Clinical, biochemical and genetic analysis of a Chinese Han pedigree with holocarboxylase synthetase deficiency: a case report

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Case report

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Abstract

Background: Holocarboxylase synthetase (HLCS) deficiency is a rare inborn disorder of biotin metabolism, which results in the defect of several biotin-dependent carboxylases and presents with metabolic ketoacidosis and skin lesions.

Case presentation : In this study, we have reported a Chinese Han pedigree with HLCS deficiency diagnosed using next-generation sequencing and validated with Sanger sequencing of HLCS and BTD gene. The Chinese proband carries a common missense mutation c.1522C>T (p.Arg508Trp) in exon 9 of HLCS gene, which generates an increased K m for biotin. A novel frameshift mutation c.1006_1007delGA (p.Glu336Thrfs*15) in exon 6 of H L CS gene produces a low Vmax and is predicted to be deleterious through PROVEAN and MutationTaster. A novel heterozygous mutation c.638_642delAACAC (p.His213Profs*4) in BTD gene is also identified. Otherwise, the proband presents abnormal BAEP suggesting hearing damage in the acute episode.

Conclusions: A Chinese proband carries a reported Arg508Trp variant, a novel 2-bp frameshift mutation c.1006_1007delGA (p.Glu336Thr) expanding mutational spectrum of HLCS gene, and a novel heterozygous mutation c.638_642delAACAC (p.His213Profs*4) expanding mutational spectrum of BTD gene. Furthermore, the reversible hearing damage is rarely reported in the patients with HLCS deficiency, which deserves for further discussion.

Background

Multiple carboxylase deficiency (MCD), a rare autosomal recessive disorder, is divided into two types depending on the pattern of enzyme deficiency: holocarboxylase synthetase deficiency (HLCS deficiency, OMIM #253270) and biotinidase deficiency (BTD, OMIM #253260). HLCS (EC 6.3.4.10) is an enzyme that catalyzes biotinylation of four biotin-dependent carboxylases including pyruvate carboxylase (EC 6.4.1.1), acetyl-CoA carboxylase (EC 6.4.1.2), propionyl-CoA carboxylase (EC 6.4.1.3), and methylcrotonyl-CoA carboxylase (EC 6.4.1.4) (1,2). HLCS gene is located on chromosome 21q22.1 in which pathogenic variants produce severe metabolic decompensation (3). Clinically, patients with HLCS deficiency usually present with poor feeding, respiratory distress, lethargy, vomiting, hypotonia, seizures, developmental delay, alopecia and skin rash (4–7).

Different phenotypes may correlate with mutation spectrum in HLCS gene and molecular genetic analysis is helpful for definitive diagnosis (8). Recently, more and more novel pathogenic variants have been reported. For example, Donti et al. have revealed 5 cases of HLCS deficiency with broad differences in initial presentation and phenotype, and also have introduced 6 novel pathogenic variants including c.500A > C (p.Tyr167Ser), c.1532A > T (p.Asn511Ile), c.2078G > C (p.Gly693Ala), c.977G > A (p.Gly326Glu), c.1710C > G (p.Asn570Lys) and c.1519 + 5G > A (9). Otherwise, Quinonez et al. have reported of a novel heterozygous variant c.996G > C (p.Gln332His), and a paracentric inversion on chromosome 21 utilizing cytogenetic analysis (10). In this report, we have investigated a Chinese Han pedigree with HLCS deficiency

and elaborated the relationship of molecular mutation and clinical manifestation. Additionally, this HLCS deficiency boy has presented an unusual clinical symptom of hearing damage during acute attack.

Case Presentation

Subjects

The proband was a one-year old male patient. His nonconsanguineous parents and elder sister were also included in this study. The patient underwent thorough physical examinations and other tests, including blood gas analysis, blood ammonia, plasma acylcarnitine profile, urinary organic acids, brainstem auditory evoked potential (BAEP) study and genetic testing. His families only underwent physical examination and genetic testing. Filter-paper dried blood-spot sample was pretreated with NeoBase Non-derivatized MS/MS Kit (Perkin Elmer Life and Analytical Sciences, Turku, Finland), and the acylcarnitine profile was analyzed with liquid chromatography-tandem mass spectrometry (Acquity TQD, Waters, Milford, MA, USA). The urinary organic acid was analyzed with gas chromatography-tandem mass spectrometry (7890B/5977A, Agilent Technologies, Santa Clara, CA, USA). Information consent for data collection and publication were obtained from the parents. The present study was approved by the Ethical Committee, Quanzhou Children's Hospital of Fujian. The study was prepared in accordance with the Health Insurance Portability and Accountability Act (HIPAA) regulations.

Clinical presentation

The proband, a Chinese Han boy, was first brought to dermatology department for skin rash around the periorbital and perioral areas and treated as eczema at one year old. Three weeks later, he was referred to PICU immediately for serious tachypnea, moaning and heart failure. And the rash was expanded to limbs, neck and groin. A capillary blood gas analysis showed metabolic acidosis with pH 6.98, base excess −26 mmol/L, bicarbonate level 3.9 mmol/L, anion gap 25.2 mmol/L, elevated lactate 13.1 mmol/L (normal < 2 mmol/L), and elevated ammonia 152.0 μmol/L (normal < 47 μmol/L). Biochemical labs on the day of admission showed low ornithine (15.99 μmol/L, normal 42–325 μmol/L), and plasma acylcarnitine profile with low free carnitine and multiple increases of C5-OH, C5-OH/C0,C5-OH/C8C3/C2 and C3/C0. Urinary organic acids profile displayed multiple excrements of 3-hydroxyisovaleric acid, acetylglycine, propionylglycine, 3-methylcrotonylglycine, methylcrotonylglycine, 3-hydroxybutyric acid, pyruvic acid and lactic acid. Increases of 2-keto-3-methyl pentanoic acid and 2-keto-isocaproic acid suggested a metabolic disorder of branched-chain amino acid. Based on the characteristic plasma acylcarnitine profile and urinary excretion pattern, he was presumptively diagnosed as MCD and treated with biotin 20 mg bid immediately. The skin rash was eliminated and the normal acid-base balance was restored 5 days later. Afterwards, the level of C5-OH decreased gradually and urinary organic acid profile showed undetectable acetylglycine, propionylglycine, 3-methylcrotonylglycine and methylcrotonylglycine (See Table 1).

Table 1
Metabolites in plasma and urine.

	13 months measurement (μmol/L)	15 months measurement (μmol/L)	17 months measurement (μmol/L)	24 months measurement (μmol/L)	Ref. range (μmol/L)
Acylcarnitine in plasma					
C5OH	3.88	1.66	0.53	0.37	0.07–0.5
C0	4.86	30.25	26.87	26.43	9.5–50
C2	7.99	9.87	11.88	10.27	3.4–45
C3	4.35	0.62	1.13	1.3	0.2–4.5
C5OH/C0	0.80	0.055	0.02	0.01	0-0.02
C5OH/C8	77.6	33.2	17.67	7.4	1.22-18
C3/C2	0.54	0.063	0.095	0.13	0.01–0.2
C3/C0	0.90	0.02	0.042	0.05	0.01–0.2
Organic acids in urine					
3-methylcrotonylglycine	24.70	0.00	-	0.00	0
3-hydroxyisovaleric acid	3.63	3.00	-	1.46	0-2.3
methylcrotonylglycine	17.51	0.00	-	0.00	0
acetylglycine	6.13	0.00	-	0.00	0-0.1
propionylglycine	36.71	0.00	-	0.00	0
lactic acid	247.95	4.23	-	4.81	0-4.7
pyruvic acid	58.14	6.45	-	3.42	0-24.1
3-hydroxybutyric acid	966.37	2.52	-	0.00	0-3.7
2-keto-3-methyl pentanoic acid	6.16	0.00	-	0.00	0
2-keto-isocaproic acid	6.16	0.00	-	0.00	0

During the acute episode, BAEP study showed that wave latency and - interpeak latency intervals were all significantly prolonged. Additionally, bilateral thresholds were also increased: the left to 50 dbnhl, the

right to 60 dbnhl. The above results declared that his bilateral hearing was impaired. And the hearing impairment was predicted to be sensorineural. Then cattle encephalon glycoside and ignotin injection was immediately supplied. After biotin therapy for 43 days, the repeated BAEP results showed that the bilateral thresholds were both decreased to 30 dbnhl, which indicated that the hearing damage was reversible. (See Fig. S1)

DNA sequencing analysis

Peripheral whole blood or dried blood spots were collected from the proband and his family members. Genomic DNA was extracted using Qiagen Blood DNA mini kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions and stored at -80℃ until further use. All exons and adjacent noncoding regions of abnormal C5OH related genes were amplified by polymerase chain reaction (PCR) and subsequently screened via next-generation sequencing (NGS) with NextSeq 500/550 Buffer Cartridge v2 Sequencing Kit on high-throughput sequencing instrument (Illumina Nextseq 500). The sequence analyses were performed by using BWA, GATK, Annovar and etc.

The identified variants HLCS c.1522C > T, c.1006_1007delGA and BTD c.638_642delAACAC were validated by Sanger sequencing of samples from all of the family members. HLCS exon 9 and exon 6 sequences were amplified by PCR using the following primers, respectively: forward 1, 5'-CTCACAGAAGCAGAACATTAT-3' and reverse 1, 5'-GAAAACTCCGAGAGCACT-3'; forward 2, 5'-TGTAAAACGACGGCCAGTTAGTGCT ATCTTTCCCCTTC-3' and reverse 2, 5'-CAGGAAACAGCTATGACCGATGATTTCCAAA CCCG-3'. BTD exon 4 was amplified by the following primers: forward 3, 5'-TGTAAAACGACGGCCAGTTTTAGTTGAGATGGGGTTT-3' and reverse 3, 5'-CAGGAAACAGCTATGACCCTCCAGAGGGGTGTGTAT-3'. Sanger sequencing was performed utilizing an ABI Prism 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and the results were analyzed with DNASTAR software (http://www.dnastar.com/).

Genetic sequencing results reveal that the patient carries a maternal missense mutation c.1522C > T (p.Arg508Trp) in exon 9 of HLCS gene, a novel paternal 2-bp deletion c. 1006_1007delGA (p.Glu336Thrfs*15) in exon 6 of HLCS gene, and even a novel paternal 5-bp deletion c.638_642delAACAC (p.His213Profs*4) in exon 4 of BTD gene (Fig. 1E). Furthermore, his parents' genotypes have been confirmed at a heterozygous level by Sanger sequencing (Fig. 1, B and C). And his healthy elder sister carries the same mutations with his father (Fig. 1D). The pedigree has been shown in Fig. 1A. The p.Arg508Trp mutation has been widely reported to be pathogenic (11,12). The p.Glu336Thrfs*15 and p.His213Profs*4 mutations could not be found in the literature, 1000 Genome, ESP6500, ExAC or dbSNP databases, and not be detected in 100 healthy individuals. The effect of these mutations on protein function is predicated to be deleterious by PROVEAN and MutationTaster (See Table 2). Additionally, the p.Glu336Thrfs*15 resides in a conserved stretch of amino acids (Fig. 2A) and leads to truncated proteins lacking the HLCS conserved domains (Fig. 2B). Taken together, the c. 1006_1007delGA (p.Glu336Thrfs*15) mutation is considered to be deleterious and likely pathogenic.

Discussion And Conclusions

HLCS protein possesses three structural domains: two in C-terminal region and one in N-terminal half (13,14). As reported, most of HLCS mutations are located on the C-terminal catalytic region and produce an increased K_m for biotin. Additionally, the mutations in the N-terminal region are located outside the catalytic domain and reduce V_{max} of HLCS enzyme (11).

As mentioned above, this Chinese boy carries a heterozygous c.1522C > T (p.Arg508Trp) and a novel frameshift mutation c.1006_1007delGA (p.Glu336Thrfs*15). Arg508Trp mutation is located on the C-terminal region and generates an elevated K_m value. Patients bearing the K_m mutants present late-onset form and respond well to the biotin therapy (15,16). Clinically, he becomes symptomatic at the age of 11 months, which is late-onset form. Moreover, a novel frameshift mutation c.1006_1007delGA has been identified. It is predicted to induce an amino acid substitution of glutamate with threonine at position 336 (p.Glu336Thr). And from the position 336 onwards, a termination condon is formed at the fifteenth amino acid, which is predicted to be deleterious through PROVEAN and MutationTaster. The novel Glu336Thr mutation is located outside the biotin-binding region and produces a low Vmax. It's reported that patients carrying Vmax mutation present partial biotin responsiveness (17). Along with the biotin-responsive allele (Arg508Trp), our patient is considered to be well responsive to biotin administration (18) and his clinical presentation supports the deduction.

The BAEP study shows that the patient's bilateral hearing has been slightly impaired. As we know, a patient with HLCS deficiency presenting abnormal BAEP is rarely reported. And the reason is worthy of discussion. Slavin et al. have reported a girl with HLCS deficiency presenting conductive hearing loss due to cerumen impaction (7), which is different from the situation of our patient. Otherwise, our patient's hearing impairment has recovered after 43 days of biotin therapy, which is different with the irreversible hearing loss of biotinidase deficiency. Hence, we speculate that it may be secondary to the primary disease of HLCS deficiency. However, it is just a hypothesis because there is no literature or experimental data. Anyway, there are nearly no reports that patients with HLCS deficiency present hearing impairment, so we believe that the case is noteworthy and the reason needs to be further confirmed.

In conclusion, we report a Chinese Han boy who shows consistent clinical features and biochemical parameters with genetic data and presents definitely HLCS deficiency. He carries a common Arg508Trp variant, which is corresponding with biotin-responsive and late-onset presentation. A novel 2-bp frameshift mutation c.1006_1007delGA (p.Glu336Thr) would expand mutational spectrum of HLCS gene. Moreover, a rare association of bilateral hearing damage with HLCS deficiency patient happens. However, this needs to be further discussion.

Abbreviations

MCD
Multiple carboxylase deficiency; HLCS:Holocarboxylase synthetase; BTD:Biotinidase deficiency; BAEP:Brainstem auditory evoked potential; HIPAA:Health Insurance Portability and Accountability Act; PCR:Polymerase chain reaction; NGS:Next-generation sequencing;

Declarations

Ethics approval and consent to participate

This study is approved by the Ethical Committee, Quanzhou Children's Hospital of Fujian. The patient's parents have provided informed consent for data collection and publication.

Consent for publication

Written consent for publication was obtained from the patient's parents.

Availability of data and materials

The data for present study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

ZZ, GY and MZ cared for patients and collected the clinical data. YL and FZ collected the biochemical parameters. MJ and LZ performed the mutation analysis. ZZ and LZ participated in study design, data analysis, and the first draft of the manuscript. And QF guided the whole research process. All authors read and approved the final manuscript.

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Tables

Due to technical limitations, tables are only available as a download in the supplemental files section.

Figures

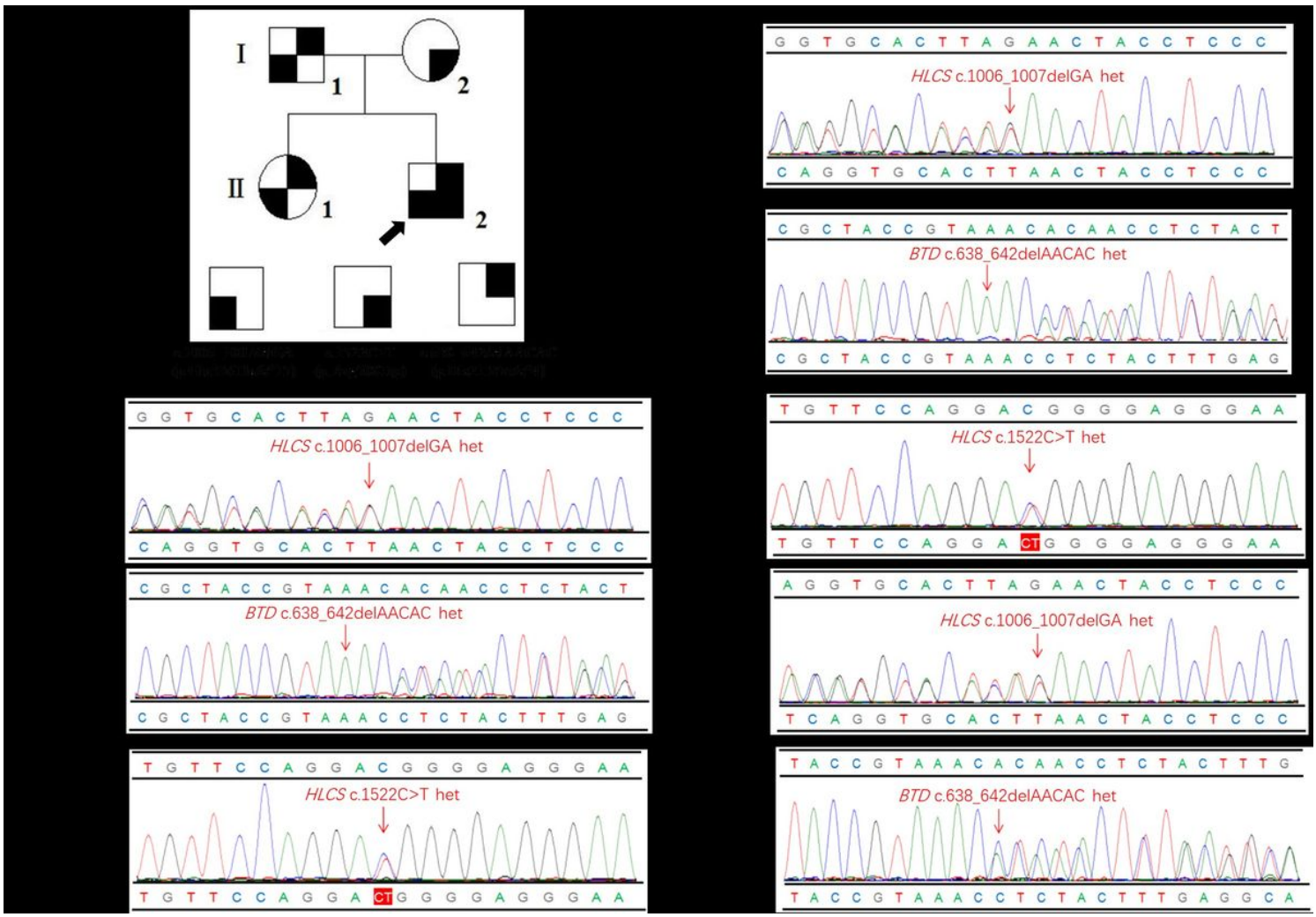

Figure 1

A. Pedigree of the family. The black arrow denotes the proband. B-E. Validation of the HLCS and BTD gene mutations by Sanger sequencing. Heterozygous mutations c.1522C>T, c.1006_1007delGA and c.638_642delAACAC were identified in the proband (Ⅱ:2), separately inherited from his father (Ⅰ:1) and

mother (Ⅱ:2), his older sister (Ⅱ:1) has the same mutations with the father (the variant is indicated by a red arrow).

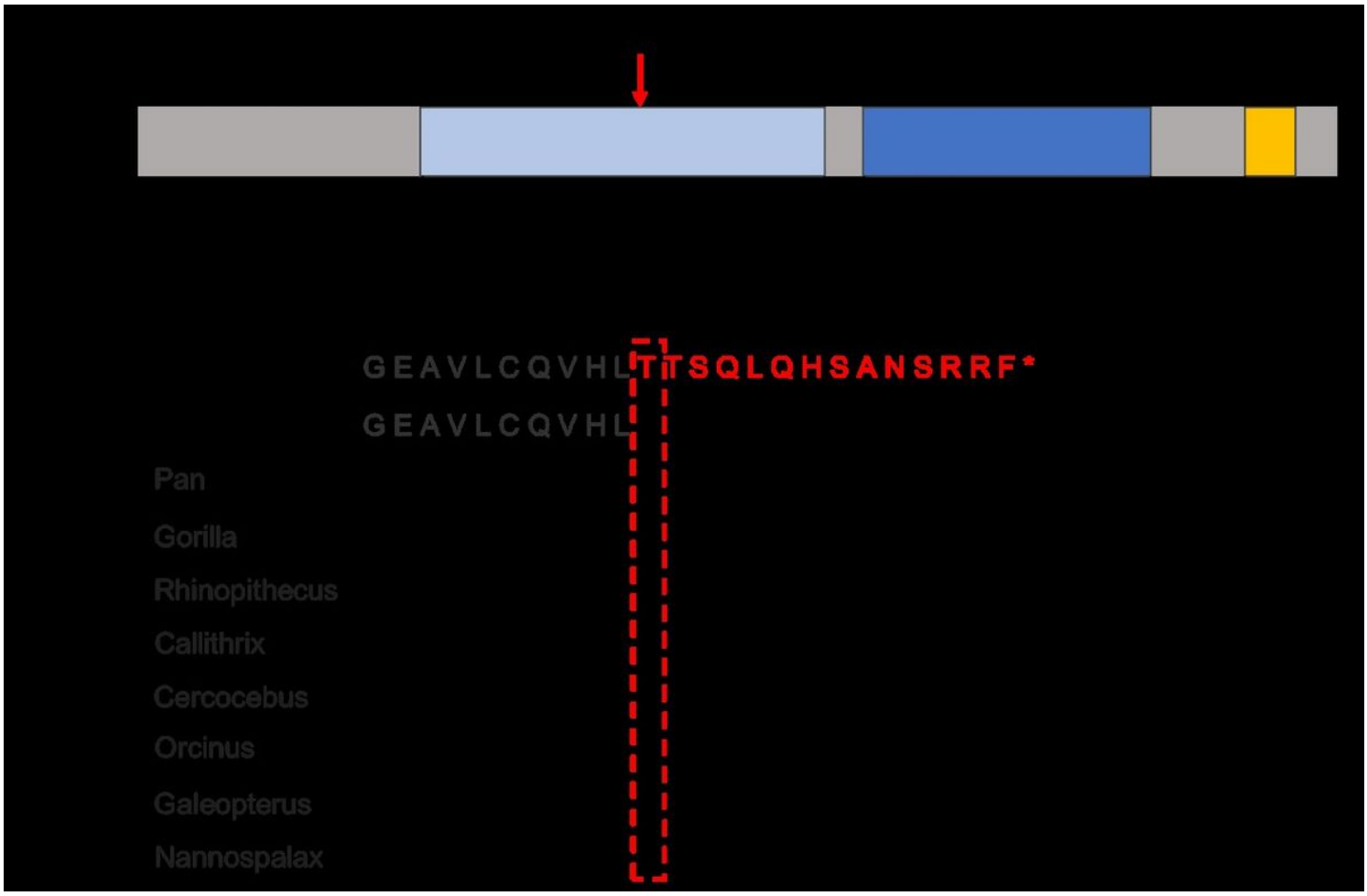

Figure 2

A. Domains structure of HLCS protein. The novel frameshift mutation identified in the proband is indicated by a red arrow. B. Conserved amino acid sequences of HLCS (amino acid 336, highlighted by a red box) and the predicted truncated HLCS caused by the frameshift mutation (c.1006_1007delGA) identified in this proband.

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