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Identification of 11 potentially relevant gene mutations involved in growth retardation, intellectual disability, joint contracture, and hepatopathy

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Abstract

The multisystemic clinical characteristics of growth retardation, intellectual disability, joint contracture, and hepatopathy in humans are rare and there are no clear diagnoses of these conditions. However, previous studies using exome sequencing have suggested that they are caused by gene mutations, and some related pathogenic gene variants have been found.

Here, we performed resequencing and genome-wide variation analysis of 3 individuals (an affected proband and unaffected parents) from a consanguineous family using Solexa sequencing technology to identify mutated genes.

The following genetic features were identified: 3,586,775 single-nucleotide polymorphisms (SNPs), 583,416 insertion/deletion polymorphisms (InDels), and 8579 structural variations (SVs) in the genome of the father; 3,624,800 SNPs, 608,685 InDels, and 8,827 SVs in the genome of the mother; 3,574,431 SNPs, 571,196 InDels, and 8371 SVs in the genome of the proband. Variations between samples were determined by comparative analysis of authentic collections of SNPs and were functionally annotated. Variations in several important genes, including *SEC22B*, *FLG*, *ZNF717*, *MUC4*, *TRIL*, *CTAGE4*, *FOXG1*, *LOC100287399*, *KRTAP1-3*, and *LRRC37A3*, were surveyed by alignment analysis.

The results present new evidence that mutations in 11 genes may be associated with characteristic clinical growth retardation, intellectual disability, joint contracture, and hepatopathy.

Abbreviations: aCGH = comparative genomic hybridization, FS = frame shifts, IGF = insulin growth factor, InDels = insertion/deletion polymorphisms, NSyn CDS = nonsynonymous coding sequences, PCR = polymerase chain reaction, SG = stop gained, SNPs = single-nucleotide polymorphisms, SSA = splice site acceptor, SSD = splice site donor, SVs = structural variations.

Keywords: gene mutation, growth retardation, hepatopathy, intellectual disability, joint contracture, whole genome resequencing

1. Introduction

Conditions that present with growth retardation, intellectual disability, joint contracture, and hepatopathy together are rare. However, these clinical characteristics have been reported in Japan, Austria, Germany, and Poland.[1,2] To summarize the characteristics of the disease, there are some outstanding resemblances that are noteworthy. First, onset of the disease occurs at infancy. Even when the infant reaches full-term delivery, the birth weight and head circumference are lower than average. Second, the parents are unaffected, and not all siblings are affected. Additionally, there is no apparent direct inheritance or collateral inheritance. Third, the process of pregnancy is normal except for intrauterine growth retardation. Importantly, other factors that could cause abnormalities are excluded, such as uterine infection, intake of drugs, and consanguineous marriages. Fourth, the clinical characteristics of growth retardation, intellectual disability, joint contracture, and hepatopathy did not onset at the same time, but rather emerged with age. In addition, few individuals had other clinical phenotypes such as encephalopathy, diabetes, zinc deficiency, and respiratory infection. However, after medical treatment, these symptoms could be relieved or cured.

Here, we investigated the case of a two-generation family. The proband, a boy, was born at 38 weeks gestational age to nonconsanguineous Han ethnicity parents. Birth weight and head circumference were low and growth retardation, intellectual disability, joint contracture, and hepatopathy onset in succession with age. Among them, hepatopathy and growth retardation were most severe. A younger brother presented with clinical characters that were very similar to the proband. In addition, the younger brother suffered from zinc deficiency. However, neither of their parents, nor direct or collateral relatives were affected.

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Whole genome resequencing is a powerful and cost-effective tool for dissecting the genetic basis of diseases. The availability of high-throughput sequencing technology not only increases sequencing throughput, but additionally identifies point mutations in critical genes responsible for Mendelian disorders using a small number of individuals.[3] The widely used, high-throughput sequencing technologies have been used to identify gene mutations underlying diseases[4] and many such mutated genes causing disorders have been identified. Several pathogenic genes causing the clinical characteristics of growth retardation, intellectual disability, joint contracture, and hepatopathy have been reported.[1,2] However, the current lack of information on genetic variation over the entire genome has limited further research into this disorder. In the present study, we conducted resequencing and genome-wide variation analysis of the proband, father, and mother using Solexa sequencing technology. Analysis of the mutations identified has the potential to provide valuable information for future genetic studies and to improve diagnosis and treatment.

2. Methods

2.1. Human samples

We enrolled 3 members of a family (the proband and his unaffected parents) in the study. The diagnosis of disease was based on typical clinical characteristics, physical examination, intellectual examination, hematological parameters, and imaging examination at the Second Clinical Medical College, Jinan University in 2006. Now, the proband was 12 years old and has mild-to-moderate intellectual disability and mildly abnormal liver function. The proband has been receiving medication and mental training. The study was approved by the review board of ethics committee of the Second Clinical Medical College, Jinan University in China and complied with the principles of the Declaration of Helsinki. Written informed consent was obtained from the proband's parents for publication of his data and any accompanying images

2.2. DNA extracting and genome sequencing

Peripheral blood samples from the proband and his parents were obtained with informed consent. Genomic DNA was obtained from peripheral blood leukocytes using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The DNA of each sample was then randomly fragmented. After electrophoresis, DNA fragments of the desired length were gel purified. Adapter ligation and DNA cluster preparation were performed and subjected to Solexa sequencing.

2.3. Library construction

Each sequenced sample was prepared according to the Illumina TruSeq DNA sample preparation guide to obtain a final library of ~300 to 400 base pair (bp) average insert size. Around 1 μg (TruSeq DNA PCR-free library) or 100 ng (TruSeq Nano DNA library) of genomic DNA was fragmented using a Covaris sonicator, which generated dsDNA fragments with 3′ or 5′ overhangs. The double-stranded DNA fragments with 3′ or 5′ overhangs were converted into blunt ends using an End Repair Mix. The 3′ to 5′ exonuclease removes the 3′ overhangs, and the polymerase fills in the 5′ overhangs. Following the end repair, the appropriate library size was selected using different ratios of the sample purification beads. A single "A" nucleotide was added to the 3′ ends of the blunted fragments to prevent them from ligating to one another during the adapter ligation reaction. A corresponding single "T" nucleotide on the 3′ end of the adapter provides a complementary overhang for ligating the adapter to the fragment. Multiple indexing adapters were ligated to the ends of the DNA fragments to prepare them for hybridization onto a flow cell. Polymerase chain reaction (PCR) was used to amplify the enriched DNA library for sequencing. PCR was performed with a PCR primer solution, which annealed sequences to the ends of each adapter. Macrogen performed quality control analysis on the sample library and quantification of the DNA library templates.

2.4. Detection and annotation of SNPs, InDels, and SVs

SNPs and InDels were detected by GATK software (https://software.broadinstitute.org/gatk/). SVs were detected using BreakdancerMax.pl software (http://breakdancer.sourceforge.net/pipeline.html) with its default parameters. The annotations of SNPs and InDels were performed by SnpEff software (http://snpeff.sourceforge.net/). The distribution of SNPs, InDels, and SVs was located based on the annotation of reference genome databases. The polymorphisms in the gene region and other genome regions were annotated as genic and intergenic. The SNPs, InDels, and SVs were classified according to their localizations. The classification of synonymous or nonsynonymous status of SNPs within the synonymous coding sequence was conducted using Genewise version 30 (https://www.ebi.ac.uk/Tools/psa/genewise/).

2.5. Variations between samples

The SNPs detected for each individual line were further compared between samples to identify the shared and unique SNP/InDel loci. Only the loci for which at least one effective sequence read was mapped for every individual were selected for comparison. The specific SNPs were compared to OMIM (http://omim.org/) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) databases to identify relevant diseases.

3. Results

3.1. Clinical features of the proband

The proband was one of 2 sons of nonconsanguineous parents of Han descent. He was born at 38 weeks of gestation by natural delivery. The birth weight was 2200 g, and the head circumference was 29.6 cm. The infant was primarily fed by breast feeding, and the nutritional status was normal. All hematological parameters (heart, liver, kidney, thyroid, and endocrine functions) and imaging examination were normal at age 1 to 6 months, except that a brain MRI showed a decrease of cerebral white matter at the age of 4 months. However, low weight, low height, and mental retardation were evident from birth. From 1 to 2 years of age, additional clinical characteristics emerged in succession. At the age of 2 years, the body weight was 7000 g and the height was 72.5 cm. Determination of bone age indicated that the age was equivalent to that of a 1.2-year-old. At the same time, torticollis and joint contracture were apparent and accompanied by mild weakness of muscle tension. Development of intellect was limited and proband could only speak several words. At 2 years of age, growth retardation was noticeable. Physical development was delayed (sitting at 15 months of age, walking at 2 years of age) with no progress in speech. Hormones and insulin growth

factor (IGF) were normal. The proband underwent a basic metabolic evaluation, which did not reveal a diagnosis. Array comparative genomic hybridization (aCGH) exanimation excluded chromosome deletion and repetition. The most notable characteristic was abnormal liver function, with a high level of transaminase activities (ALT, AST). The levels of ALT and AST were 30 times greater than normal, but γGT, ALP, and bilirubin levels were normal. Additionally, imaging examination (ultrasound, CT, and MRI) found nonspecific changes in the liver. Based on these phenotypes, the proband has received intellectual training and psychomotor and body therapy. Additionally, the proband has visited a hospital regularly and accepted medical treatment, including treatment for his liver and reduction of liver enzymes. Between the ages of 2 to 6 years, multiple clinically severe infections occurred, especially in the respiratory system. At the time of this writing, the proband is 12 years old and has mild-to-moderate intellectual disability, moderate elevation of liver enzymes, and is vulnerable to infection. However, his weight and height are normal. He understands instructions, interacts with others, and can participate in activities. The younger brother of the proband has very similarly clinical characteristics as the proband. In addition, the younger brother has a zinc deficiency.

3.2. Genome sequencing

The genomes of the proband, father, and mother were analyzed with approximately 10-fold coverage by genome sequencing using Solexa sequencing technology. According to the protocol, 3 DNA libraries were constructed and 398G bp were generated. After cleaning, 122.550 G (proband), 130.376 G (father), and 145.073 G (mother) clean bases were generated and 99.3%, 99.5%, and 98.7% of the reads were mapped to the genomic reference sequence. The overall effective depth coverage of proband, father, and mother was 43×, 46× and 51×, respectively (Table 1).

3.3. Characteristics of SNPs, InDels, and SVs

SNPs, InDels, and SVs were then examined with SOAPsnp1 and SOAPsv (http://soap.genomics.org.cn/) using a conservative quality filter pipeline, yielding 3,574,431 SNPs in the genome of the proband, 3,586,775 SNPs in that of the father and 3,624,800 SNPs in that of the mother. 571,196, 583,416, and 608,685 InDels were found in the genomes of the proband, father, and mother, respectively. The number of SVs in the genomes of the proband, father, and mother were 8,371, 8,579, and 8,827, respectively (Table 1).

Table 1

Summary of the original sequencing data.

Sample	Father	Mother	Proband
Base (G)	130.376	140.073	122.550
Depth	46	51	43
Coverage (%)	99.5	98.7	99.3
Num SNPs	3,586,775	3,624,800	3,574,431
Num InDels	583,416	608,685	571,196
Num CNV	899	963	838
Num SVs	8579	8827	8371

Depth: Approximation of how many times each base in the genome has been sequenced. Coverage: How many regions of the genome's DNA were detected in this study. SNPs: The individual DNA bases that were found to mutated. If the mutation occurred at a critical region of the gene, it may have had a significant effect on gene function. The most important and the most common type of genomic variation. InDels: The insertion or deletion of small fragments of DNA.CNV: The number of copies of the DNA segments more than 1000 bp. SVs: A variety of mutations occurred at the chromosome level of the genome, including translocation, inversion and fusion.

3.4. Variations in SNPs between proband, father, and mother

An analysis of the SNP variations and their distributions in the genomes was performed. Synteny analysis of variations revealed 118 shared SNPs (Table S1, http://links.lww.com/MD/C603) and 192 different SNPs between the proband and the father (Table S2, http://links.lww.com/MD/C604), 93 shared SNPs (Table S3, http://links.lww.com/MD/C605) and 209 different SNPs between the proband and the mother (Table S4, http://links.lww.com/MD/C606), and 11 shared SNPs (Table S5, http://links.lww.com/MD/C607) and 384 different SNPs between father and mother (Table S6, http://links.lww.com/MD/C608). The SNPs in coding regions were analyzed to gain further insights into the potential functional effects of the detected SNPs. Between the father and the proband, 84 shared SNPs were found in coding regions, of which 78 were nonsynonymous coding sequences (NSyn CDS) and 6 were frame shifts (FS) (Table S7, http://links.lww.com/MD/C609) and 73 different SNPs were found in coding regions, of which 62 were NSyn CDS, 7 were FS, 2 were Stop Gained (SG), one was Splice Site Acceptor (SSA), and one was Splice Site Donor (SSD) (Table S8, http://links.lww.com/MD/C610). Between the mother and the proband, 82 shared SNPs were found in coding regions, of which 76 were NSyn CDS and 6 were FS (Table S9, http://links.lww.com/MD/C611) and 88 different SNPs were found in coding regions, of which 81 were NSyn CDS, 3 were FS, 2 were SG, one was SSA, and one was SSD (Table S10, http://links.lww.com/MD/C612). Between the father and the mother, 59 shared SNPs were found in coding regions, of which 56 NSyn CDS and 3 were FS (Table S11, http://links.lww.com/MD/C613) and 141 different SNPs were found in coding regions, of which 127 were NSyn CDS, eight were FS, 4 were SG, one was SSA, and one was SSD (Table S12, http://links.lww.com/MD/C614).

3.5. Variation analysis on mutational genes

Based on the analysis on the mode of the pedigree, the variants of SNPs between the proband and the father and between the proband and the mother were then prioritized for further comparative analysis. A total of 11 SNPs were detected among the proband, father, and mother, which included 10 *de novo* heterozygous mutations (*FLG*: chr1, c. 152277966G>T, p. S3132R; *ZNF717*: chr3, c. 75790805T>A, p. Y47F; *MUC4*: chr3, c. 195509423G>T, p. P3010T; *TRIL*: chr7, c. 28997411C>T, p. S84N; *CTAGE4*: chr7, c. 143964657T>C, p. S563G; *CTAGE4*: chr7, c. 143964658G>T, p. S562R;*FOXG1*: chr14, c. 29236567G>T, p. V28F; *LOC100287399*: chr15, c. 21071520G>A, p. R31C; *KRTAP1-3*: chr17, c. 39191003C>G, p. C24S; *LRRC37A3*: chr17, c. 62892031C>T, p. V449I) and one Splice Site Acceptor (*SEC22B*: chr1, c. 145116306TTTTTGTTTTTTTG>T). Most of the mutated genes were recorded in OMIM (https://www.ncbi.nlm.nih.gov/omim) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) databases and are known to be associated with various diseases (Table 2).

4. Discussion

This study reports a rare disease with multisystemic clinical characteristics of growth retardation, intellectual disability, joint contracture, and hepatopathy. We found previous reports of

Table 2

Variation analysis of mutated genes.

Chrom	Location	Ref	Alt	Quality	Het/hom	Read number	Type	Base change	Aa change	Gene	NCBI id	OMIM ID
chr1	145116306	TTTTTGT TTTTTTG	T	604	0/1	64,18	SSA			*SEC22B*	NM_004892.5	604029
chr1	152277966	G	T	33	0/1	1,2	NSyn CDS	agC/agA	S3132R	*FLG*	NM_002016.1	135940
chr3	75790805	T	A	148	0/1	27,18	NSyn CDS	tAc/tTc	Y47F	*ZNF717*	NM_001128223.1	.
chr3	195509423	G	T	42	0/1	9,3	NSyn CDS	Cct/Act	P3010T	*MUC4*	NM_018406.6	158372
chr7	28997411	C	T	145	0/1	17,12	NSyn CDS	aGc/aAc	S84N	*TRIL*	NM_014817.3	613356
chr7	143964657	T	C	36	0/1	3,4	NSyn CDS	Agc/Ggc	S563G	*CTAGE4*	NM_198495.2.2	608910
chr7	143964658	G	T	36	0/1	3,4	NSyn CDS	agC/agA	S562R	*CTAGE4*	NM_198495.2.2	608910
chr14	29236567	G	T	32	0/1	7,2	NSyn CDS	Gtc/Ttc	V28F	*FOXG1*	NM_005249.4	164874
chr15	21071520	G	A	55	0/1	8,8	NSyn CDS	Cgc/Tgc	R31C	*LOC100287399*	NM_001277303.1	.
chr17	39191003	C	G	57	0/1	31,7	NSyn CDS	tGc/tCc	C24S	*KRTAP1-3*	NM_030966.1	608820
chr17	62892031	C	T	116	0/1	10,8	NSyn CDS	Gta/Ata	V449I	*LRRC37A3*	NM_199340.2	.

Chrom: The variation of chromosome number. Location: The chromosome on which the mutation occurred. Ref: The base on the reference genome. Alt: The base after the mutation. Quality: The quality of the variation. The higher the quality, the more reliable the variation. Het/hom: 0/1 indicates a heterozygous mutation, 1/1 indicates a homozygous mutation. Read number: The left number represents the type of mutant, the right number represents wild-type. The sum of the left and right values is the number of tests. Type: Variation type, including nonsynonymous coding, stop gained and splice site acceptor. Base change: The base mutation of the codon. Aa change: amino acid change in the gene site. Gene: The genes that were mutated. NCBI id: The gene number in the NCBI database, OMIM id: The gene number in the OMIM database.

similar clinical characteristics.[1,2] Some studies have reported similar individual phenotypes, but not with the combination of growth retardation, intellectual disability, joint contracture, and hepatopathy. Pedersen et al. recognized phenotypes of hypotonia, encephalopathy, growth retardation, and intellectual disability in families from Iraq, Morocco, and Norway.[5] Windpassinger et al[6] identified phenotypes of growth retardation, spine malformations, and developmental delay in families from Turkey, Algeria, Tunisia, and Saudi Arabia. In both of these studies, the clinical characteristics were studied by whole genome resequencing or exome sequencing to identify potentially relevant gene mutations. Among the clinical characteristics in this study, growth retardation has been reported to be associated with intellectual disability in several previous studies. The remaining individual features include epilepsy, sensorineural hearing loss, diabetes, myopathic, scoliosis, abnormal face shape, and abnormal fat distribution under the skin.

Smigiel et al[1] and Kopajtich et al[2] reported individuals that have the same clinical characteristics of growth retardation, intellectual disability, joint contracture, and hepatopathy. Significant growth delay was found in all of the reported subjects. However, weight and height become normal with age. The cause for growth delay is unknown and might improve with age and/or growth hormone (GH) therapy. There was no evident abnormality of endocrine hormones, including GH, in the proband from infancy to childhood. We hypothesize that GH played a role in growth, but could not be a determining factor.

Liver dysfunction was seen in 5 previously reported individuals. Liver function was normal with age after medical treatment in 3 individuals. However, one individual had liver function that was seriously impaired, progressing to acute liver failure. Two individuals reported in studies were diagnosed with cholestasis, and 4 individuals were diagnosed with hepatic fibrosis through liver biopsy. Zinc deficiency was detected in 4 individuals, who were additionally vulnerable to infection.[1,2] In this study, abnormal liver function was assessed by elevated levels of AST and ALT, though γGT, ALP, and bilirubin levels were normal. Imaging failed to detect liver fibrosis. There was no evidence that could be used to evaluate liver function. Liver function varies from individual to individual. However, early treatment of abnormal liver function can slow the progression of liver failure and even cure it. Liver disease is known to be associated with zinc deficiency.[7] The abnormal liver function led to low immunoglobulinemia, resulting in the proband being vulnerable to infection. This may explain why zinc supplementation was initiated at infancy in some individuals, resulting fewer infections with age.

The 5 individuals presented in the literature and reported here had an evident intellectual disability. However, their intellectual disability improved with mental and physical training. Some individuals were able to interact with others, perform some activities, and studied in school.[1,2,8] Current data indicate that Ewards syndrome and Down syndrome are the most common causes of intellectual disability. The cause of Edwards syndrome is congenital chromosomal changes.[9,10] Just like Edwards syndrome and Down syndrome, the patients reported in the literature showed intellectual disability throughout their life. Although we excluded chromosomal changes in proband in this study, we suspect that intellectual disability was congenital and caused by gene mutations and the additional phenotypes did not contribute to the intellectual disability.

Our study uncovered new evidence that recessive mutations in *SEC22B*, *FLG*, *ZNF717*, *MUC4*, *TRIL*, *CTAGE4*, *FOXG1*, *LOC100287399*, *KRTAP1-3,* and *LRRC37A3* genes are associated with growth retardation, intellectual disability, joint contracture, and hepatopathy. However, in recent years, exome sequencing studies have been used to investigate similar clinical phenotypes, revealing mutations genes in *IARS*.[1,2,8] Other similarly multisystemic clinical characteristics such as hypotonia, encephalopathy, growth retardation, and intellectual disability were revealed to be associated with mutations in *UNC80*[5] and growth retardation, spine malformations, and developmental delay to be related to mutations in *CDK10*.[6] The clinical characteristics were multisystemic and we speculate that the disease was caused by mutations in multiple genes rather than only in one. Individual clinical characteristics could be the result of either one or multiple gene mutations. Additionally, one mutated gene could be associated with one or multiple clinical characteristics. There are currently no reports of mutations in *SEC22B*, *FLG*, *ZNF717*, *MUC4*, *TRIL*, *CTAGE4*, *FOXG1*, *LOC100287399*, *KRTAP1-3,* or *LRRC37A3* genes associated with growth retardation, intellectual disability, joint contracture, and hepatopathy. This may be a result of the differences in individual genes and the limitations in the number of individuals

for whom these genes have been sequenced. We cannot conclude for certain that the disease was caused by the mutations in the genes reported here. Because the proband suffers from this rare disease, the study of the mutated genes reported here could help to enrich the database of etiological genes.

5. Conclusion

This study delineated mutations in *SEC22B*, *FLG*, *ZNF717*, *MUC4*, *TRIL*, *CTAGE4*, *FOXG1*, *LOC100287399*, *KRTAP1-3*, and *LRRC37A3* genes underlying a multisystemic disease presenting with growth retardation, intellectual disability, joint contracture, and hepatopathy. The mutated genes reported here and in other studies will be valuable for future studies on the pathogenesis of the disease and for its diagnosis and treatment.

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