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Exome analysis for a case of cronkhite-canada syndrome

Li ZD *et al*. Exome analysis for cronkhite-canada syndrome

Abstract

BACKGROUND

Cronkhite-Canada syndrome (CCS) is a rare, non-genetic disorder characterized by multiple gastrointestinal polyps, as well as ectodermal lesions like alopecia, fingernail atrophy, and skin mucosal pigmentation. Unfortunately, its pathogenesis is currently unknown.

CASE SUMMARY

Here, we describe the case of an elderly female with diarrhea, fatigue, and hair loss, who experienced abdominal pain for over half a year and was found to have multiple gastrointestinal polyps. She was diagnosed with CCS and was provided with albumin supplementation and prednisone, while correcting her electrolyte imbalance. Following these treatments, her symptoms improved significantly. To elucidate the role of potential genetic events in the pathogenesis of CCS, we performed exome sequencing with an extract of her colorectal adenoma.

CONCLUSION

Our data revealed multiple somatic mutations and copy number variations. Our findings provide a novel insight into the potential mechanisms of CCS etiology.

Key Words: Whole exome sequencing; Cronkhite-Canada syndrome; Somatic mutations; Case report

Li ZD, Li R, He YJ, Ji YZ, Li X, Zhou SF, Li XA. Exome analysis for a case of Cronkhite-Canada syndrome. *World J Clin Cases* 2022; In press

Core Tip: Cronkhite-Canada syndrome is a rare, non-genetic disorder characterized by multiple gastrointestinal polyps, ectodermal lesions including alopecia, fingernail atrophy, and skin mucosal pigmentation. But its pathogenesis is not clear. We performed exome sequencing on an elderly female patient and obtained somatic mutation results in the hope that these data could provide a genetic perspective on the pathogenesis of the disease.

INTRODUCTION

Cronkite and Canada reported the first case of the Cronkhite-Canada syndrome in 1955, which was characterized by multiple gastrointestinal polyps, ectodermal lesions including alopecia, fingernail atrophy, and skin mucosal pigmentation[1]. Unfortunately, despite much research, Cronkhite-Canada syndrome (CCS) pathogenesis remains unknown. Some studies reported an association between CCS and immune factors, especially infiltration of the IgG4-positive plasma cells[2]. Thus far, there is no standard treatment for CCS, however, some studies recommended glucocorticoid therapy for partial symptoms relief[3]. Herein, we describe the case of an elderly female with CCS diagnosis. As part of her treatment regimen, we provided her with albumin supplementation and prednisone, while correcting her electrolyte disturbance. After15 days, her symptoms, namely, hypokalemia and diarrhea, improved significantly. To elucidate the role of potential genetic events in the pathogenesis of CCS, we performed exome sequencing with the DNA extract of her colorectal adenoma. Our data revealed multiple somatic mutations and copy number variations. We were the first to identify 3 novel genetic mutations (*USP24*, *KCNQ5*, and *FKBP10*) in a CCS patient. Moreover, we demonstrated that the *HPSE2*, *SPATA7*, and *ZC3H18* genes had markedly elevated copy

numbers. Given these evidences, we hypothesized that these specific gene mutations and copy number variations are associated with CCS pathogenesis.

CASE PRESENTATION

Chief complaints

An elderly female patient sought treatment for diarrhea, fatigue, and hair loss, as well as abdominal pain for half a year at the Department of Gastroenterology on October 11, 2019.

History of present illness

She reported diarrhea 4-5 times daily, watery stools, no blood, and abdominal pain under the xiphoid process, not related to eating. Physical examination revealed obvious emaciation, alopecic, and nail atrophy

2

History of past illness

Her past medical history is unremarkable.

Personal and family history

Her family history is unremarkable.

Physical examination

Physical examination revealed obvious emaciation, alopecic, and nail atrophy (Figure 1).

Laboratory examinations

Laboratory tests revealed the following: (routine blood examination) white blood cells: 10.03×10^9 / L, hemoglobin: 95g/L, and platelets: 151×10^9 / L; (liver function) albumin 25g/L; (electrolyte) Na^+ :128 mmol/L, K^+: 2.8 mmo/L.

Imaging examinations

She underwent a gastroenteroscopy, which revealed multiple small polyps in the gastrointestinal tract (Figure 2).

Materials and methods

DNA samples: This study was performed in accordance with the guidelines of the Ethics Committee of the Mianyang Central Hospital. DNA was extracted from a sample of the patient's colonic adenomas and normal colon tissue. The samples were obtained *via* endoscopic mucosal resection and was preserved in liquid nitrogen.

Whole exome sequencing: Genomic DNA ≥ 1.5 μg from colonic adenomas and normal colon tissue was used for the whole exome sequencing library construction. Meanwhile, the Agilent liquid chip capture system was employed for the efficient enrichment of human DNA in all exon regions. Upon library construction, Qubit2.0 was used for preliminary quantification, and the library was diluted to 1ng/μL. Subsequently, Agilent 2100 was utilized for the library insert size detection to ensure library quality (the effective concentration of the library >2 nmol/L). Next, the PE150 high-throughput sequencing was performed, based on the Illumina Hiseq platform. Finally, the library and capture experiment were constructed using the Agilent SureSelect v5 kit, according to manufacturer's instructions.

Quality control: Upon collection of the original sequenced reads (Sequenced Reads), the cutadapt software was used to remove the adapter and reads with N (N denotes that the base information cannot be determined) ratio ≥ 10%. In addition, the read pairs were discarded if the low-quality bases (quality score ≤ 5) accounted for 50% of the entire single read. Furthermore, quality controls (including, sequencing error rate distribution, base quality distribution, and GC content distribution) were performed, based on the select filtered reads using the above methods.

Data analysis: The BWA (v0.7.15) software was used to map the sequencing read to the human reference genome GRCh37 (hg19). The Picard software was used to remove the sequence generated by PCR-duplication. The somatic single nucleotide variations (SNVs) and InDel detection were performed using the GATK (v4.1.0.0) and muTect2 software, respectively. The ANNOVAR software (Mon, 17 Jul 2017) was used to annotate gene mutations. Lastly, the control-free software (v11.4) was used for copy number variation analysis.

Exon sequencing results

Somatic mutation analysis, based on samples from the CCS patient, identified 47 SNVs. 75% of them were nonsynonymous SNVs. In addition, about 70% of them occurred in exonic regions (Figure 3). The mutations of the *USP24*, *KCNQ5*, and *FKBP10* genes were identified as deleterious *via* SIFT, LRT, Polyphen2, and Mutation Taster softwares (Supplementary Table 1). Based on the COSMIC data, the three mutations in genes *USP24*, *KCNQ5*, and *FKBP10* may be novel in CCS. The mutated genes are associated with the regulation of DNA-templated transcription (Figure 4). Our analysis showed that the *HPSE2*, *SPATA7*, and *ZC3H18* genes had remarkably elevated copy numbers, while other significant altered fragments were located in the intergenic regions (Table 1).

FINAL DIAGNOSIS

She was thus diagnosed with CCS.

TREATMENT

As part of her treatment regimen, she was provided with albumin supplementation 10 g per day and prednisone 10 mg per day, while correcting her electrolyte imbalance.

OUTCOME AND FOLLOW-UP

The treatment lasted for 15 d and the diarrhea and hypokalemia improved significantly, as well as the abdominal pain.

DISCUSSION

About 500 cases of CCS are currently described in literature, with an estimated incidence of 1 in 1 million per year. The average age of CCS diagnosis is early 60s and it is predominantly diagnosed in males[4]. At present, there is no definite treatment plan for CCS. Prior literature reported that glucocorticoids and immunosuppression exert certain benefits, and some studies reported that anti-TNF therapy is effective in CCS patients[5]. Our treatment plan and effect are consistent with these published works. However, there are limited studies on CCS pathogenesis. Therefore, based on the fact that CCS is an acquired non-genetic disease, we performed exon sequencing on the excised diseased and non-diseased tissues from our CCS patient, in an attempt to elucidate CCS pathogenesis. Based on our exon sequencing results, three genes, namely, *USP24*, *KCNQ5*, and *FKBP10* may be related to CCS pathogenesis.

We searched HGMD and found that *USP24* belongs to a large family of cysteine proteases that function as deubiquitinating enzymes. *USP24* stabilizes bromine domain protein and promotes malignant lung cancer[6]. In addition, Zhang L found that *USP24* deubiquitinase regulates DNA damage by directly targeting the tumor suppressor gene p53[7]. Also, the *USP24-Mcl-1* axis may represent a novel strategy in treating acute T cell lymphoma[8], whereas functional studies of the *FKBP10* mutation reported an association with osteogenesis imperfecta[9]. The above two genes are both related to tumor formation and body development. Combined with the clinicopathological characteristics of CCS, we speculated that gene mutations are involved in the formation of multiple intestinal adenomas. KCNQ family protein activates slowly during depolarization and forms heterogeneous channels with the protein encoded by KCNQ5 gene. KCN5 dependent potassium channels play an important role in airway smooth muscle relaxation[10]. Given the symptoms of diarrhea and difficult-to-correct hypokalemia of CCS, the KNQ3 mutation seems to suggest an association.

In terms of copy variation, *ZC3H18* copy number losses are known to contribute to homologous recombination defects in high-grade serous ovarian cancers[11]. *HPSE2* was reported to play an inhibitory role in bladder cancer[12]. Mutations in *SPATA7* are associated with fundus macular degeneration[13]. CCS patients have multiple clinicopathological manifestations, such as, multiple gastrointestinal adenomas, nail atrophy, skin pigmentation, and alopecia, which may be related to the increased copy number of the three genes mentioned above.

However, due to the isolation of individual cases, the sample size of phenotypic alterations, caused by the above these gene mutations, needs to be further expanded.

CCS is a rare disease and its etiology is not clear. The autoimmune etiology of CCS was previously proposed, and case reports described beneficial responses to immunosuppressive therapies like azathioprine, anti-tumor necrosis factor antibodies, cyclosporine, and sirolimus[14,15]. Interestingly, Brigid S. Boland also conducted exome sequencing on tissue from a CCS patient who responded effectively to infliximab, and found that *PRKDC* mutations may be involved. However, this gene was not covered in our analysis[14]. This suggests that the data on these mutations need to be further validated using tissues from a large CCS patient population.

CONCLUSION

In conclusion, herein, we reported a classical case of CCS, which was effectively treated with parenteral nutritional support and glucocorticoids, and we explored the pathogenesis of CCS from the perspective of gene mutation. Based on our analysis, we identified several gene mutations and alterations in gene copy numbers. However, we acknowledge that much genetic and epidemiological work remains to be done to understand the complex pathogenesis of this rare but highly fatal disease.

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