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Archivio della ricerca – postprint

The Challenge of Next Generation Sequencing in a Boy With Severe Mononucleosis and EBV-related Lymphoma

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Summary: A severe course of infectious mononucleosis should always lead up to the suspicion of a primary immunodeficiency. We describe the case of a boy with severe mononucleosis accompanied by the development of hemophagocytic lymphohistiocytosis and lymphoma. By whole exome sequencing, we identified a mutation of uncertain significance in *CTPS2*, a gene closely related to *CTPS1*, which is involved in a primary immune deficiency with susceptibility to herpesviruses. We discuss the challenge of a correct interpretation of data from whole exome sequencing, questioning whether the *CTPS2* variant found in our patient is just an incidental finding or a mutation with variable penetrance.

Key Words: Epstein Barr virus, lymphoma, hemophagocytic lymphohistiocytosis, whole exome sequencing, *CTPS2*

Infectious mononucleosis is a common disorder caused by Epstein Barr virus (EBV), usually characterized by a benign, often unnoticed course.[1] However, EBV infection can present a severe course in subjects with primary immunodeficiency diseases (PIDs), with acquired immunodeficiency syndrome or malnutrition.[2,3] Moreover, several PIDs may present with quite selective susceptibility to EBV infection in otherwise healthy people, as reviewed elsewhere.[2] We present a boy who developed an EBV infection complicated with hemophagocytic lymphohistiocytosis and B-cell lymphoma and discussed the challenging results of exome sequencing performed to detect genetic factors associated with disease susceptibility.

CASE REPORT

We report the case of a 15 year-old boy who presented to the pediatric emergency room because of inability to feed, swallowing pain, fever (> 39°C) and weight loss (−7 kg, starting from 78 kg). Past medical history was unremarkable except for recurrent otitis media and nocturnal apneas leading to adenoidectomy and tonsillectomy at 5 years of age, and recurrent herpes labialis infections. There was no familial history of severe infections or cancers. At medical examination, the boy was pale and in poor general condition. He presented pharyngitis, cervical lymphadenopathy, and palpable spleen. Laboratory examinations showed mild thrombocytopenia, raised liver enzymes (aspartate aminotransferase 256 U/L, alanine aminotransferase 361 U/L, gamma-glutamyl transferase 210 U/L) and slightly increased acute phase reactants (C-reactive protein 32.5 mg/L, erythrocyte sedimentation rate 40 mm/1st hour). A positive test for serum viral capsid antigen-immunoglobulinM and IgG allowed diagnosing a primary EBV infection. The boy was therefore started on prednisone (50 mg/d). Nevertheless, after a few days without any benefit, a pulmonary involvement become evident (crackles on right pulmonary apex, x-ray findings of right upper lobe opacity and ipsilateral hilum enlargement). Ultrasound and computed tomographic scans showed multiple bilateral cervical and supraclavicular enlarged lymph nodes, hepatic, and spleen enlargement, as well as the presence of an anterior mediastinal mass (50×65 mm) in the preaortic region and a pulmonary lesion of 25 mm in the right middle lobe, associated with atelectasis of the right upper lobe (Fig. 1). Bone marrow aspirate cytology showed hemophagocytosis.

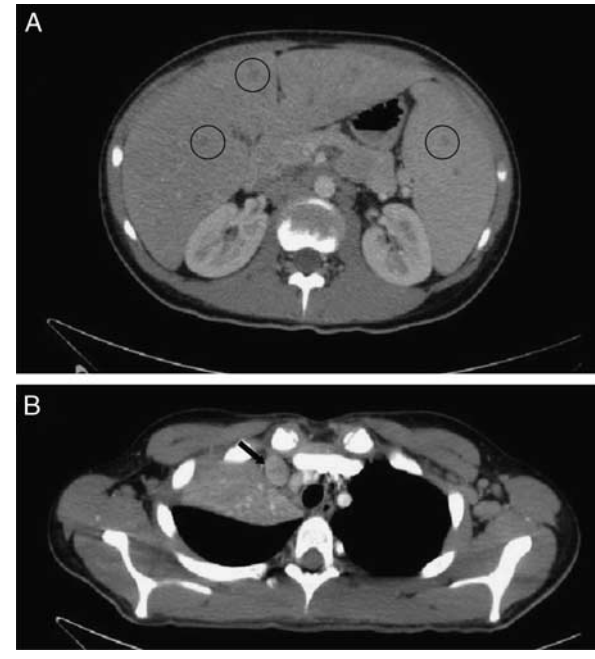

FIGURE 1. A, Multiple nodular lesions in liver and spleen on CT scan. B, Anterior mediastinal mass in the periaortic region with atelectasis of the right upper pulmonary lobe on CT scan. Anterior mediastinal mass in the periaortic region with atelectasis of the right upper pulmonary lobe on CT scan. CT indicates computed tomography.

Accepted June 13, 2017. From the Departments of *Pediatrics; †Advanced Diagnostic and Clinical Trials, Institute for Maternal and Child Health, IRCCS Burlo Garofolo; and ‡Department of Medical Surgical and Health Sciences, University of Trieste, Trieste, Italy.
F.V. and E.V. are co-first authors.
Supported by the "IRCCS Burlo Garofolo," the "Associazione Azzurra Malattie Rare" and the "Beneficientia Stiftung in Vaduz," grant #17/14.
The patient parents gave their informed consent for anonymized publication of data about their son.
The authors declare no conflict of interest.
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Given the persistence of fever and splenomegaly, together with hyperferritinemia (13,412 ng/mL) and cytopenia (platelets 97,000/μL, neutrophils 940/μL), hemophagocytic lymphohistiocytosis was diagnosed. Liver, nodal, and marrow histologic findings were diagnostic for diffuse large B-cell lymphoma, EBV-related. EBV-encoded RNA (EBER) was detected by in situ hybridization in the nuclei of malignant cells. No abnormalities were found in cerebrospinal fluid. High-titer EBV positivity (2×10^5/μL copies) was found on both marrow and peripheral blood samples by polymerase chain reaction. Despite glucocorticoids, high-dose intravenous immunoglobulins and broad spectrum prophylactic antibiotics, clinical symptoms worsened requiring oxygen support and blood and plasma transfusions. The patient was enrolled in the ongoing protocol for diffuse large B-cell lymphoma, stage 4, central nervous system negative. Accordingly, treatment was started with combined chemotherapy (prephase with vincristine, cyclophosphamide, prednisolone, then 2 cyclophosphamide, vincristine, prednisone, doxorubicin and high-dose methotrexate cycles, 2 cytarabine, etoposide cycles, 1 maintenance cycle with vicristine, high-dose methotrexate, cyclophosphamide, doxorubicin, prednisolone). After an initial lack of response to the treatment, a progressive reduction in the number and size of lesions was observed, with complete clinical response at the end of first-line protocol. After suspension of treatment the disease relapsed with a mediastinal paratracheal mass with subtotal tracheal compression and other disease localizations.

The patient therefore underwent a second-line treatment (cycle R-ICE: rituximab, ifosfamide, carboplatin, etoposide) that resulted in complete remission of disease, with residual permanent bone marrow aplasia requiring transfusion support.

Multiple infectious complications occurred, such as *Stenotrophomonas maltophilia* sinusitis, *Staphylococcus epidermidis*, and *Candida albicans* bloodstream infections, thoracic *Herpes zoster* and pulmonary aspergillosis, requiring prolonged hospitalization. Finally, given the severity of the lymphoma, the suspicion of an underlying primary immune deficiency and the bone marrow aplasia the boy underwent allogeneic bone marrow transplant from an unrelated donor. At last follow-up, 240 days after transplantation, he was in complete remission.

Immunologic and Genetic Analysis

Because of the clinical history, there was a strong suspicion of a PID, but natural killer degranulation assay, expression of perforin, SAP and XIAP were normal. X-linked lymphoproliferative disease was also excluded by sequencing *SH2D1A* and *XIAP* genes. Immunophenotyping, showed a severely reduced percentage of recent thymic emigrants (CD3+ CD4+ CD45RA+ CD31+ cells, RTE, Fig. 2A), but these data could be influenced by the large dose of glucocorticoids administered to the boy. Expression of activation antigens (CD25 and CD69) on T lymphocytes, after 5 μg/mL phytohemagglutinin treatment of peripheral blood mononuclear cell, was partially reduced (Fig. 2B). The percentage of RTE in the maternal grandfather and in the brother, bearing the same *CTPS2* mutation as the patient, was not significantly reduced (Table 1).

Considering the clinical severity and the potential implication of a bone marrow transplantation, a whole exome sequencing (WES) analysis with a trio experiment (proband and parents) was performed. We used the Ion Proton technology to perform the WES analysis (Life Technologies, CA) with an average base coverage depth ranging from 96.24 to 90.48 and a target base coverage at 20× ranging from 90.23% to 86.58% in the 3 samples. Initially 33 genes associated to susceptibility to EBV were analyzed (*AKT1*, *BCL10*, *BCL6*, *CARD11*, *CD27*, *CD27L*, *CORO1A*, *CTLA4*, *CTPS1*, *TNFRSF6*, *FCGR3A*, *GATA2*, *ITK*, *KLHDC8B*, *LRBA*, *MAGT1*, *MALT1*, *MCM4*, *NLRC4*, *PIK3CD*, *PIK3R1*, *PRF1*, *PRKCD*, *RMRP*, *SH2D1A*, *STAT3*, *STK4*, *STX11*, *STXBP2*, *UNC13D*, *WAS*, *BIRC4*). Despite the high coverage (only 0.75% of candidate genes-targeted bases have not been covered at all) the analysis performed detected no possible causative mutations. A subsequent analysis looking for all the possible pattern of inheritance: (1) dominant de novo; (2) autosomal recessive; and (3) X-linked recessive was started. Despite no de novo or recessive possible mutations were found, the c.C1108T; p.P370S (NM_001144002) variant in the *CTPS2* gene on the X-chromosome was detected. This variant has never been found in 1000 g or ESP6500 databases and has once been reported in a hemizygous state in 86674 alleles in Exac database. Moreover, it affects a conserved site (GERP++ 5,18) and all the consulted webtools (Mutation Taster, PolyPhen2, SIFT, PROVEAN) predicted this variant as pathogenic. The study of family segregation showed a heterozygous healthy mother and hemizygous maternal grandfather and brother, who never developed severe infections with herpetic viruses. Serologic investigation showed that both subjects had developed memory antibodies against EBV. The maternal X-chromosome inactivation (XCI) performed in peripheral blood lymphocytes, as already described,[4] and in selected RTE population isolated using CD4+ recent thymic emigrant isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) showed a random inactivation (Fig. 3).

DISCUSSION

The notion that in predisposed individuals EBV infection can evolve with severe outcomes dates to 40 years ago, when Purtilo et al[5] described the different outcomes of EBV infection in X-linked lymphoproliferative disease, with dysgammaglobulinemia, fatal infectious mononucleosis, lymphohistiocytosis, and lymphoma. Since then, several different PIDs, mostly involving defects in T cells or natural killer cytotoxicity, have been associated with susceptibility to EBV. Moreover, the use of next generation sequencing techniques is expanding the list.[2,3,6] Thus, children with complicated EBV infection deserve an extensive immunologic and genetic evaluation, as prompt detection of a PID can allow a better cure and a proper familial counseling. To accomplish the tasks of a prompt diagnosis, next generation sequencing panels have been proposed, which can allow the simultaneous examination of several genes.[3,7]

The clinical picture of our patient was suggestive of a PID, as he developed within a few weeks an infectious mononucleosis, hemophagocytic lymphohistiocytosis, and an EBV-associated B-cell lymphoma. Therefore, we urged to search for a genetic immune defect. However, due to the high number of potential genes involved and the lack of a suitable sample for immunologic analyses, we decided to perform WES soon after ruling out the more common EBV-associated PIDs.

We found a possibly pathogenic variant in C*TPS2*, encoding the cytidine 5-prime triphosphate synthase 2 that catalyzes the formation of CTP from UTP with the concomitant deamination of glutamine to glutamate. *CTPS2* is closely related to *CTPS1* gene, encoding the cytidine 5-prime triphosphate synthase 1, and responsible for the catalytic conversion of UTP to CTP. Interestingly, the *CTPS1* gene has a central role in lymphocyte proliferation and biallelic mutations in this gene are responsible for an autosomal recessive immunodeficiency characterized by impaired capacity of activated T and B cells to proliferate in response to antigen-driven activation.[6] Patients affected by the CTPS1 deficiency (Immunodeficiency 24, MIM #615897) have an early onset of severe chronic viral infections, mostly caused by herpesviruses, including EBV and varicella zoster virus. Furthermore, immune analysis in some patients with CTPS1 deficiency showed a normal CD4:CD8 ratio, also during infections and we note these unusual data in our patient.

Unfortunately, we could not obtain peripheral blood samples suitable to conduct extensive functional studies, such as antigen-driven proliferation assays, as the patient needed prompt chemotherapy and subsequently hematopoietic stem cell transplantation.

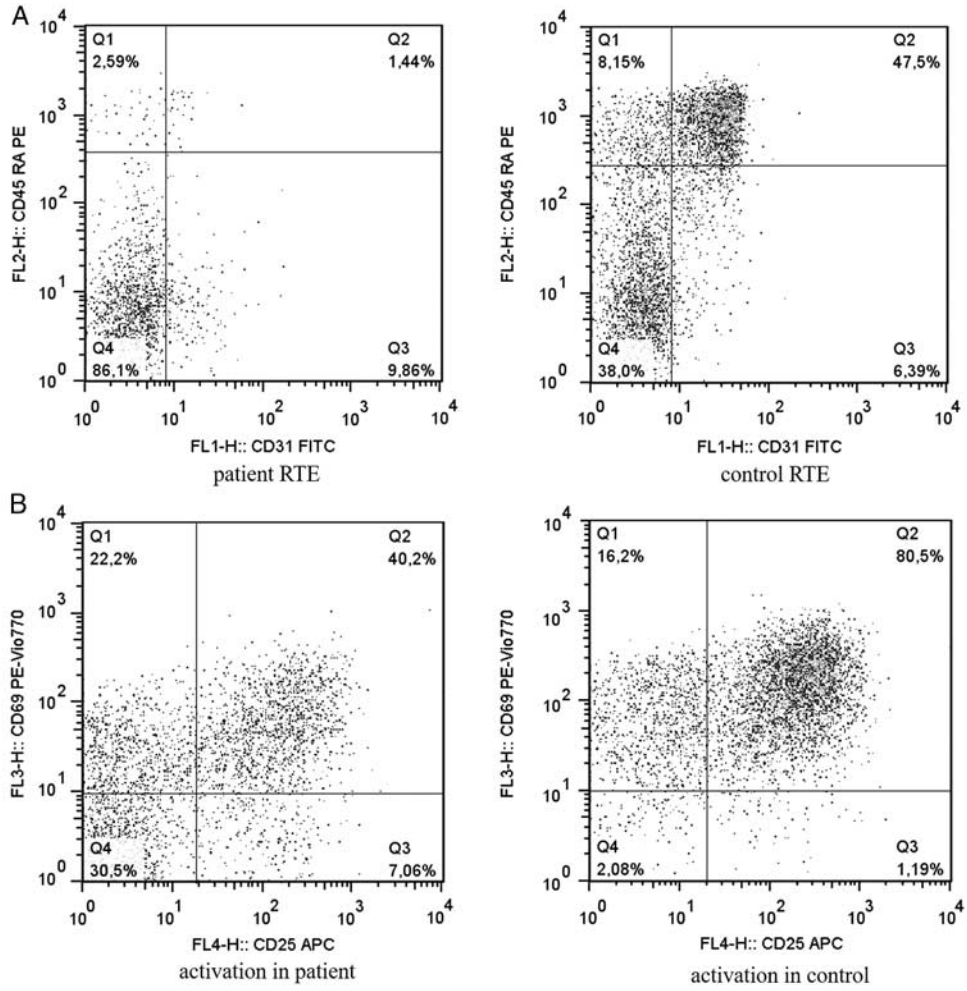

FIGURE 2. A, Analysis of RTE in the patient and in a healthy control. RTE are identified by the presence of CD45RA and CD31 on CD4 T cells (upper right quadrant). B, Expression of activation markers on peripheral blood T lymphocytes after stimulation with 5 μg/mL phytohemagglutinin. Completely activated cells, positive both for CD69 and CD25 are displayed in the upper right quadrant. RTE indicates recent thymic emigrant.

Thus, the *CTPS2* variant that we found in our patient remains of uncertain significance. In particular, the fact that both the grandfather and the brother encountered EBV without developing severe consequences may be concerning. It can suggest either that the *CTPS2* variant is actually innocent, or that it is a variant associated with a nonmendelian risk of disease.

In conclusion, although our data are not conclusive, they support the possible association between CTPS2 deficiency and susceptibility to herpesviruses. On the basis of the recent scientific literature and our experience, *CTPS2* might be introduced in panels for EBV-related PIDs to find similar cases to define the role of this gene in the response to EBV.

TABLE 1. Immunophenotypic Analysis With RTE Values in the Patient, His Grandfather, and Brother

Lymphocyte Subsets	Patient	Grandfather	Brother	Normal Values
T cells (CD45++CD3+) % of lymphocytes-cells/μL	62%-310	64.8%-907.2	64.2%-1630	59-83%-780-3000
Helper T cells (CD45++CD3+CD4+) % of T cells-cells/μL	51%-158.1	81.3%-737.5	56.4%-919	31-59%-500-2000
Suppressor T cells (CD45++CD3+CD8+) % of T cells-cells/μL	39%-120.9	ND-ND	ND-ND	12-39%-200-1200
Recent thymic emigrants (CD45++CD3+CD4+CD45RA+CD31+) % of helper T cells-cells/μL	1.4%-2.2	14%-103.2	21.6%-198.6	6.4-51%-50-2400
Double negative T cells (CD45++CD3+CD4-CD8-TCRa/b+) % of T cells-cells/μL	0.56%-1.7	ND-ND	ND-ND	< 2.5%- < 23
NK cells (CD45++CD3-CD16/56+) % of lymphocytes-cells/μL	18.5%-92.5	ND-ND	ND-ND	6-27%-100-1200
B cells (CD45++CD19+) % of lymphocytes-cells/μL	11%-55	ND-ND	ND-ND	2.8-17.4%-64-820

NK indicates natural killer; RTE, recent thymic emigrant.

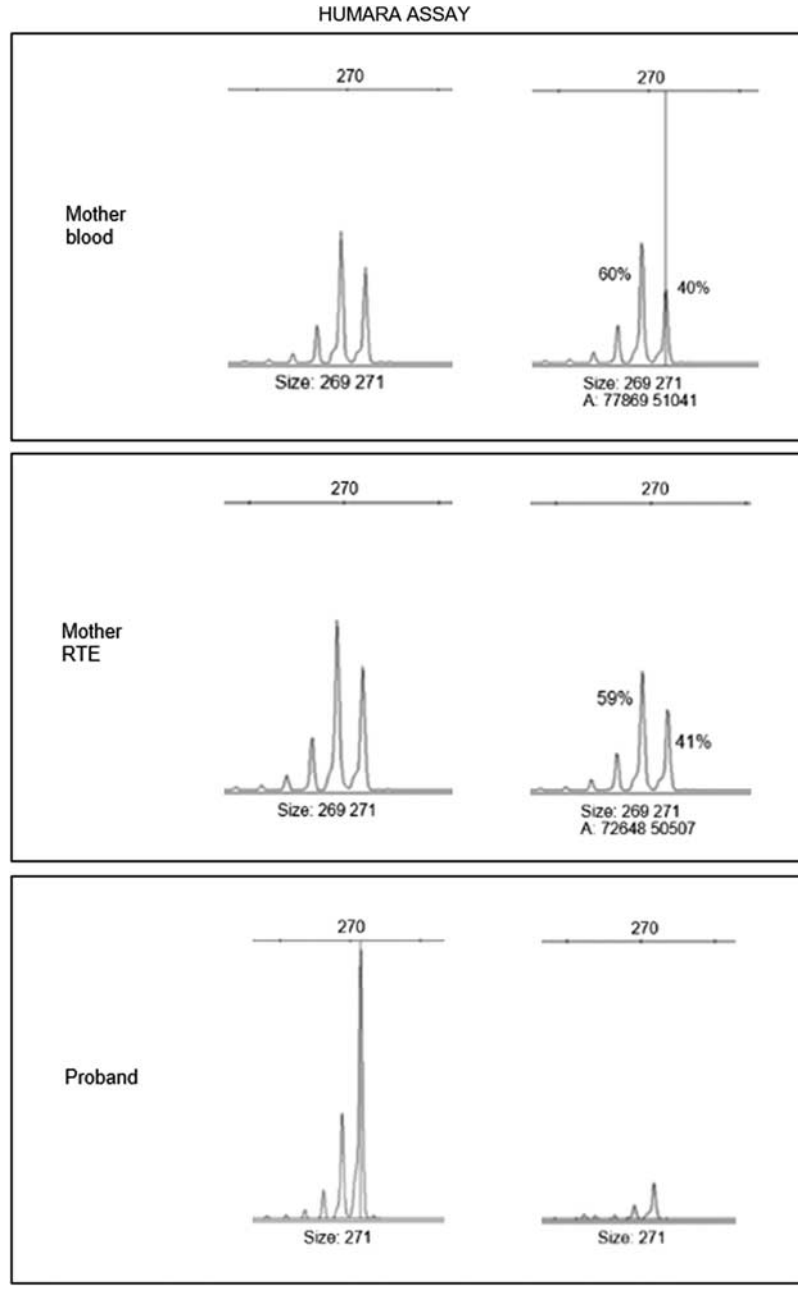

FIGURE 3. Analysis of the Humara polymorphic marker in genomic DNA (from mother whole blood cells and mother RTE cells) digested with HpaII and in DNA digested with RsaI. The graphs suggest a balanced X inactivation in both total lymphocytes and in RTE. RTE indicates recent thymic emigrant.

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