A Child with Juvenile Myelomonocytic Leukemia Possessing a Concurrent Germline CBL Mutation and a NF1 Variant of Uncertain Significance: A Rare Case with a Common Problem in the Era of High-throughput Sequencing.

Wei-Hao Wang[1], Meng-Yao Lu[1], Cheng-Hong Tsai[1], Shih-Chung Wang[2], Shu-Wei Chou[1], and Shiann-Tarng Jou[1]

[1]National Taiwan University Hospital
[2]Changhua Christian Medical Foundation Changhua Christian Hospital

May 26, 2020

Abstract

Genetic changes in juvenile myelomonocytic leukemia (JMML) determine distinct subtypes, treatments and outcomes. JMML with germline CBL mutation and somatic NRAS mutation possibly achieves spontaneous remission, but hematopoietic stem cell transplantation is indicated for other subtypes of JMML. We hereby report a child with JMML harboring a germline CBL mutation (c.1111T>C) and an NF1 variant (c.3352A>G) concurrently. After evaluation, we considered the NF1 variant not the major contributor. After one year of observation, this case had no signs of disease progression. This case highlights the importance of combining available evidence and clinical findings in caring patients with unusual genomic variations.

Introduction

Juvenile myelomonocytic leukemia (JMML) is one of the rarest pediatric hematologic malignancies, affecting around 1.2 per million children per year[1]. It usually occurs in early childhood and possesses a dismal prognosis[2]. Ninety percent of cases with JMML could be attributed to alterations involving *RAS/MAPK* signaling pathway, including *KRAS* , *NRAS* , *PTPN11* ,*NF1* , and *CBL* [3]. Hematopoietic stem cell transplantation (HSCT) has been regarded as the only cure for patients with JMML[2]. However, some specific subtypes, such as germline *CBL* -mutated JMML or JMML with clonal *RAS*mutations, portrayed a less aggressive picture and even displayed spontaneous remission[4-7]. As a result, determining the culpable molecular aberrancy is curial in the management of patients with JMML. With the booming development of next-generation sequencing (NGS) in the field of hematologic malignancies, the underlying genomic alterations of neoplastic diseases are more easily accessible nowadays. However, NGS also discovers genetic changes with unknown influences on protein function or clinical pathogenicity, termed variant of uncertain significance (VUS)[8]. In clinical practices, a VUS usually brings challenges to the physicians because of the inadequacy of clinical information for decision-making. We report our experience in taking care of a child with JMML concurrently harboring a germline*CBL* pathogenic mutation and a germline *NF1* VUS.

Case Presentation

A two-year-old boy was referred to our hospital for unexplained anemia and thrombocytopenia lasting for 6 months. His height was 81.8cm (3^{rd} to 10^{th} percentile; weight was 11.3kg (25^{th} to 50^{th} percentile). The development was normal without hearing or visual impairment. Physical examination findings were unremarkable except splenomegaly. There was no Cafe au lait spot nor hyperpigmentation on the skin.

No low-set ears, webbed neck nor hypertelorism was noted. Echocardiogram revealed no cardiac structural anomaly. Splenic longitudinal length measured by sonography was 11.2cm (Suggested upper limit for 2 to 4 years old children was 9cm)[9]. Complete blood count showed monocytosis and the presence of myeloid and erythroid precursors. The fetal hemoglobin was 3.6% (reference range: less than 2%). Bone marrow studies revealed the blast cells were less than 20%. He had a normal karyotype (46; XY). The bone marrow mononuclear cells were identified to have a pathogenic *CBL* mutation (c.1111T>C; p.Tyr371His; variant allele frequency 97.3%) via NGS (Oncomine Myeloid Research Assay, Thermofisher). The diagnosis of JMML was established. Additionally, a single-nucleotide variant in*NF1* (c.3352A>G; p.Ser1118Gly, variant allele frequency 49.62%) was also found. According to the classification in ClinVar, an openly accessible database for reports of interpreting the relationships between variants and medical conditions[10], this was a VUS of *NF1* .

For confirmation, we obtained his buccal swab and did Sanger sequencing for the *CBL* gene. The same variant was found on one allele, and the other remained wide type, confirming a germline heterozygous mutation. For inheritance investigation, we took blood samples for Sanger sequencing from both his parents. The result affirmed neither of them carried the mutation (Fig. 1). In a nutshell, this patient has a*de novo CBL* germline mutation. Given that JMML with germline heterozygous *CBL* mutation often experiences spontaneous regression[4-7]. We considered adopting observation for this patient. Yet, concerns regarding the *NF1* variant raised, because HSCT is indicated for JMML patients with *NF1*mutations[5]. As a complementary diagnostic test, we draw his parents' peripheral blood for *NF1* Sanger sequencing. The result showed that the *NF1* variant was inherited from his mother (Fig. 1). This patient's mother was asymptomatic with a normal hemogram. There was no medical history of hematological malignancies or skin tumors among maternal relatives. We thus considered that this *NF1*variant was not the driver gene for the JMML in this case. Based on these findings, close observation with regular follow-up was suggested.

After the diagnosis, this boy was once hospitalized owing to left leg cellulitis and resolved after antibiotics. We re-evaluated this child one year after the diagnosis. His growth was fair with a height velocity of 8cm within the pasting year. Developmental milestones were in accordance with his age. There were no signs of autoimmune disorders, and the antinuclear antibody and anti-double stranded DNA antibodies were within the normal range. Hemogram of peripheral blood and bone marrow examination showed no signs of disease progressing according to the criteria for response evaluation of JMML[11]. The results of serial tests during the following up period were illustrated in Table 1.

Discussion

The treatment outcome of JMML is discouraging. HSCT was the only known curative option, but merely about half of the patients achieved long-term survival even if they received the transplantation[2,12]. However, Niemeyer et al[4]. found some patients with germline*CBL*- mutated JMML often experienced spontaneous regression. An international survey also found HSCT did not improve the outcome of*CBL* -mutated JMML, thus active surveillance rather than immediate HSCT was suggested for patients initially diagnosed with*CBL* -mutated JMML[5]. Nevertheless, individuals harboring germline heterozygous *CBL* mutations had a higher risk of growth retardation, development delay, cardiovascular disease, optic or auditory problems, and autoimmune-related diseases[4,13,14] in addition to hematopoietic disorders. Hence, the treatment principles of *CBL* -driven JMML are distinct from other subtypes of JMML.

The *CBL* mutation, p.Tyr371His, found in our case, was confirmed as pathogenic and highly recurrent in JMML[15]. However, the myeloid NGS panel also disclosed a VUS of *NF1* in this case and put us into a difficult spot: should we initiate the HSCT plan for our patient? For *CBL*- mutant JMML was often self-resolving, but*NF1*- driven JMML needed intensive therapies and bridged to transplantation[5]. We did *NF1* sequencing for both this case's parents and recognized this variant was inherited from his mother. The maternal family members were without*NF1* -associated hematological or dermatological diseases after the survey. For these reasons, we speculated that the *CBL* mutation was the driver mutation of his JMML, and observation with close monitoring of the signs of disease progressing was suggested. In the following year, no evidence of disease deterioration was noted, which was supporting our speculation.

With the wide application of high throughput screening techniques, comprehensive genetic testing would become commonplace for the management of rare diseases and neoplastic malignancies. The detection of VUS is not uncommon. More than 30% of patients with breast cancer were found with at least one VUS by a 25 gene panel for cancer susceptibility[16]. Defining the implication of a VUS is an arduous task, and while a variant is suspected pathogenic, re-evaluation with orthogonal methods is suggested[8]. Designing computational prediction models or functional studies of the gene is also helpful yet usually infeasible in clinical setting[8]. Clinical evaluation is therefore an indispensable part. In this case, we performed serial physical exams for phenotype assessment, took family history to analyze the pattern of inheritance, validated the NGS result with Sanger sequencing, and most importantly, monitored the response after management. Combining laboratory findings and bedside information, we could tailor a personalized treatment strategy for patients with a rare disease and genetic alterations with unknown effects.

In summary, we presented how we approached a rare case diagnosed with *CBL* -mutated JMML with concurrent germline *NF1* VUS. Because lacking information about the penetrance of these variants in adolescence and adulthood, long term follow-up is warranted.

Conflict of Interest

The authors declare that they have no conflict of interest.

Reference

1. Chan RJ, Cooper T, Kratz CP, Weiss B, Loh ML. Juvenile myelomonocytic leukemia: a report from the 2nd International JMML Symposium. *Leuk Res.* 2009;33(3):355-362.

2. Locatelli F, Nollke P, Zecca M, et al. Hematopoietic stem cell transplantation (HSCT) in children with juvenile myelomonocytic leukemia (JMML): results of the EWOG-MDS/EBMT trial. *Blood.*2005;105(1):410-419.

3. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood.* 2016;127(20):2391-2405.

4. Niemeyer CM, Kang MW, Shin DH, et al. Germline CBL mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia. *Nat Genet.* 2010;42(9):794-800.

5. Locatelli F, Niemeyer CM. How I treat juvenile myelomonocytic leukemia. *Blood.* 2015;125(7):1083-1090.

6. Matsuda K, Taira C, Sakashita K, et al. Long-term survival after nonintensive chemotherapy in some juvenile myelomonocytic leukemia patients with CBL mutations, and the possible presence of healthy persons with the mutations. *Blood.* 2010;115(26):5429-5431.

7. Muraoka M, Okuma C, Kanamitsu K, et al. Adults with germline CBL mutation complicated with juvenile myelomonocytic leukemia at infancy.*J Hum Genet.* 2016;61(6):523-526.

8. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.*2015;17(5):405-424.

9. Rosenberg HK, Markowitz RI, Kolberg H, Park C, Hubbard A, Bellah RD. Normal splenic size in infants and children: sonographic measurements.*AJR Am J Roentgenol.* 1991;157(1):119-121.

10. Landrum MJ, Lee JM, Benson M, et al. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res.* 2016;44(D1):D862-868.

11. Niemeyer CM, Loh ML, Cseh A, et al. Criteria for evaluating response and outcome in clinical trials for children with juvenile myelomonocytic leukemia. *Haematologica.* 2015;100(1):17-22.

12. Bergstraesser E, Hasle H, Rogge T, et al. Non-hematopoietic stem cell transplantation treatment of juvenile myelomonocytic leukemia: A retrospective analysis and definition of response criteria.*Pediatric Blood*

& Cancer. 2007;49(5):629-633.

13. Martinelli S, De Luca A, Stellacci E, et al. Heterozygous germline mutations in the CBL tumor-suppressor gene cause a Noonan syndrome-like phenotype. *Am J Hum Genet.* 2010;87(2):250-257.

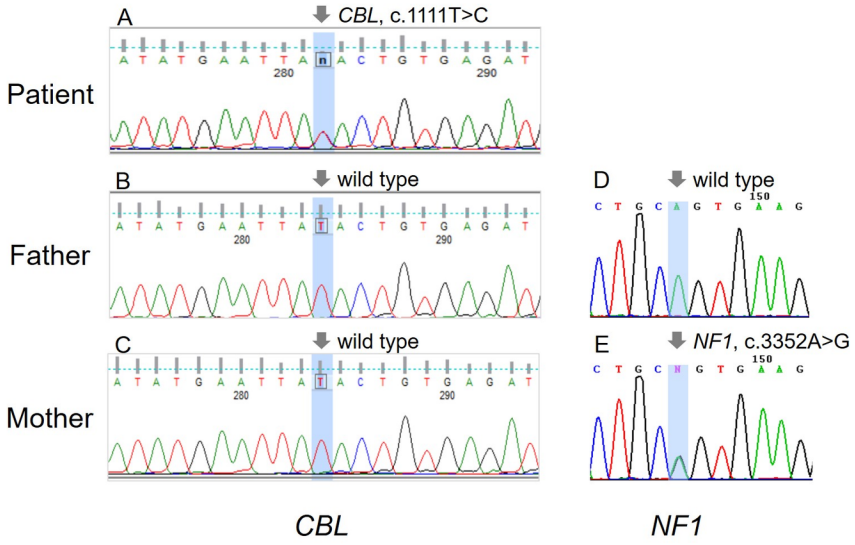
14. Cortellazzo Wiel L, Pastore S, Taddio A, Tommasini A. A Case of Uveitis in a Patient With Juvenile Myelomonocytic Leukemia Successfully Treated With Adalimumab. *J Pediatr Hematol Oncol.* 2019.

15. Chang TY, Dvorak CC, Loh ML. Bedside to bench in juvenile myelomonocytic leukemia: insights into leukemogenesis from a rare pediatric leukemia. *Blood.* 2014;124(16):2487-2497.

16. Tung N, Lin NU, Kidd J, et al. Frequency of Germline Mutations in 25 Cancer Susceptibility Genes in a Sequential Series of Patients With Breast Cancer. *J Clin Oncol.* 2016;34(13):1460-1468.

Figure Legends

Figure 1. Direct sequencing of *CBL* and *NF1* for this case and his parents. (A): Sequencing result of the DNA from this patient's buccal smear cells, revealing a heterozygous mutation of *CBL*(c.1111T>C; p.Tyr371His). (B) and (C): Sequencing results of *CBL* from the DNA from paternal and maternal peripheral blood mononuclear cells (PBMC), respectively; both wild type. (D) and (E): Sequencing results of *NF1* from the DNA from paternal and maternal PBMC, respectively; identifying a heterozygous *NF1* variable site (c.3352A>G; p.Ser1118Gly) in maternal PBMC.



Hosted file

`Table 1.docx` available at https://authorea.com/users/325898/articles/453890-a-child-with-juvenile-myelomonocytic-leukemia-possessing-a-concurrent-germline-cbl-mutation-and-a-nf1-variant-of-uncertain-significance-a-rare-case-with-a-common-problem-in-the-era-of-high-throughput-sequencing