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A novel mutation c.2090_2091del in NECRC of a 18.5-month-old boy: a case report and short literature review

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ABSTRACT

BACKGROUND

Neurodevelopmental-Craniofacial Syndrome with Variable Renal and Cardiac Abnormalities (NECRC) is a rare, autosomal, dominant neurological disorder caused by mutations in Zinc Finger MYM-Type Containing 2 (ZMYM2) gene. To date, the clinical and functional characteristics of the novel ZMYM2 mutation c.2090_2091del have not yet been reported.

CASE SUMMARY

A 18.5-month-old Chinese boy with motor and language delay, microcephaly, facial dysmorphism, moderate malnutrition, single palmar crease on left, synpolydactyly of the right foot, hypotonia and feeding problems. The boy diagnosed with NECRC was enrolled from the First Affiliated Hospital, Henan University of Chinese Medicine, the clinical data was collected. In Whole-exon sequencing (WES) data, the pathogenic SNVs/InDels were identified, and the molecular finding was characterized. WES revealed that the heterozygous variant in ZMYM2 gene of the child (c.2090_2091del, p.Ser697TrpfsTer3), a frameshift mutation, which is a NECRC-related gene mutation.

CONCLUSIONS

We performed a systematic literature review to identify, appraise and synthesize NECRC, substantial evidence from the literature that the patients with ZMYM2 gene mutation showed different degrees of intellectually disability, motor and language retardation, facial dysmorphism, and a few suffered from congenital heart defects, kidney and urinary tract abnormalities. Subsequently, early diagnosis and prompt management with comprehensive rehabilitation training are beneficial, but may not improve long-term outcomes.

Keywords: ZMYM2; NECRC; frameshift mutation; global developmental delay; case report; literature review

Core Tip: We describe a patient with NECRC caused by ZMYM2 mutation, bioinformatics analysis suggested that the presence of a novel complex heterozygous variant in ZMYM2 gene.

INTRODUCTION

Neurodevelopmental-Craniofacial Syndrome with Variable Renal and Cardiac Abnormalities

is an autosomal dominant disorder characterized by craniofacial dysmorphology associated with mild developmental delay, mildly impaired intellectual development or learning difficulties, speech delay, and behavioral abnormalities, approximately half of patients have congenital abnomalies of the kidney and urinary tract (CAKUT) and/or congenital cardiac defects, including septal defects. We identified a patient with a novel de novo frameshift variant exhibiting the combinational phenotype of developmental delay and facial dysmorphism, medication combined with the comprehensive rehabilitation training was not effective. Meanwhile, we carry out a literature review, including the medical history, clinical symptoms and genetic features of NECRC patients with ZMYM2 mutations, which further specify the phenotype and genotype of such patients. This case report was approved the publication by the Ethics Committees of the First Affiliated Hospital, Henan University of Chinese Medicine, and written informed consent was obtained from the patient's parents and his family.

CASE PRESENTATION

Chief complaints

The patient, a 18.5-month-old boy, was admitted to the First Affiliated Hospital, Henan University of Chinese Medicine on August 31, 2022, due to developmental delay being experienced for more than 1 year.

History of present illness

He is developmental delay, communication disability, attention deficit hyperactivity disorder, sluggish response, clumsy in movement and behavioral concerns, he exhibited decreased appetite, sleep deterioration, and ingestion of a liquid diet. Poor effect on the patient during treatment with lysine hydrochloride and zinc gluconate granules 35mg twice daily (ineffective) on June 18, 2022. Oxiracetam capsules 400mg twice daily (a little effective) was added on July 21, 2022.

History of past illness

At the age of 6 months, the case presented his initial symptom of motor retardation, he had a history of poor motor and language development milestones: he could not sit steadily until 12 months, could walk with help at 17 months, could not walks smoothly until 18.5 months, and only say single tone. The patient has been diagnosed as "global developmental delay" at his 6.9 months old.

Personal and family history

The patient had abnormal birth history: his mother, a 29-year-old woman, was hospitalized for 2 weeks because of small fetal heart at 35 weeks of gestation, the boy was delivered by cesarean section at 37 weeks of pregnancy because of oligohydramnios, he was born with birth weight of 3000 g (-1SD), birth length of 51.0 cm (0.80SD) and no history of asphyxia or hypoxia. The boy is the second child of healthy non-consanguineous parents, his elder sister can only say "baba" and "mama" at the age of 2-year-old, whose language is slightly delay, until now, his elder sister is 4-year-7-month-old, her intelligence, height, weight and facial features are normal. There is no family history of intellectually disability, motor or language retardation.

Physical examination

So far, he is 18.5 months old, his height is 85.5 cm (0.50SD), weight is 9.2 kg(-2SD), and head circumference is 45.5cm (-0.5SD). He was observed with less-full forehead, protruding ears, wide interpupillary distance, broad nasal bridge, thin lips, single palmar crease on left, synpolydactyly of the right foot (figure 1). There were no obvious abnormalities in heart and lung auscultation and abdominal diagnosis, no pigmentation on the skin, no cafe-au-lait-spots, withered and yellow hair, normal hair distribution, no change in physiological curvature of the spine, hypotonia, and normal patellar tendon reflex.

Laboratory examinations

The child has been conducted the Gesell Developmental Schedule (GDS) for four times at different ages (Table 1). At his 18.5 months, the AIMS scores of Albertalnfant Motor Scale (AIMS): prone position: 21, supine position: 9, seat position: 12, standing position: 9, total score: 51, AIMS percentile:＜5, equivalent age: 11.5 months, indicating that motor development is obviously delay. Electromyographic evoked potential: visual evoked potentials(VEP): the latency of bilateral P100 is normal, and brainstem auditory evoked potentials(BAEP): the peak latencies of bilateral Ⅰ, Ⅲ and Ⅴ waves and the interpeak latencies of Ⅰ-Ⅲ waves, Ⅲ-Ⅴ waves are normal.

Further examinations, including 25-hydroxyvitamin D: 54.4ng/ml(normal range:≥20 ng/ml) at the age of 6.9 months. Thyroid function: T3: 2.590 nmol/L(normal range: 1.32-4.07 nmol/L), T4: 146.200 nmol/L(normal range: 73-206 nmol/L), TSH: 5.530 mIU/L(normal range: 0.73-8.35 nmol/L), belong to the normal range at the age of 8.7 months. At his 14.5-month-old, neuromuscular function showed that the activity of surface electromyographic signal(sEMG)

about gastrocnemius and adductor muscle was normal; test of sensory functions in infants showed that there was sensory processing disorder in young children; chromosome karyotype analysis showed no obvious abnormalities; and blood metabolic screening (blood amino acids and acyl carnitine) was normal, urinary metabolic screening(urine organic acid analysis) showed that 2-hydroxyisobutyric acid-2: 1.5(normal range: 0.0-0.5), oxalic acid-2: 9.3(normal range: 0.0-1.0), phosphate-3: 132.3(normal range: 0.0-72.7), palmitic acid-1: 60.8(normal range: 0.0-23.3); otoacoustic emissions(OAE) showed that both left and right ears passed; acoustic impedance testing: left ear: As, right ear: As; serum vitamin A: 0.34 mg/L(normal range: 0.30-0.70 mg/L), serum vitamin E:11.9 mg/L(normal range: 5.0-20.0 mg/L).

Imaging examinations

At the age of his 14-month-old, cardiac color ultrasound showed that there was no obvious abnormality in intracardiac structure, normal range of left ventricular systolic function. At his 18.5 months old, color ultrasound of urinary system showed that there were no obvious abnormalities in kidneys, ureter and bladder. At his 12.1-month-old, DR of the right foot showed that synpolydactyly of the right foot. At his 14.5-month-old, magnetic resonance imaging (MRI) for brain showed that the bilateral frontotemporal subarachnoid space was slightly wider.

FURTHER DIAGNOSTIC WORK-UP

A retrospective case study was performed, the boy diagnosed with NECRC was recruited at August 2022. He underwent a neurological examination, GDS, AIMS, VEP, BAEP, MRI for brain, sEMG, OAE, blood and urinary metabolic screening. His intellectual disability was estimated according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-V). The clinical data of family members was obtained, investigated and independently reviewed by two neurologists.

Genomic DNA extraction: Genomic DNA sample was extracted from the EDTA-treated peripheral blood were collected with informed consent of the patients using QIAamp R Blood Mini Kit (Qiagen, Hilden, Germany).

Process of WES: The extracted DNA sample was subjected to detect by 0.8% agarose gel electrophoresis analysis, and confirming that Genomic DNA sample is free from serious degradation and impurity pollution was evaluated by NanoDrop2000 and Qubit3.0 to testing the concentration and purity of DNA, DNA was sheared with M220 Focused-ultrasonicator (Covaris,

Woburn, MA, USA), the genomic DNA was fragmented to the length range of 150-300bp, with the main peak at 250bp. The purified product was fragment screened by AMPureXP magnetic beads, with the length of the screened fragment is 400bp. DNA target region was captured by hybridizing the genomic DNA sample library with the XGen® Exome Research Panel kit (IDT, USA), which can specifically enrich the exon region of the genome for further processing. Purify the amplified product, quantify the library with Qubit and the inspection with Bioanalyzer2100 to detect the fragment distribution of the library for sequencing. The captured and amplified DNA sample was sequenced using Illumina NovaSeq6000 (Illumina, San Diego, CA, USA) with 150 base-paired end reads.

Detection of SNVs/Indels: Sequencing data was analyzed to identify disease-associated SNVs/Indels according to an in-house pipeline. Both public software and commercial packages were implemented during bioinformatics analysis. Raw data was processed with FASTP for adapters removing and low-quality reads filtering. The paired-end reads were then performed against the Ensemble GRCh37/hg19 reference genome with Burrows-Wheeler Aligner(BWA). Base quality score recalibration together with SNVs/Indels were conducted by HaplotypeCaller tool of GATK after the necessary post-processes on primary alignment. SNVs/Indels were screened according to the sequence depth and variant quality that high quality and reliable variants were obtained. Notably, the online system independently was used to annotate database-based minor allele frequencies (MAFs), and American College of Medical Genetics and Genomics(ACMG) practice guideline-based pathogenicity of every yielded gene variant for conservative analysis and protein product structure prediction. Each variant was compared against several public databases, dbSNP, gnomAD, 1000 genomes project, Exome Aggregation Consortium (ExAC), Chigene in-house MAFs database and NHLBI Exome Sequencing Project 6500 (ESP6500) to achieve allele frequency in the general population. Mutationtaster, Provean, Sift, M-Cap, Polypen2_hdiv, Polypen2_hvar, and Revel software packages were used to predict protein product structure variation.

Genetic analysis: WES was performed to identify disease-causing variants, a novel heterozygous variant (NM_197968.3: c.2090_2091del, p.Ser697TrpfsTer3) of ZMYM2 gene that occurs in exon 11, as evidenced by the deletion of nucleotides from 2090 to 2091 of the ZMYM2 gene(c.2090_2091del), resulting in the change of serine at position 697 into tryptophan and

downstream amino acid at position 3 becomes the stop codon (p.Ser697TrpfsTer3). Through Pedigree of the family, it was found that none of the parents carried the variant, was wild type and had no obvious clinical phenotype (figure 2). The clinical phenotype associated with ZMYM2 gene mutation is NECRC, which has not been reported in the control population (gnomAD and ClinVar).

This frameshift variant was detected according to ACMG from the following evidence: (i) there is frameshift variant in the ZMYM2 gene where loss of function is a known mechanism of disease, PVS1; (ii) all sequence variants in the proband and parental samples were confirmed by Sanger sequencing analysis that the variant was de novo, and the phenotype was in accordance with the ZMYM2 gene, PS2_Moderate; (iii) the variant is absent from the control population in Exome Sequencing Project, gnomAD, 1000 Genomes Project or Exome Aggregation Consortium, PM2_Supporting. With the evidence of PVS1 + PS2 + PM2, the class of this variant was curated as pathogenetic.

FINAL DIAGNOSIS

The patient was diagnosed with NECRC accompanied by the ZMYM2 gene (c.2090_2091del, p.Ser697TrpfsTer3) mutation.

TREATMENT

The early comprehensive rehabilitation training was conducted on the basis of routine treatment might not improve the long-term outcome of NECRC patients.

OUTCOME AND FOLLOW-UP

Half a year later, detailed history-taking and follow-up examinations showed that the patient currently lagging behind in motor and language developmental.

DISCUSSION

The data in our study indicated that disruptions of ZMYM2 as the cause of NECRC, and the mutation site of c.2090_2091del (p.Ser697TrpfsTer3) in ZMYM2 gene is a novel frameshift mutation, which is reported for the first time in a NECRC case, the ZMYM2 (c.2090_2091del) mutation in pediatric patient expand the genotype and phenotypic spectrum of NECRC.

ZMYM2, also known as ZNF198, FIM or RAMP, has been characterized member of the family of MYM-type zinc fnger proteins. ZMYM2 is a cellular transcription factor with a zinc

finger structure coding gene which localizes to the nucleus (specifically the PML body), is a novel B-MYB binding protein that contains 1377 amino acids with a molecular mass of 150 kDa. ZMYM2 is encoded by the zinc-fnger protein that harbors 2 putative nuclear localization signals (NLS) and 10 MYM type zinc fingers, and the zinc finger domain acts as a transcription factor that it mainly binds to the targets DNA and RNA, the zinc finger structure also mediate protein-protein interactions to regulate the efficiency of binding nucleic acids[1]. N-terminal of ZMYM2 includes 3 action sites related to Small Ubiquitin Like Modifier, including a MYM zinc finger domain and a proline/valine-rich domain, is related to the formation and stabilization of PML nuclear bodies (PML-NBs), C-terminal acidic domain contains a putative NLS and a region similar to Cre-likedomain[1-4], and mutant mRNA transcripts are predicted to undergo nonsense-mediated decay, which prevents its translation, so as to ZMYM2 haploinsufficiency and loss of function (LOF)[5].

ZMYM2 has a specialized role in pronephric development in a subset of regions, LoF variants in ZMYM2 induces CAKUT-like defects. Quantification of sides with morpholino oligonucleotides knockdown of ZMYM2 demonstrated that a loss of posterior atp1a1 signal in 30% of embryos, human disease features are replicated in X. tropicalis larvae with morpholino knockdowns, in which expression of truncated ZMYM2 proteins, based on individual mutations, failed to rescue renal and craniofacial defects, and heterozygous ZMYM2-deficient mice recapitulated features of CAKUT with high penetrance. The discoverys of monogenic causes of CAKUT, indicating that potential mutation and LOF in ZMYM2 in its interaction, which provides a new way to identify the cause, study the pathogenesis of this kind of disease and develop relevant treatment regimens.

We also reviewed the literature[5] and this example, there are 15 different heterozygous nonsense or frameshift mutations of ZMYM2 in 16 unrelated families, and 19 affected individuals with CAKUT and/or syndromic extra-renal features, the heterozygous truncated mutations affected reproductive function. The genes related to the single gene form of CAKUT accounted for 14%-20% of the cases[6-8], neurological manifestations were noted in 17 affected individuals in 15 unrelated families, including 5 individuals were intellectually disability, 10 individuals were motor retardation, 5 individuals were speech delay, 8 individuals were urinary system abnormality, 6 individuals were heart abnormality, 4 individuals were hypodystonia and 5 individuals were

microcephaly.

ZMYM2 selectively binds to the LSD1–CoREST–HDAC1 ternary complex, which is characterized as a corepressor of transcription by interacting with different nuclear receptors, and associated with LSD1-containing corepressor complexes, the LSD1-CoREST-HDAC1 complex on chromatin to regulate gene expression[5,9]. There are multiple ZMYM2 interactors, including members of the LSD1–CoREST–HDAC1 pathway, suggesting that the broader ZMYM2 interactome, which include DNA binding transcription factors, transcriptional-corepressors, proteins linked to chromatin regulation and organization represent potential candidate genes in urinary tract malformation[3].

A wider range of ZMYM2 interaction groups, including DNA-binding transcription factors, transcriptional corepressors, and proteins related to chromatin regulation and tissue, represent potential candidates for urinary tract malformations[3]. FOXP transcription factors play important roles in neurodevelopment, FOXP cooperatively regulates gene expression by forming homo- and hetero-dimers with each other. ZMYM2 is a novel FOXP-interacting transcription factor[10], other genes in this interaction group can also be considered candidates for participation in the disease due to FOXP1 or ZMYM2 loss-of-function mutations. Most pathogenic pathways of ZMYM2-like proteins remain elusive, further work is required to clarify the role of potential interactions in the pathogenesis of NECRC caused by ZMYM2 mutations.

CONCLUSION

Relatively few genetic findings have reached the clinic, in the wide array of inherited metabolic disorders, NECRC has attracted increasing attention due to the neurological damage it causes. The ZMYM2 gene (c.2090_2091del, p.Ser697TrpfsTer3) mutation induces a variety of clinical phenotype, for patients with neurodevelopmental disorders, intellectual disability, autism spectrum disorder, schizophrenia, congenital heart defects, hydroureter, duplex and cystic kidneys, and suspected of NECRC, accurate molecular diagnosis can be provided promptly for children carrying the genetic mutation, which is of great significance for early intervention, precise treatment and family genetic counseling of NECRC.

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