

Received Date : 02-Nov-2016

Revised Date : 18-Nov-2016

Accepted Date : 25-Nov-2016

Article type : Letter to the Editor

To the Editor:

Late-onset combined immune-deficiency due to LIGIV mutations in a 12 years old patient

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/pai.12684

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Key words: Atypical DNA ligase IV deficiency, Radiosensitivity, Immunodeficiency, Normal development

The non-homologous end joining (NHEJ) pathway is involved in the repair of the DNA double-strand breaks. Defects in NHEJ factors result in IR sensitivity, and in defects in V(D)J recombination leading to immunodeficiency. DNA Ligase IV is an essential component that performs the final 'end processing' step of NHEJ. Mutations in DNA Ligase IV was initially reported in a 14 years old-boy who exhibited severe radiosensitivity during treatment for leukemia [1,2]. Subsequently, *LIGIV* mutations were identified in patients with growth and/or mental retardation, microcephaly, pancytopenia, increased predisposition to leukaemia and variable degrees of immunodeficiency, as well as in early-onset SCID without overt developmental delays [3,4].

More recently, studies have broadened the phenotypic variability associated with Ligase IV mutations, including adult patients with mild clinical presentation and clinically asymptomatic subjects [5,6]. We report a developmentally normal 12 years old male patient with two heterozygous mutations in *LIG IV* gene, who presented initially with prevalent humoral immune dysfunction and low B cells, followed by evolution into a Combined Immunodeficiency (CID).

A six-year-old caucasian boy, born at term from non-consanguineous parents, was referred to our Unit of Immunology and Infectious Disease of a tertiary care Hospital for a recent history of relapsing pneumonia with pleural effusion in the last three months and dysgammaglobulinemia.

Since 1 year of age the child had suffered from recurrent upper respiratory tract infections, mainly otitis, for which at 5 years old he underwent adenoidectomy with bilateral transtympanic drainage.

Immunological investigations confirmed dysgammaglobulinemia with absent IgA and IgM and normal value of IgG. Evaluation of antibody response to a range of antigens was analyzed and the results indicated a lack of IgG responsiveness to pneumococcus, tetanus toxoid, measles, mumps and rubella for which he had been immunized as well as absent titers of isohemoagglutinins. In addition, he showed incomplete seroconversion against EBV (IgG anti-VCA present, IgM anti-VCA absent, IgG anti-EBNA absent). Flow cytometry analysis on his peripheral blood mononuclear cells (PBMC) revealed mild decreased number of T cells and reduced B cells. In particular memory B cells were reduced with poor response to CpG stimulation and exclusive production of IgM (data not shown).

Other investigations including autoimmunity, complement tests, total IgE, alpha-fetoprotein, sweat test and nasal brushing were all negative.

A chest CT scan revealed lingular bronchiectasis and normal thymus. Pulmonary function tests did not show abnormality. Considering the complex immunologic phenotype, regular intravenous immunoglobulin replacement therapy was started. Sanger sequencing was performed for BTK, XLP, ICOS, TACI, RAG1 and RAG2 and no mutations were identified. Furthermore Western Blot analysis demonstrated normal expression of BTK, SYK, LYN and BLNK.

During the follow up the patient's clinical condition remains stable on regular IVIG infusion with nor recurrence of pneumonia or other severe infections, however a progressive worsening of lymphocytes counts was observed. Further investigations showed progressive reduction of CD3⁺ T cells , mainly CD4⁺ in which naive cells were nearly absent. In vitro T-cell proliferation to PHA and anti OKT3 were slightly decreased. Evaluation of thymic output by TRECC analysis on CD4⁺ and CD8⁺ resulted undetectable and the spectratyping analysis for TCRV β of the CDR3 region revealed oligoclonal expansion in the most of peripheral CD8⁺ and CD4⁺ T cells (Tab.1). HLA typing of the patient and his mother revealed no evidence of maternal engraftment. In the suspect of a double strands break repair defect, radiosensitivity test was performed, showing an increased number of chromatid aberrations induced in EBV transformed B lymphoblastoid cell lines (LCSs) exposed to x-rays (Figure 1).

The increase of radiosensitivity detected in fibroblast, confirmed previously result (data not shown)

Sanger sequencing was performed, and it revealed two compound heterozygous mutations in the DNA Ligase IV gene, previously described: c.1271_1275delAAAGA p.Lys424fsX20 and c.833G>A p.R278H. DNA analysis of both parents and a healthy brother demonstrated that the c.833G>A p.R278H mutation had ben inherited from the mother, and the c.1271_1275delAAAGA p.Lys424fsX20 mutation from the father. The two brother were found to be heterozygous for the mutations (Figure 2). Homozygosity for the p.R278H mutation has been already reported in two *LIG4* deficient patients with distinct phenotype, since one developed leukemia, while the second patients exhibited a developmental delay and mild immunodeficiency. This mutation affects the catalytic domain and is contiguous to the highly conserved ligase motif (aa 273-278) leading to a dramatically reduction of the in vitro ligase activity and protein expression [2,3].

The p.Lys424fsX20 was reported in two patients with profound lymphocytopenia and microcephaly, leads to a deletion within the N-terminal 650 aa of *LIG4* abolishing its catalytic activity, the 5-bp deletion leads to a putative truncated non-functional protein lacking the two BRCT domains and the XRCC4 interaction domain [4]. The *LIGIV* syndrome is an autosomal recessive genetic disorder caused by hypomorphic mutations in *LIGIV* gene. Complete absence of *LIGIV* activity leads to embryonic death due to impaired neuronal development [9].

Of note, the phenotype of *LIGIV* deficiency correlates with the mutation site thus, clinical and immunological manifestations of the disease are variable ranging from leukemia to radiosensitive severe combined immunodeficiency [1]. We report a case with a combination of clinical and immunological data, which were not related to a common *LIGIV* syndrome and at date only two case of “developmental normal” patients to be reported [7,8].

Normally patients with *LIGIV* deficiency present clinical phenotypes resembling the one of Nijmegen breakage syndrome patients and includes microcephaly, developmental delay, short stature, pancytopenia, severe cellular radiosensitivity with variable degree of combined immunodeficiency phenotype. Increased risk of malignances and chromosomal instability are also reported. [1,3,4, 10].

Based on the dysgammaglobulinemia with absent IgA and IgM, lack of IgG responsiveness to several antigens and reduced B cells, we first hypothesize a B cell defects.

Later revaluation of T compartment due to the progressive worsening of naïve CD4 T cells prompt has to consider the patient’s atypical presentation of the disease and to extend the clinical variability associated with *LIGIV* mutations.

ACKNOWLEDGEMENTS

The authors thank Ospedale Pediatrico Bambino Gesù staff for their contribution to this study; the patient and his family.

CONFLICT OF INTEREST

All authors declare no conflict of interest

FUNDING

This work was supported by: the European Commission - Advanced Cell-based Therapies for the treatment of Primary Immuno-Deficiency (HEALTH-F5-2010-261387, CELL-PID) and Ospedale Pediatrico Bambino Gesù Ricerca Corrente (201602P003708).

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Table 1. Haematological and Immunological profile of our patient.

Parameter	Patient's results (age 7 y)	Patient's results (age 9 y)	Reference value
Complete blood count			
White blood cell (10^3 /uL)	3,8	3,84	4.00 - 13.50
Red blood cells (10^6 /uL)	4,7	4,24	3.80 - 4.80
Neutrophils (10^3 /uL)	2,1	1,99	1.32 - 7.97
Lymphocyte (10^3 /uL)	1,4	1,34	1.04 - 6.48
Platelets (10^3 /uL)	258	223	150 - 450
Hemoglobin (g/dL)	13,5	12,3	13- 16
Lymphocytes (%)			
CD3+	39	42,5	60-76
CD4+	15	16,1	31-43
CD8+	21	20,9	18-35
CD4+CD45RA	nd	1,0	24-53
CD4+CD45RO	nd	15,1	12-53
CD8+CD45RA	nd	9,2	15-35
CD8+CD45RO	nd	11,7	0,4-21
TCR CD3+ α/β	nd	75	44-92
TCR CD3+ γ/δ	2,5	15	2-24
CD19+ Pan B	7	2,7	13-27
CD19+CD27- (naïve)	94,6	87,7	62-94
CD19+CD27+ (memory)	5,4	12,3	9,0–35,0
CD24++CD38++ (transitional B cells)	6	7.03	4,6-8,3
CD38++CD24- (plasmablast)	nd	2,3	0,6-5,3
CD16+CD56+	46	52,4	4-17
TCR spectratyping			
CD4+	nd	Oligoclonal	Polyclonal
CD8+	nd	Oligoclonal	Polyclonal
TRECs (pg/mL)			
CD4+	nd	Absent	

CD8+	nd	Absent	
Lymphoproliferation test (10³ cpm)	2010		Normal Value
PHA (2.5 µg/mL)	33,5		> 35 cpm.
PWM (0.35 µg/mL)	34,7		>18 cpm
OKT3 (1.5 µg/mL)	16,4		> 25 cpm
Serum immunoglobulin levels (mg/dL)			
IgA	<5	nd	41-315
IgG	792	nd	633-1016
IgM	<10	nd	56-261
IgG1	730	nd	400-1080
IgG2	94,4	nd	85-410
IgG3	0,55	nd	13-142
IgG4	1,77	nd	1-189
Isohemoagglutinins Anti-A, Anti-B	Absent*	nd	≥ 1:8 present
Antibody response to vaccinations[§]			
Tetanus	0,2		<0,03 absent >0,1 present >0,6 protective >1,1 long term >1,1 long term
HBsAb (mIU/mL)	Absent		<10 absent
Measles, Mumps, Rubella (AU/mL)	Absent		<13 absent
PCP (mg/L)	10		>35 or T1 > 3T0

*Patient's blood group AB. [§]Patient's vaccinations: tetanus-diphtheria-pertussis polio-HiB-HBV (3 doses), measles-mumps-rubella (1 dose), Pneumococcus (4 doses), Meningococcus (1 dose).

FIGURES

Fig.1

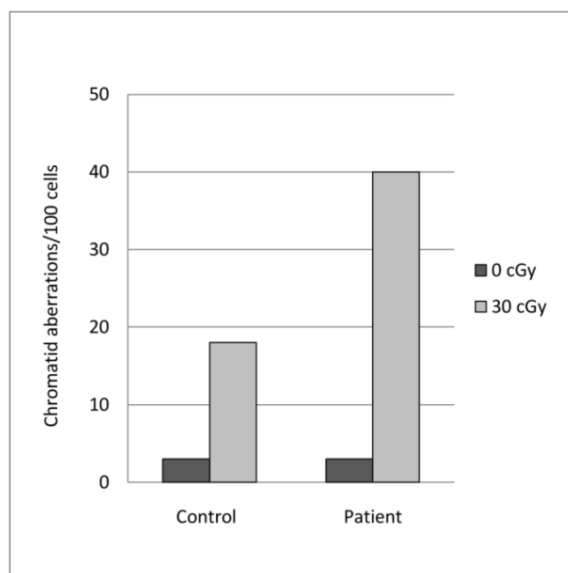
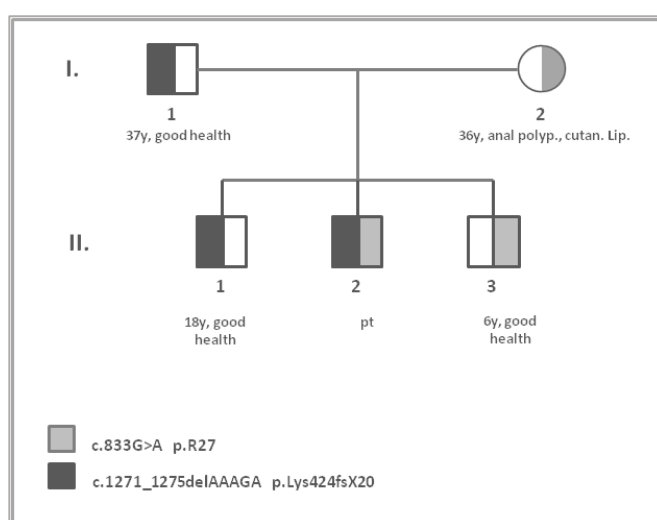


Fig.2



FIGURES LEGEND

Figure 1. Chromosome aberration test: patient's EBV transformed B lymphoblastoid cell lines (LCLs) showed a higher number of chromatid aberrations after x-ray exposure than healthy controls. The test has been confirmed twice.

Figure 2. Pedigree of the family.