequencing of 8 subjects from 6 kindreds with onset of portal hypertension of indeterminate etiology during infancy or childhood. Three subjects from two consanguineous families shared the identical rare homozygous p.N46S mutation in *DGUOK,* a deoxyguanosine kinase required for

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CONFLICT OF INTEREST

The authors declare no conflict of interest with respect to this study.

mitochondrial DNA replication; haplotype sharing demonstrated the mutation in the two families was inherited from a remote common ancestor. All three affected subjects had stable portal hypertension with non-cirrhotic liver disease for 6–16 years of follow-up. This mutation impairs ATP binding and reduces catalytic activity. Loss-of-function mutations in *DGUOK* have previously been implicated in cirrhosis and liver failure but not in isolated portal hypertension. Interestingly, treatment of patients with HIV infection with the nucleoside analog didanosine is known to cause portal hypertension in a subset of patients and lowers deoxyguanosine kinase levels in vitro; the current findings implicate these effects on deoxyguanosine kinase in the causal mechanism. CONCLUSION: Our findings provide new insight into the mechanisms mediating inherited and acquired non-cirrhotic portal hypertension, expand the phenotypic spectrum of *DGUOK* deficiency, and provide a new genetic test for a specific cause of INCPH.

Keywords

whole-exome sequencing; germ line mutations; mitochondrial kinase; DGUOK deficiency; didanosine toxicity

INTRODUCTION

Portal hypertension is a clinical syndrome defined by portal venous system pressure that is at least 5 mmHg higher than the pressure in the inferior vena cava (1). High pressure in the portal venous system leads to shunting of blood through vessels that are poorly suited to carrying large blood volumes, resulting in collateral circulation and splenomegaly. While liver cirrhosis is the most frequent cause, a variety of other liver and extra-hepatic disorders may cause elevated pressure in the portal venous system. When all known causes of portal hypertension have been ruled out, the diagnosis of idiopathic non-cirrhotic portal hypertension (INCPH) is established (2). In accord with the Baveno VI consensus conference, idiopathic portal hypertension, non-cirrhotic portal fibrosis and INCPH indicate the same clinical entity (3). These entities all feature the presence of portal hypertension in the absence of cirrhosis, known extra-hepatic diseases and splanchnic venous thrombosis, at some point in the disease process (4).

INCPH has been reported in infancy and childhood (5), and in some families more than one individual is affected (6). Since rare childhood phenotypes are promising candidates for new Mendelian traits, we posit that genetic factors may underlie the onset and development of portal hypertension in a subset of these subjects. The advent of whole exome sequencing (WES), which sequences ostensibly all ~20,000 protein-coding genes in the human genome, is an effective means to identify rare protein-coding variants in individual gene(s). Rigorous statistical analysis can identify genes that are mutated more often than expected by chance after accounting for examination of all protein-coding genes, and has the potential to identify new genes causing human diseases (7–9).

To investigate this hypothesis, we studied 8 pediatric patients with INCPH from 6 kindreds ascertained in Turkey that were not known to be related to one another. In four kindreds affected subjects were the offspring of consanguineous union.

PATIENTS and METHODS

Human subjects

The study protocol was approved by the Human Investigation Committees of Gazi University and Yale University. All patients and/or their parents provided written informed consent.

DNA isolation, exome capture and sequencing

DNA was isolated from peripheral venous blood using standard procedures. DNA fragments containing exonic sequence were captured using the Roche/Nimblegen SeqCap EZ Human Exome Library v2, and 75 base paired end sequencing on the Illumina HiSeq platform was performed as previously described (10). Coverage statistics are provided in Table 1.

Exome Sequencing Analysis

Sequence analysis was performed using a previously described pipeline (11). Protein-altering variants with MAF < 1% in the Yale (2,500 European subjects), NHLBI Exome Variant (4,300 European and 2,203 African-American subjects; last accessed March 2015), dbSNP (version 137), 1000 Genomes (1,094 subjects of various ethnicities; May 2011 data release) and ExAC databases (61,000 subjects of various ethnicities; January 2015, data release) were selected and annotated for predicted impact on encoded proteins and for conservation of the reference amino acid among vertebrate and invertebrate orthologs (removing missense mutations at weakly conserved positions among orthologues as defined as two or more species with substitutions). Autosomal-recessive inheritance was investigated and genes with rare homozygous or compound heterozygous variants were prioritized. The mutation burden per gene in the cohort of patients with INCPH was compared to that in a control cohort comprising 1,310 parents of children with autism of European ancestry (12) whose parents were not known to have autism or liver disease. These control subjects were sequenced to similar depth of coverage using the same method. Mutation burden of each gene in cases and controls was compared using Fisher's exact test.

Sanger sequencing of genomic DNA

Direct bidirectional Sanger sequencing of an identified *DGUOK* mutation, p.N46S, was performed by PCR amplification of genomic DNA of three probands (patient 1, patient 2A and patient 2B) and their parents using forward primer: 5′-CGAGCACCCTTCAGTTCCAT-3′, and reverse primer: 5′-CTCCTTTCCCAGCCTCTGTC-3′. Nomenclature of the *DGUOK* variants is based on NCBI reference sequence NM_080916.2.

Orthologs and related kinases

Full-length orthologs of deoxyguanosine kinase and related kinases (deoxycytidine kinase and thymidine linase 2) protein sequences from available species in GenBank. Protein sequences were aligned using the ClustalW algorithm.

Principal Component Analysis (PCA) and analysis of relatedness among the three subjects that share the identical homozygous p.N46S DGUOK mutation

PCA was performed as previously described (10). In brief, all tag SNP genotypes were extracted from exome sequencing data and used as inputs to perform PCA with EIGENSTRAT software along with the same SNPs from subjects in the HapMap project (13). Relatedness analysis was performed using Beagle v.3.3.2(14) and by calculating the fraction of rare (MAF <0.01%) variants shared among the three index cases harboring the recurrent p.N46S *DGUOK* mutation.

RESULTS

Exome sequencing of 8 pediatric patients with idiopathic non-cirrhotic portal hypertension

Eight subjects with INCPH from 6 families were studied. All individuals presented between the ages of 5 months and 17 years with signs of isolated portal hypertension (hepatosplenomegaly +/− esophageal varices). All subjects had liver biopsy showing no evidence of cirrhosis, and biochemical tests showed no evidence of hepatic synthetic dysfunction as defined by normal albumin, bilirubin and prothrombin time levels (Supplementary Table 1). All patients had patent abdominal vasculature as documented by abdominal doppler ultrasound, and unremarkable echocardiogram.

We performed WES of germ line DNA isolated from each of the eight subjects. Targeted bases were sequenced by a mean of 58 independent reads, with 95.5% of targeted bases having > 8 independent reads, allowing high confidence calling of homozygous and heterozygous variants across the exome (Table 1).

In four kindreds, affected subjects were the offspring of consanguineous union, suggesting autosomal recessive transmission. Hence, we first searched for genes with rare homozygous or compound heterozygous variants. For recessive transmission, we considered protein-altering alleles with minor allele frequencies (MAF) <1% in dbSNP, NHLBI, 1000Genomes, Exome Aggregation Consortium (ExAC) and Yale exome databases and sought homozygous and putative compound heterozygous genotypes. We then determined whether 1. Specific recessive genotypes recurred among affected members in more than one kindred; 2. Any genes had different recessive genotypes more often than expected by chance; 3. Any recessive genotypes were found in genes previously implicated in human recessive diseases.

Identification of a recurrent rare homozygous loss of function variant in *DGUOK*

In these analyses, we found a recurrent homozygous variant, encoding a p.N46S missense mutation, in the gene *DEOXYGUONOSINE KINASE* (*DGUOK;* NM_080916), in the two living affected siblings in one kindred and the sole affected subject from a second kindred. Sanger sequencing in each case confirmed the homozygous mutation, and the heterozygous carrier state of their parents, who were first cousins in both instances (Figure 1). This specific variant was extremely rare, with no homozygotes in any database. There were two heterozygotes for this allele among more than 129,000 alleles sequenced from diverse populations in the ExAC (15) database, and none in NHLBI and 1000 Genome databases; this allele was also absent among 894 exomes of Turkish individuals in the Yale database

(Table 2). The two subjects with this allele in ExAC were of European descent (allele frequency in this population in ExAC of 3.1×10^{-5}). Principle component analysis of the affected individuals with this homozygous mutation indicated that they cluster with people of European ancestry from the HapMap project (Figure 2).

Distant relationship of kindreds with p.N46S *DGUOK* mutation

The finding of the identical very rare homozygous mutation in affected members of two kindreds strongly suggests that these mutations are inherited identically by descent from a remote common ancestor. To test this possibility, we compared the sharing of rare and common variants between siblings in kindred 2 and between the affected members of kindred 2 and kindred 1. As expected, the affected siblings in kindred 2 shared a high fraction of all novel and rare variants: 59%,115 of 195 variants with MAF < 0.01% in dbSNP, NHLBI, 1000Genomes, ExAC, Yale and Turkish exome databases were shared by the siblings. This proportion is greater than 50% owing to consanguinity. In contrast, the subject from kindred 1 shared only four (~2%) rare and zero novel variants with either patient 2A or patient 2B, indicating that these subjects are not extremely closely related. Kinship analysis using the Beagle program (14) indicated that patients 1 and 2A shared an estimated 1.3% of their genomes, consistent with the affected subjects from these kindreds being approximately third cousins (i.e., they would share a set of great-great-grandparents).

Consistent with the inference that the mutation is inherited identically by descent in affected subjects from the two families, haplotype analysis indicates that SNPs captured in the exome data that flank the *DGUOK* mutation are homozygous and identical in affected members of the two kindreds (Figure 3 and supplementary Table 2). This segment of homozygous identity spans a minimum segment of ~1.8 Mb, strongly supporting the inference that this mutation is inherited identically by descent by these subjects (Figure 3).

Assuming 3rd cousin relationship, we can calculate the likelihood that these three affected individuals would be homozygous for the same variant by chance alone. The LOD score for linkage of this mutation to INCPH is 3.9 (likelihood ratio of 8.2×10^3 in favor of linkage), providing strong statistical support for the role of this mutation in INCPH.

DGUOK is previously implicated in liver failure

The finding of the identical very rare homozygous variant in two kindreds, with evidence of significant co-segregates of this genotype with INCPH, implicates this homozygous genotype in this disease. This observation is further supported by three additional findings. First, recessive mutations in *DGUOK* have previously been implicated in liver disease. *DGUOK* encodes deoxyguanosine kinase, which phosphorylates purine deoxyribonucleosides in the mitochondrial matrix and is required for replication of mitochondrial DNA (mtDNA). Patients with recessive *DGUOK* deficiency have mtDNA depletion syndrome type 3(16), characterized by a reduction in mtDNA copy number in affected tissues such as liver (17). They typically present with severe liver dysfunction at early age associated with neurological impairment (hepatocerebral form) (17). A less common phenotype includes isolated hepatic disease with no neurological dysfunction (18, 19). The majority of these patients do not survive beyond the first few years of life due to

progressive liver failure (18, 19) (Table 3). Nonetheless, four adult individuals with no liver involvement and primary neurological symptoms have also been reported (20), reinforcing the concept that *DGUOK* deficiency can lead to diverse clinical phenotypes (21).

Secondly, the crystal structure of DGUOK (PDB accession code 2OCP (22)) shows that N-46 is located in the catalytic domain P-loop, a canonical kinase motif that binds and orients the phosphates of the donor ATP molecule. This position is completely conserved among DGUOK orthologs as well as two more distantly related kinases (Figure 4A). In DGUOK, the side chain amide of N-46 makes a hydrogen bond with the backbone carbonyl of Leu-219 in helix α8 (Figure 4B). The p.N46S mutation results in loss of this hydrogen bond, potentially disrupting binding and catalysis. Consistent with this expectation, this mutation has been shown to result in biochemical loss of function (23); nonetheless, unlike many mutations in DGUOK associated with liver failure that are likely complete null alleles, this mutation has been shown to retain partial activity (14% of normal) (23).

Thirdly, the p.N46S mutation has previously been associated with liver disease in five individuals, including one subject with homozygous and four with compound heterozygous mutations (19, 23, 24). The homozygous infant had rapid progression to cirrhosis and liver failure at 10 months of age (24) while the other four subjects all presented with severe liver dysfunction during infancy with variable rate of disease progression. Specifically, a pair of monozygotic twins compound heterozygous for p.N46S and another DGUOK mutation have been described; one infant died of liver failure at 10 months of age whereas his twin brother had reversible liver failure and was reported alive and clinically stable at 3.5 years of age (19). Interestingly, herpes simplex virus type 1 infection occurred in the deceased, but not the surviving twin brother, suggesting the possibility of environmental modifiers of the phenotype. Additionally, a patient with compound heterozygous mutations (p.N46S and p.L266R) who initially presented with infantile cholestasis and liver fibrosis demonstrated a spontaneous unexplained gradual improvement of his liver function after 32 months of age, with subsequent normal growth and development (patient was 10 years-old at the time of last report) (23). These findings leave no doubt about the pathogenicity of the p.N46S mutation in our subjects, but indicate a surprisingly distinct clinical picture from what has previously been described with this mutation.

Clinical features of patients with homozygous DGUOK p.N46S mutation

Patient 1 is a 19 year-old male who presented at 12 years of age with intermittent right upper quadrant pain for approximately 1 year. Physical examination was remarkable for hepatosplenomegaly with no other liver-specific findings. Liver function tests were within normal limits. Abdominal ultrasound showed heterogeneous liver with patent abdominal vasculature. Additional diagnostic evaluation included serologies for viral hepatitis, EBV, TORCH (Toxoplasma gondii; Other: coxsackievirus, chickenpox, chlamydia, HIV, HTLV, syphilis; Rubella; CMV; HSV-2); tests for autoimmunity (immunoglobulin G level; antinuclear, anti-smooth muscle, anti-dsDNA, anti-liver kidney microsomal, anti-soluble liver pancreas, anti-cytosolic, anti-tissue transglutaminase and anti-thyroglobulin antibodies); metabolic tests (sweat chloride test, urine organic acids, tandem mass spectrophotometry, urine reducing substances, serum alpha-1 antitrypsin, ferritin,

ceruloplasmin and urine copper levels). All of these tests yielded results that were within normal limits. Upper endoscopy revealed small esophageal varices with no stigmata of recent bleeding. Echocardiogram was normal. Liver biopsy was obtained at 12 years of age and revealed subtle vascular changes in the absence of significant fibrosis or cirrhosis. The trichrome stain revealed several mildly enlarged portal tracts due to fibrosis and fibromuscular thickening of the portal venules – findings consistent with phlebosclerosis. The outlines of the venules were often irregular with increased muscle fibers in the wall and narrowed lumen (Supplementary Figure 1A and B). Hepatocytes occasionally showed mild swelling (hydropic change), but otherwise were essentially within normal limits (Supplementary Figure 1C). The hepatic cord pattern was preserved and central venules were patent. Bile ducts were also within normal limits.

Patient 2A is a 17 year-old male who was admitted to the hospital at 5 months of age for evaluation of abdominal distention, vomiting and fever. At that time, he was found to have hepatosplenomegaly and elevated transaminases (AST=293 U/L; ALT=105 U/L). Remaining laboratory tests, including liver synthetic function tests, as well as the battery of tests outlined above for Patient 1, were unremarkable. He is the offspring of an uncomplicated pregnancy and vaginal delivery. He has been followed for the last 16 years. At 2.5 years of age he was found to have small esophageal varices, but his platelet count was within normal limits. However, at 6 years of age, he was found to have large esophageal varices and new onset of thrombocytopenia. He was started on a beta-blocker and his platelet count has varied from 81,800–130,00mm^3 for the last 11 years. During this time, he has never been hospitalized, and his other laboratory tests, including liver function tests, have remained normal. Abdominal Doppler ultrasound showed patent abdominal vasculature. Liver biopsy at age 7 years showed a liver parenchyma without significant fibrosis or cirrhosis (Supplementary Figure 1D). The portal changes were similar to those seen in patient 1 with irregular outlines of the portal venules, narrow lumen, and smooth muscle proliferation and fibrosis of the wall (phlebosclerosis) (Supplementary Figure 1E). Areas of mild steatosis (small fat droplets/microsteatosis) and focal mild hepatocyte swelling were seen (Supplementary Figure 1F).

Patient 2B, the younger sister of patient 2A, is a 12 year-old girl who was found to have abdominal distention and hepatosplenomegaly at her 5 year-old routine exam. She was born at term following an uncomplicated pregnancy and vaginal delivery. She has been followed annually and remains clinically stable with no laboratory abnormalities. Abdominal Doppler ultrasound and echocardiogram were both unremarkable. Upper endoscopy showed no esophageal varices. Liver biopsy showed sinusoidal dilatation and periportal fibrosis in the absence of cirrhosis (Supplementary Figure 1G). Venous wall irregularities similar to patient 1 and 2A were seen. In addition, mild chronic lymphocytic infiltration in portal tracts without significant interface activity was also noted (Supplementary Figure 1H and 1I). The liver histological and laboratory features as well as a summary of clinical findings of patients 2A and 2B have been previously reported (6).

Patients 2A and 2B had two sisters who died *in utero* at 4 and 5 months of gestation and one sister who was born prematurely at 28 weeks of gestation and died 39 days after birth (Figure 1). DNA and histologic data is not available for these subjects.

DISCUSSION

Our findings define a previously unrecognized form of idiopathic non-cirrhotic portal hypertension resulting from a homozygous p.N46S mutation in *DGUOK.* In contrast to previously described patients with *DGUOK* deficiency, their clinical findings make clear that the affected subjects have portal hypertension in the absence of advanced fibrosis or cirrhosis on liver biopsy, and have no evident hepatic synthetic dysfunction. Moreover, these patients have no signs or symptoms of coagulopathy and/or cholestasis; similarly, they do not have other signs of mitochondrial disease such as myopathy or neurological impairment, and have been clinically stable for follow-up of 6 to 16 years (Table 3). It has been proposed that INCPH is likely a vascular disease of the liver, and a variety of vascular changes have been described on pathology specimens. These changes include extensive phlebosclerosis of the portal venules, obliterative venopathy of the terminal branches, variable dilatation of the portal venules, herniation of portal venules into the lobules, sinusoidal dilatation and muscularization of portal veins. Hence, the vascular changes seen in our 3 index patients are typical of the changes seen in INCPH. Interestingly, experimental data linking mitochondrial dysfunction and vascular diseases, such as in pulmonary artery hypertension (PAH), has been reported (25); and a loss-of-function variant in the mitochondria-localized deacetylase Sirtuin 3 (*SIRT3*) that suppresses mitochondrial function has also been associated with PAH (26). Moreover, in the literature, there is evidence that mitochondrial suppression leads to the hyperproliferative and antiapoptotic vascular cells seen in PAH. Further investigation will be required to elucidate how mitochondrial dysfunction due to *DGUOK* deficiency might cause phlebosclerosis and other portal venous wall abnormalities and ultimately lead to portal hypertension.

Our findings represent a clear expansion of the phenotypic spectrum resulting from *DGUOK* mutation. Such discoveries have become increasingly common as patients with unexplained diseases are subjected to genome-level sequencing. Traditional studies of Mendelian diseases have focused on the study of families that are homogeneous for phenotype. When underlying disease-causing mutations are found, it is frequently assumed that the full spectrum of clinical manifestations of disease has been described, and mutations in these genes are only sought in patients with phenotypes that match or closely resemble those previously described. Recent genetic studies have demonstrated that the phenotypic spectrum arising from mutations in many disease genes is frequently much broader than initially estimated. These phenotypic expansions - different phenotypic features resulting from the same or different disease-causing mutations in a gene - demonstrate pleiotropy resulting from disease-causing mutations (29). The explanations for this clinical variability may be attributed to genetic or environmental modifiers or stochastic effects. The finding of 3 patients with INCPH who have remained clinically stable over time who share the same homozygous *DGUOK* mutation establishes this phenotype as a distinct outcome from the rapidly progressive liver cirrhosis more commonly associated with recessive DGUOK mutation. Identification of genetic or environmental phenotypic modifiers would be very challenging, particularly with the small number of subjects available for study.

Our study explains the pathogenesis of INCPH in two of the six families studied. Among the remaining four families, we found no genes with statistically significant burden of mutation

under recessive or dominant models of transmission and no other compelling mutations in genes known to cause liver disease, shared by more than one kindred. The etiology of in these kindreds remains unexplained; while these cases may have a Mendelian genetic cause, mutation in diverse genes might account for the disease in different families. Larger cohorts will need to be studied to address this question. Our success in finding one new gene for this trait from the study of this relatively small cohort should motivate such efforts, and underscores the general importance of studying patients with unexplained clinical phenotypes.

Given our finding of portal hypertension due to genetic deficiency of purine nucleotide precursors for DNA replication and repair, it is interesting that many reports link the onset of non-cirrhotic portal hypertension with exposure to pharmacologic inhibition of purine nucleotide biosynthesis, including didanosine, azathioprine and 6-thioguanine. In the case of didanosine (2′,3′-dideoxyinosine) (30–34), a nucleoside analogue used in the treatment of human immunodeficiency virus (HIV) infection, the Food and Drug Administration updated the labeling of this drug in February 2010 to include non-cirrhotic portal hypertension as a rare but potentially life-threatening side effect. In vitro studies have shown that incubation of U2OS cells with 20μM didanosine for 3 days leads to approximately 60% reduction of deoxyguanosine kinase protein levels (35), suggesting that didanosine treatment in vivo causes *DGUOK* insufficiency. Our findings provide strong support to this effect as the mechanism of portal hypertension related to didanosine therapy, with the drug producing a pharmacologic phenocopy of the genetic deficiency we describe herein. Further studies may reveal individual genetic predisposition to non-cirrhotic portal hypertension due to didanosine and/or azathioprine and 6-thioguanine, perhaps via reduced baseline deoxyguanosine kinase enzymatic activity due to heterozygous loss of function mutations in *DGUOK*, or other mutations with similar effects.

Collectively, our study provides new insight into the mechanisms mediating INCPH, and expands the phenotypic spectrum of *DGUOK* deficiency. Furthermore, these results provide a simple genetic test for a specific cause of INCPH in a subset of patients and motivate further studies to enable identification of patients at increased risk for drug-induced non-cirrhotic portal hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

INCPH	idiopathic non-cirrhotic portal hypertension
WES	whole-exome sequencing
NHLBI	National Heart, Lung, and Blood Institute
ExAC	Exome Aggregation Consortium
DGUOK	deoxyguanosine kinase
HIV	human immunodeficiency virus

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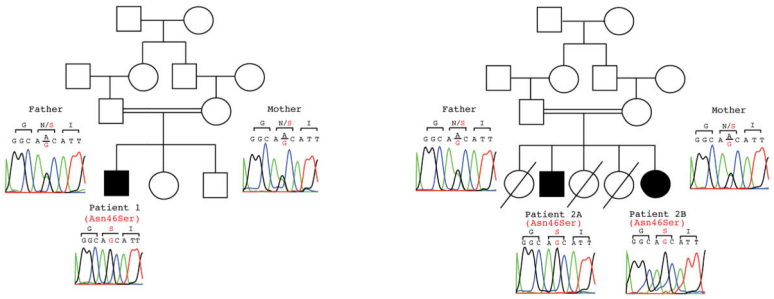

Figure 1.

Kindreds with non-cirrhotic portal hypertension with recurrent homozygous p.N46S *DGUOK* mutation are shown. Studied subjects with non-cirrhotic portal hypertension are shown as black filled symbols. Corresponding Sanger sequencing of the three index cases and their parents are depicted and labeled. Mutations are homozygous in affected subjects and heterozygous in unaffected parents.

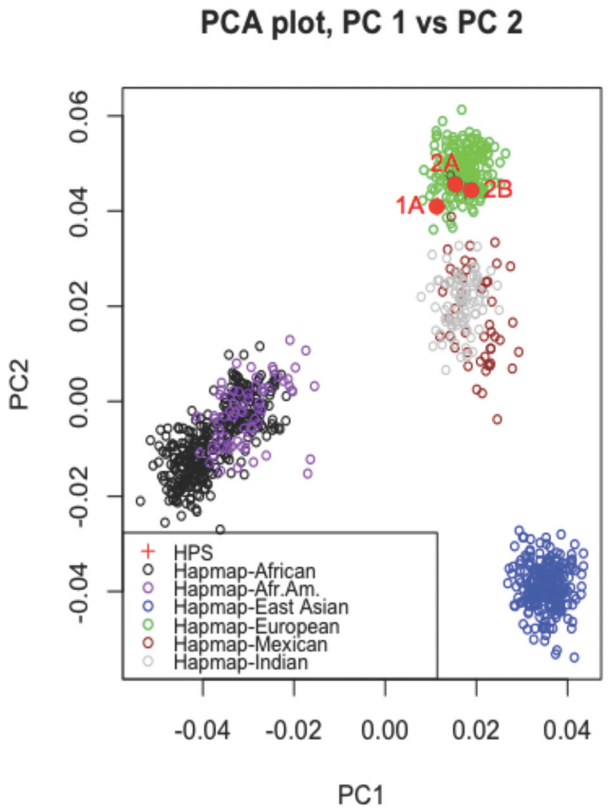

Figure 2.

Principal component analysis of the three index cases with recurrent p.N46S mutation in *DGUOK* (red filled circles) shows they cluster with people of European ancestry from the HapMap project (green circles).

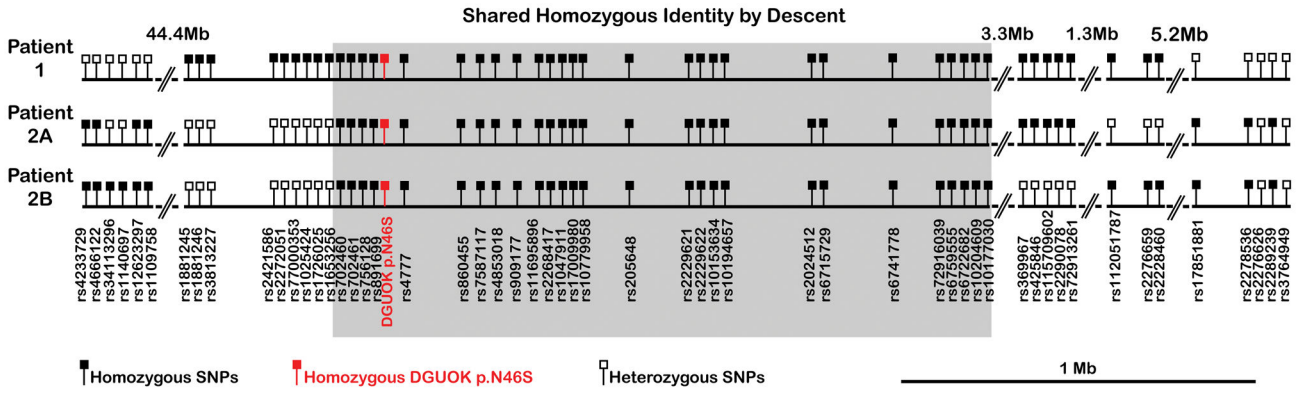

Figure 3.

Shared haplotypes among subjects with homozygous *DGUOK* mutation. Homozygous SNPs (filled symbols) and heterozygous SNPs (unfilled symbols) are shown across the segment of chromosome 2 containing DGUOK in the affected members of kindreds 1 and 2. The segment in which SNP genotypes are identical among all three subjects is shaded in gray. This haplotype identity establishes that the DGUOK mutation is inherited from a remote common ancestor in these families. The minimum identical homozygous segment spans 27 SNPs and 1.8 Mb (shaded in gray). rs numbers for SNPs are indicated (see also Supplementary Table 2).

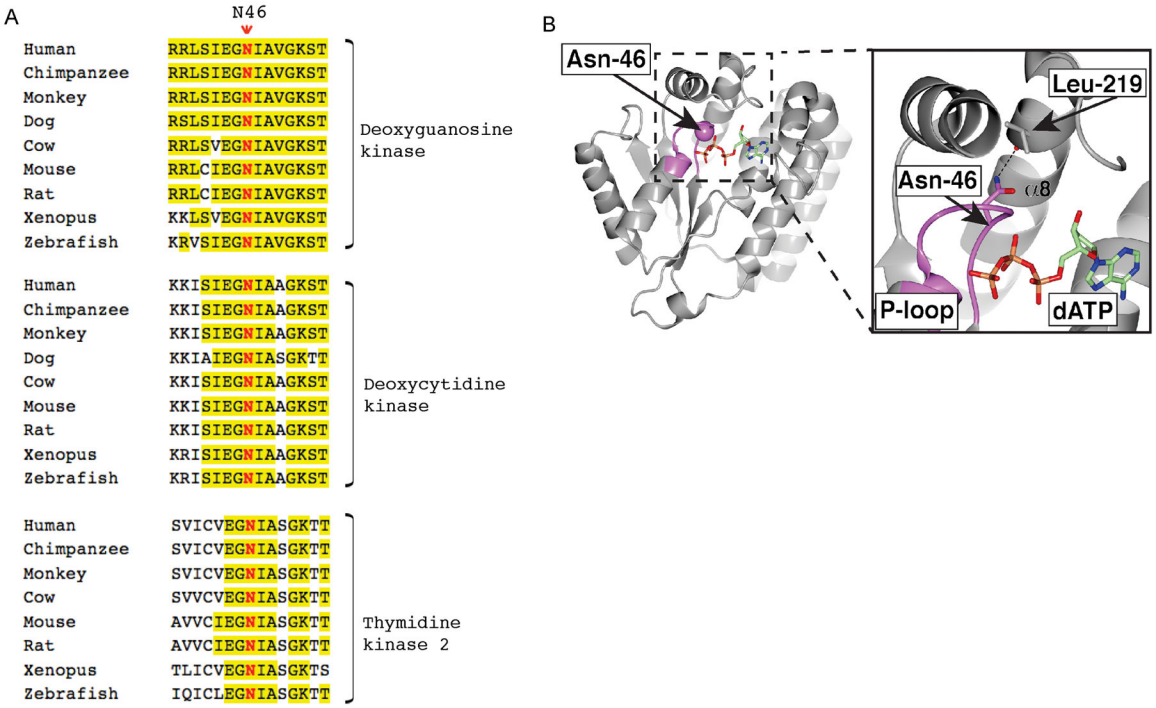

Figure 4.

Conservation and role of p.N46 in *DGUOK.* (A) Conservation of N-46 in *DGUOK* orthologs and related kinases (deoxycitidine kinase and thymidine kinase 2). The amino acid sequences of a segment including the P-loop of deoxyguanosine kinase, deoxycitidine kinase and thymidine kinase 2 are shown, demonstrating that asparagine is completely conserved at the homologous position across all vertebrate species. (B) Ribbon diagram of the crystal structure of deoxyguanosine kinase with bound dATP (PDB accession code 2OCP (22)). The P-loop of the kinase is shown in pink, and the positions of Asn-46 and its hydrogen-bonding partner Leu-219 are indicated by arrows.

Table 1

Sequencing statistics of 8 exomes.

Mean independent reads per targeted base	57.5
% of bases mapping to genome	90.9
% of targeted bases with ≥ 8 independent reads	95.5
Mean error rate (%)	0.47

Table 2

Frequency of the p.N46S variant in *DGUOK* in NHLBI, 1000G, ExAC, Yale and Turkish Exomes databases.

Patient ID	Chr:position (hg19)	Gene (Accession number)	AA change, Protein Length, cDNA change	NHLBI (4,300 European subjects)	1000G (1,097 subjects)	Yale DB (2,500 European subjects)	Turkish DB (894 subjects)	ExAC (31,911 European subjects)
1	2:74154174	*DGUOK* (NM_080916)	N46S, 46/277, c.137A>G	0	0	0	0	$3.134e^{-05}$
2A								
2B								

Chr, chromosome; AA, amino acid; NHLBI, National Heart, Lung, and Blood Institute Exome Sequencing Project database; 1000G, 1000 Genome database; DB, database; ExAC, Exome Aggregation Consortium database.

Table 3

Comparison of the clinical features among three subjects with NCPH in the absence of liver synthetic dysfunction and who harbor a recurrent recessive p.N46S mutation in *DGUOK*, and patients suffering from mtDNA depletion syndrome 3 (OMIM#251880) due to *DGUOK* deficiency.

	Patient 1	Patient 2A	Patient 2B	mtDNA depletion syndrome 3 (hepatocerebral type)*
Age at Presentation	12 yo	5 m	5 yo	infancy and early childhood
AST / ALT	WNL	WNL to 8x ULN	WNL	WNL to 20x ULN
Conjugated hyperbilirrubinemia	–	–	–	+
Coagulopathy	–	–	–	+
Cirrhosis / Liver failure	–	–	–	+
Portal hypertension findings in the absence of cirrhosis or liver failure	+[1]	+[2]	+[3]	–
Neurological impairment	–	–	–	+
Follow-up Time /Prognosis	6 y / stable with no liver or neurological dysfunction	16 y / stable with no liver or neurological dysfunction	6 y / stable with no liver or neurological dysfunction	N/A / poor prognosis due to progressive liver +/− neurological dysfunction

yo, years old; m, months; y, years; N/A, not applicable; WNL, within normal limit; ULN, upper limit of normal; +, present; -, absent. Phenotypic differences between the patients in our study with N46S recessive *DGUOK* mutation and the subjects with mtDNA depletion syndrome 3 (hepatocerebral type) associated with *DGUOK* deficiency are depicted by gray shading.

*Phenotype OMIM number 251880.

[1]Splenomegaly and small non-bleeding esophageal varices.

[2]Splenomegaly and large non-bleeding esophageal varices.

[3]Splenomegaly.