Reviewer #2:

The article found that a younger girl had a rare recessive genetic disorder, and did a detailed clinical examination and whole exon sequencing, and found that she had inherited the disease-causing mutation from both parents.

Additionally, the identification of the c.5683+1G>C variant in the OBSL1 gene is noteworthy, as it has not been previously reported in public databases.

In addition to her own genetic disease, she also complicated with spinal deformity is also the first report.

Although this study shows a novel locus in 3MS, there are some problems listed as follow:

1. What is the basis for dividing 3MS into 3 types?
2. Does having two mutation sites at the same time lead to more severe disease manifestations?
3. How to take effective treatment measures against gene mutation?
4. Is there a link between scoliosis and mutated genes?
5. The use of punctuation in some parts of the article is not standard, and it is recommended to modify it carefully.
6. What is the full name of MVV in the resulting paragraph?

This case report is novel and has sufficient sequencing and pedigree verification, so I recommend publishing this article.

Thank you very much for your valuable comments. We have revised them one by one.

1. Because of the different mutated genes, 3MS is categorized into three distinct types: type 1, type 2, and type 3, with incidence rates of 77.5%, 16.3%, and 6%, respectively.
2. It is rare to have two mutations simultaneously, which may also lead to more severe developmental abnormalities; however, the patient's intellectual development remains unaffected.
3. In this case, growth hormone (GH) therapy remains controversial owing to individual differences and the treatment effectiveness [10]. Although the patient had normal GH levels, GH therapy should be considered to address the severe short stature commonly associated with 3MS despite the absence of growth restriction in this patient.
4. Similarly, determining whether a direct relationship exists between scoliosis and genetic mutations in patients requires additional case data.
5. We invited editage to polish the language and upload the English Editing Certificate.
6. The full name of MVV is Maximum voluntary ventilation

Reviewer #1:

Manuscript 89801 A Case Report of China 3M Syndrome Patient with a Novel Mutation Review comments In general,

English should be improved along the manuscript to correct grammar issues and spelling mistakes and improve the use of English. Along the manuscript, authors should comply with the proper nomenclature for genes and proteins, e.g., gene symbols should be italicized, with all letters in uppercase, and proteins should be designated as the gene symbol but not italicized, with all letters in uppercase. For instance, the authors say, “In this case, we employed whole exon sequencing to sequence the CUL7⋯ genes”.

1 Title. The title can be improved to be grammatically correct; instead of ⋯China 3M Syndrome.. should say ⋯ Chinese 3M Syndrome ⋯

4 Background The background section is missing information describing the function of the 3M complex under physiological conditions together with the molecular basis for the malfunction of the mutations in OBSL1, Cullin 7 and CCDC8 in disease. A description of the predicted domain(s) or protein fold of OBSL1 could be helpful to understand the effect of the mutations. A brief description of the already-known mutations in OBSL1, Cullin 7, and CCDC8 is desired. A small attempt to include this information was presented in the Discussion section, but it is scant and limited mainly to what can be found in the corresponding Uniprot section.

5 Methods. In the methodology section regarding “Basic information,” some details are missing along the text. For example, it mentions that family medical records and consanguinity were extracted from hospital records, but no information is mentioned in the text.

6 Results. The head of the table and the figure legends should be improved, keeping in mind these should be auto-consistent and should be understandable on their own without reading the manuscript. For example, the head of Table 1 says, “Clinical characteristics and molecular findings of the patient”; however, out of context of the manuscript, this title does not inform the type of clinical patient. The symbology used along the table could be improved, for instance, “long slender tubular bones, +.” Authors could expand this information somewhere in the table. How much is the + symbol referring to in order to be considered +? Does the “– “ symbol mean that the feature is typical? If so, why should all the typical features be included in the table, making reading long and tedious? A phrase in the text mentioning that other features were within the normal standard would suffice.

Inconsistencies referring to the patient as 15- or 16-year-old are along the text and should be corrected.

The authors' main contribution is a novel mutation in the OBSL1 gene corresponding to c.5683+1G>C together with a second already known mutation c.3341G>A. The authors mention that the c.5683+1G>C variant potentially affects mRNA splicing, leading to decreased OBSL1 protein expression and loss of protein function, which in turn contributes to disease occurrence. Since the authors performed whole exome sequencing, it is assumed that this mutation is present in an exome. Which one? At the coding level, authors should state the outcome of the mutation. Is it an insertion, a deletion, or a frameshift that introduces of a stop codon that truncates the protein? Exactly what does “potentially affects mRNA splicing” mean? The phrase seems ambiguous as it stands. Can the authors perform a northern blot or an immune blot to sustain their claim? At the coding level, the second

mutation corresponds to p.Trp1114Ter which truncates the protein in the IGc2 domain. What does IGc2 domain refer to? Authors never mentioned in the text that OBSL1 consists of a protein with 4 tandem N-terminal immunoglobulin (Ig)-like domains, a central fibronectin domain, and 13 C-terminal Ig domains. Hence, this type of information needs to be mentioned in the manuscript. What is the function of each domain of the protein?
7 Discussion Authors mention that disruption of this complex results in microtubule damage, abnormal chromosome separation, and cell death. What does microtubule damage mean? By "chromosome separation," do the authors mean chromosome segregation? The narrative in the discussion section is messy, disorganised, and difficult to follow. For example, the authors say, "As orthopedists, it is important for us to provide symptomatic treatment and follow-up care, considering the high likelihood of encountering patients with this syndrome." 3MS is a rare disease, with approximately 200 cases reported worldwide. The purpose of the paragraph about the growth hormone (GH) therapy section is unclear, as they mention that their patient does not show growth abnormalities. Finally, in the concluding remarks, the authors state that sequencing of CUL7, OBSL1, and CCDC8 genes is necessary to confirm the clinical diagnosis and provide appropriate genetic counseling. Although true, the authors should be more specific to aid the audience, e.g. i

Thank you very much for your valuable comments. We have revised them one by one.
1. We changed the title to "A 3M Syndrome Patient with a Novel Mutation: A case report from China".
4. CUL7 is responsible for encoding the CUL7 protein, which serves as a scaffold protein and is a vital component of an E3 ubiquitin ligase enzyme. Nonsense or missense mutations in the CUL7 gene prevent the substrate from ubiquitinating, degrading, and accumulating in the body. OBSL1, this particular protein encodes a cytoskeletal adaptor protein that is primarily localized within the prenuclear region. The exact function of OBSL1 is still under investigation, however, recent studies have highlighted its interaction with the protein encoded by CCDC8. This interaction is essential for p54-mediated apoptosis in cells. The function of CCDC8 remains largely unknown. Unraveling the precise mechanisms governing this intricate relationship is crucial for understanding the physiological and pathological implications associated with these proteins.
5. The patient provided informed consent. The study protocols were approved by ethics committees at the First Affiliated Hospital of Nanjing Medical University (2022-SR-661). We identify the committee that approved the research, confirm that all research was performed in accordance with relevant guidelines/regulations, confirming that informed consent was obtained from all participants and/or their legal guardians.
6. Table1. Clinical characteristics and molecular findings of the 3MS patient.
Fig1. Clinical features of the 3MS patient.
- represents a negative result, + represents a positive result
We unified the age of the patients to 15 years old
The nonsense mutation c.3341G>A (p.Trp1114Ter) is located in exon10, the IGc2 domain of the protein (amino acid positions 1095 to 1160). The mutation prematurely terminates the translation of the protein and prevents subsequent amino acid synthesis, which includes several domains. The IG domain may be involved in a variety of functions in proteins, such as

intercellular recognition, cell surface receptors, muscle structure, and the immune system, so the mutation may affect the normal physiological function of the protein.

The c.5683+1G>C mutation is located on the intron, which is located at the classical splicing site and is predicted by the software to potentially affects mRNA splicing, which leads to decreased OBSL1 protein expression and loss of protein function, contributing to disease occurrence.

We added the following: OBSL1 consists of a protein with 4 tandem N-terminal immunoglobulin (Ig)-like domains, a central fibronectin domain, and 13 C-terminal Ig domains.

7. It is evident that there is still much to be discovered about the intricate functions and interactions of CUL7, OBSL1, and CCDC8. Moreover, the detailed mechanisms responsible for the growth impairments observed in the 3M syndrome remain largely unclear.