Dysregulated immune system secondary to novel heterozygous mutation of CIITA gene presenting with recurrent infections and Systemic lupus erythematosus

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Abstract

The expression of Major Histocompatibility Complex (MHC) molecule is essential for homeostasis of the immune system. Tissue-specific expression of MHC-II is regulated at the level of transcription. The master regulator for transcription of the MHC-II gene is CIITA. Homozygous mutations affecting the CIITA gene results in bare lymphocyte syndrome type-II. The clinical manifestations of heterozygous mutations are not well reported. Hence, this case report aims to provide more insight into the clinical features associated with heterozygous mutations of CIITA. We report a 5-year-old child who had presented with recurrent infections in infancy and systemic lupus erythematosus (SLE) in toddler age.

Tables: Nil

Figures: 2

Abbreviations	
MHC	*Major Histocompatibility Complex*
SLE	*Systemic lupus erythematosus*
ELISA	*Enzyme Linked Immunosorbant Assay*
HIV	*Human Immunodeficiency Virus*
CVID	*Common Variable Immunodeficiency*
EULAR/ACR	*European League Against Rheumatism/American College of Rheumatology*
HLA	*Human Leukocyte Antigen*
NGS	*Next Generation Sequencing*
BLS	*Bare Lymphocyte Syndrome*
ANA	*Antinuclear Antibody*

Abstract

The expression of Major Histocompatibility Complex (MHC) molecule is essential for homeostasis of the immune system. Tissue-specific expression of MHC-II is regulated at the level of transcription. The master regulator for transcription of the MHC-II gene is CIITA. Homozygous mutations affecting the CIITA gene results in bare lymphocyte syndrome type-II[1]. The clinical manifestations of heterozygous mutations are not

well reported. Hence, this case report aims to provide more insight into the clinical features associated with heterozygous mutations of CIITA. We report a 5-year-old child who had presented with recurrent infections in infancy and systemic lupus erythematosus (SLE) in toddler age.

Case Description

Our index child was a sixth-order child born to a third-degree consanguineous couple with a birth weight of 3 kg. Two female siblings succumbed to severe sepsis and meningitis before 1 year of age. A male sibling had a history of recurrent episodes of severe anemia requiring blood transfusion since the age of 18 months and finally succumbed by 2 years of age. The index child presented to us at 15 months of age with a stormy infantile period with recurrent episodes of lower respiratory tract infection, persistent diarrhea and failure to thrive since 9 months of age. None of the signature organisms pertaining to immunodeficiency could be isolated during the evaluation of these episodes. There was no organomegaly, lymphopenia, thrombocytopenia or neutropenia. As part of workup for failure to thrive, a duodenal biopsy was performed through upper gastrointestinal endoscopy, which revealed features consistent with eosinophilic enteritis. The colonic biopsy also revealed similar results. Evaluation for immunodeficiency disorders was performed at 18 months of age. Lymphocyte subset analysis by flow cytometry revealed: absolute lymphocyte count- 2700/μL, helper T cells-12% (322/μL), cytotoxic T cells-43% (1154μL), B cells-7% (188/μL), NK cells-14% (376/μL). Serum immunoglobulin evaluation by nephelometry showed: IgG-18.6 g/L, IgM- 1.01g/L, IgA- <0.246g/L, IgE- <4.4 IU/mL. Thus, the helper T cell count and serum immunoglobulin A levels were reduced for his age. Human immunodeficiency virus (HIV) ELISA was negative. A possibility of combined immunodeficiency or Common Variable Immunodeficiency (CVID) was considered. However, further evaluation for immunodeficiency could not be performed as the child was lost to follow-up.

The child presented again at four years of age with severe pallor (hemoglobin 3.6 gm/L) and congestive cardiac failure. There was significant failure to thrive and hepatosplenomegaly. His mother reported intermitted episodes of persistent diarrhea and bronchopneumonia in the last three years, which were managed conservatively by his primary care physician. There were features suggestive of intravascular hemolysis (spherocytes in peripheral smear, hemoglobinuria, elevated reticulocyte count and indirect hyperbilirubinemia). Direct Coomb's test was positive for IgG. Evans syndrome was initially diagnosed in view of severe thrombocytopenia (9000/μL) and autoimmune hemolytic anemia. The child also had stage-I hypertension and proteinuria (1+) with low serum C3 levels (42 mg/dL). Hence, a possibility of SLE was entertained and worked up. Serum antinuclear antibody (ANA) and anti-double-stranded DNA (dsDNA) were positive (4+). However, the serum albumin (3.3g/dL), serum cholesterol (122 mg/dL), serum creatinine (0.53 mg/dL) and blood urea (39 mg/dL) were within normal limits. Renal biopsy (done in view of hypertension with sub-nephrotic proteinuria) showed evidence of class-II lupus nephritis. He did not have other systemic manifestations of SLE i.e cutaneous domain, arthritis, neurological domain, serositis domain, antiphospholipid antibody domains were all negative. The absence of cutaneous and neurological manifestations excluded complement deficiency-induced lupus as our differential diagnosis. EULAR/ACR 2019 criteria to diagnose systemic lupus erythematosus (SLE) were satisfied (score=25). The child was treated with intravenous methylprednisolone pulse (30mg/kg/dose) for 3 days followed by oral prednisolone (2mg/kg/day) with oral mycophenolate mofetil (925mg/m^2) for 4 weeks. Laboratory evaluation (complete hemogram and urine analysis) after 4 weeks confirmed that the child was under remission. The dose of prednisolone was tapered gradually and mycophenolate mofetil was continued with oral hydroxychloroquine.

During the third month of the steroid taper, there was a hematological flare of SLE (hemoglobin- 3.3gm/L). Renal function tests and urine analysis did not reveal any features suggestive of active nephritis. The anti-ds DNA antibodies were negative. Treatment was initiated with intravenous methylprednisolone pulses for 3 days and 4 weekly doses of injection Rituximab (350mg/m^2). Tapering dose of oral prednisolone, mycophenolate mofetil, and hydroxychloroquine were continued for the next 6 months. He was in clinical remission status during this period.

Since the index child had presented with features of combined immunodeficiency disorder initially and features of SLE later, further evaluation was done to identify the immunodeficiency disorder. Flow cytometric

evaluation for Human Leucocyte Antigen (HLA)-DR expression on monocytes was performed. 87% of the monocytes expressed HLA-DR antigen (Fig. 1A and 1B). Whole-exome sequencing by Next Generation Sequencing (NGS) was performed using the Illumina sequencing platform. The sequences obtained were aligned to the human reference genome (GRCh37/hg19) using the Sentieon aligner and analyzed. Sentieon-haplotype-caller has been used to identify variants that were relevant to the clinical indication. Gene annotation of the variants was performed using VEP program against the Ensemble release 91 human gene model. The NGS testing revealed a **heterozygous missense variation in exon 13 of the CIITA gene (chr16:g.11004088G>C; Depth:73x)** resulting in the amino acid substitution of Leucine for Valine at codon 94 (p.val954Leu; ENST00000324288.8). The child was initiated on monthly Intravenous immunoglobulin (IVIG) replacement therapy, daily cotrimoxazole and acyclovir prophylaxis for the past one year as a bridge till stem cell transplantation is performed.

Discussion

Four transacting genes control and coordinate the MHC-II expression- CIITA, RFX5, RFXANK, RFXAP. Homozygous mutations in any of these four genes result in Bare Lymphocyte Syndrome type-II, a rare genetic disorder characterized by a lack of expression of MHC-II antigens[2]. However, manifestations of heterozygous mutations of these genes have not been well described.

Our case adds insight to the clinical manifestations of heterozygous CIITA gene mutation. This child had most of the features of MHC-II deficiency- failure to thrive, recurrent respiratory tract infections, protracted diarrhea, hypogammaglobulinemia, and CD4 lymphopenia. There have been reports of cases with recurrent infections secondary to heterozygous mutation of one of the four transacting regulators of MHC-II expression[3,4]. All of these cases lacked expression of HLA-DR on monocytes. These cases highlight the quantitative defect of MHC-II protein due to CIITA mutation. Additionally, CIITA gene is also responsible for the qualitative function of MHC-II[5]. A single amino acid deletion has been reported to be sufficient to abolish the activity of CIITA in vivo[6]. The HLA-DR expression was normal in our patient, which confirms the quantitative presence of MHC-II. Thus, we hypothesize these immunodeficiency manifestations in our index child are secondary to CIITA mutation, causing functionally defective MHC-II protein. However, due to resource limitations, we could not perform an IFN[?] stimulated HLA-DR expression, which was a limitation in our case.

Autoimmune cytopenias have been described in cases with heterozygous mutations of CIITA, but features of SLE have not been reported so far[7]. The presence of MHC-II is paramount for central tolerance. Three mechanisms have been described to silence developing auto-reactive cells at the first checkpoint in bone marrow - deletion, anergy, and receptor editing. Defective MHC function leads to poor presentation of tissue-restricted antigens to medullary thymic epithelial cells. This leads to the failure of negative selection of self-reactive T-cells[8]. The CD4 T cells that have escaped the normal selection process have been observed to be present in patients with BLS. Alternate unusual selection mechanisms would have been more prominent in these patients, thereby leading to defective anergy and central tolerance. All these factors together could have triggered SLE in this patient[9]. This has been emphasized by the fact that CIITA mutation has been identified as one of the susceptible genes for SLE by genome-wide association studies[10].

Most children with homozygous mutation of the CIITA gene succumbed to underlying infection by 5 years of age without bone marrow transplantation[2]. Since our index child managed to survive till 5 years without bone marrow transplant, we hypothesize that heterozygous mutations, though significant to cause immunodeficiency, maybe less lethal.

Conclusion

Homozygous mutations of CIITA have been well known to cause primary immunodeficiency. But heterozygous mutation causing clinically significant immunodeficiency is less reported. In this case, a novel presentation of primary immunodeficiency with autoimmunity adds a new facet to existing literature.

Conflict of interest: The authors declare that they have no conflict of interest.

Legends for figures

Figure 1A- Peripheral flow cytometry -CD14 gated monocytes- 87% express HLA-DR

1B- Peripheral flow cytometry - CD64 gated monocytes- 84% express HLA-DR

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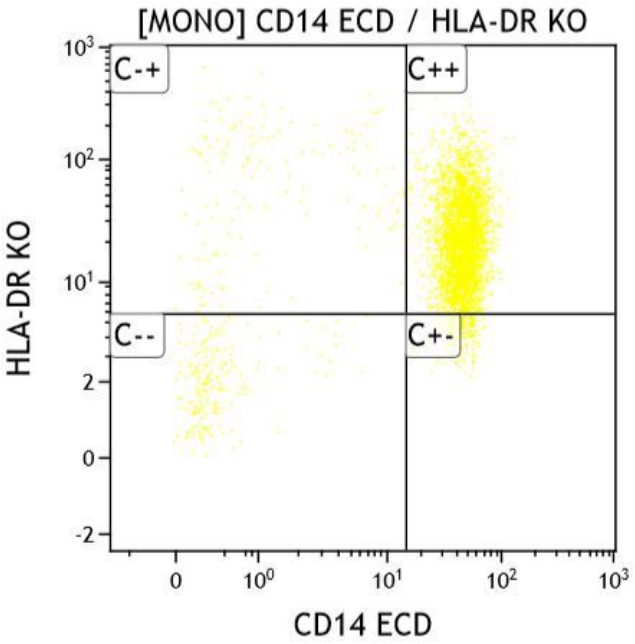
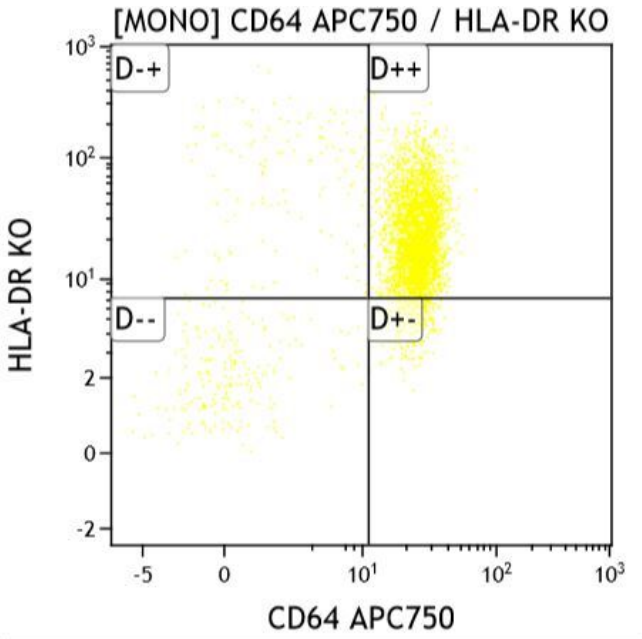


Gate	Number	%Total	%Gated
All	4,411	4.4110	100.0000
C--	198	0.1980	4.4888
C-+	144	0.1440	3.2646
C+-	200	0.2000	4.5341
C++	3,869	3.8690	87.7125



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D--	196	0.1960	4.4434
D-+	128	0.1280	2.9018
D+-	370	0.3700	8.3881
D++	3,717	3.7170	84.2666