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## LETTER TO THE EDITOR

# Altered germinal center reaction and abnormal B cell peripheral maturation in PI3KR1-mutated patients presenting with HIGM-like phenotype☆

**KEYWORDS**
PIK3R1;
Hyper-IgM syndrome;
B cells;
Germinal Center (GC) reaction

*Dear Editor,*

Heterozygous gain-of-function mutations in *PIK3CD* [1,2] were recently reported to be responsible for a novel form of primary immunodeficiency (activated PI3 kinase delta syndrome- APDS). The immunological presentation of patients affected with APDS may include lymphopenia, elevated IgM serum levels, low IgG serum levels, viral infections, lymph node enlargement and elevated risk to develop lymphomas [1–4]. *PIK3CD* encodes for the p110δ, one of the catalytic subunits of the Class IA PI3K molecules. In turn, this catalytic subunit is maintained stable by a regulatory subunit formed by different proteins, among which p85α encoded by *PIK3R1*. The PI3K pathway is important for B and T cell homeostasis. Deleterious homozygous mutations in p85α result in agammaglobulinemia and absence of peripheral B cells [5]. Recently, heterozygous gain-of-function splice site mutations in *PIK3R1* encoding for p85α were shown to be responsible for a novel form of immunodeficiency in 8 patients from 6 unrelated families resembling APDS [6,7]. We report on 4 novel unrelated patients with an immunological phenotype resembling the Hyper-IgM syndrome that harbour heterozygous splice site mutations in *PIK3R1* and present novel immunological data regarding the impact of these mutations on peripheral B cell maturation and the germinal center reaction.

Patient 1, a female born to unrelated Albanian parents, came to our attention due to pneumonia and poor growth. Immunological work-up at 9 months of age showed normal absolute neutrophil and lymphocyte counts. Hypogammaglobulinemia with elevated IgM serum levels (IgG 10 mg/dl, IgA 0 mg/dl, IgM 444 mg/dl) and reduction of switched memory B cells mimicked Hyper-IgM syndrome (Fig. 1A). The patient developed progressive lymphnode enlargement. Patient 2, a female born to unrelated Italian parents, came to our attention at 19 months of age with a history of respiratory infections, poor growth and hypogammaglobulinemia with elevated IgM serum levels (IgG 20 mg/dl, IgA 10 mg/dl, IgM 158 mg/dl) and absence of switched memory B cells (Fig. 1A). Absolute neutrophil and lymhocyte counts were normal. The patient developed lymphnode enlargment and severe tonsillar hypertrophy (resembling AID deficiency). Patient 3, a male born to non consanguineous Italian parents, presented recurrent respiratory infections, lymph node elargement and tonsillar hypertrophy, the latter leading to tonsillectomy due to sleep apnea episodes. Immunological work-up at 36 months showed hypogammaglobulinemia with Hyper-IgM (IgG 85 mg/dl, IgA $<5$ mg/dl, IgM 523 mg/dl) and absence of switched memory B cells, as seen in patients 1 and 2. Patient 4, a female born to unrelated Swedish parents, came to our attention due to recurrent infections in the first year of life, poor growth and mild hepatosplenomegaly. Immunological work-up at 44 months showed lymphopenia and hypogammaglobulinemia with elevated IgM serum levels (IgG 30 mg/dl, IgA 0 mg/dl, IgM 120 mg/dl) (Fig. 1A).

Due to the HIGM-like phenotype in Patient 1, genetic analysis for *CD40*, *AICDA*, *UNG* mutations was undertaken, but resulted negative. Whole exome sequencing analysis was then performed revealing the previously reported [6,7] splice site mutation c.1425 + 1G > A in *PIK3R1* (Fig. 1A and 1B). Genetic analysis for mutations in *PIK3R1* was then undertaken in 102 patients with a HIGM phenotype without mutations in known causative genes revealing *de novo* splice site mutations in 3 additional patients (index patients 2,3 and 4) (Fig. 1A and 1B).

A more extended immunological evaluation for Patients 2 and 3 showed an abnormal expansion of immature B cells

*Abbreviations:* GC, germinal center; HIGM, hyper-IgM; APDS, activated PI3 kinase delta syndrome.

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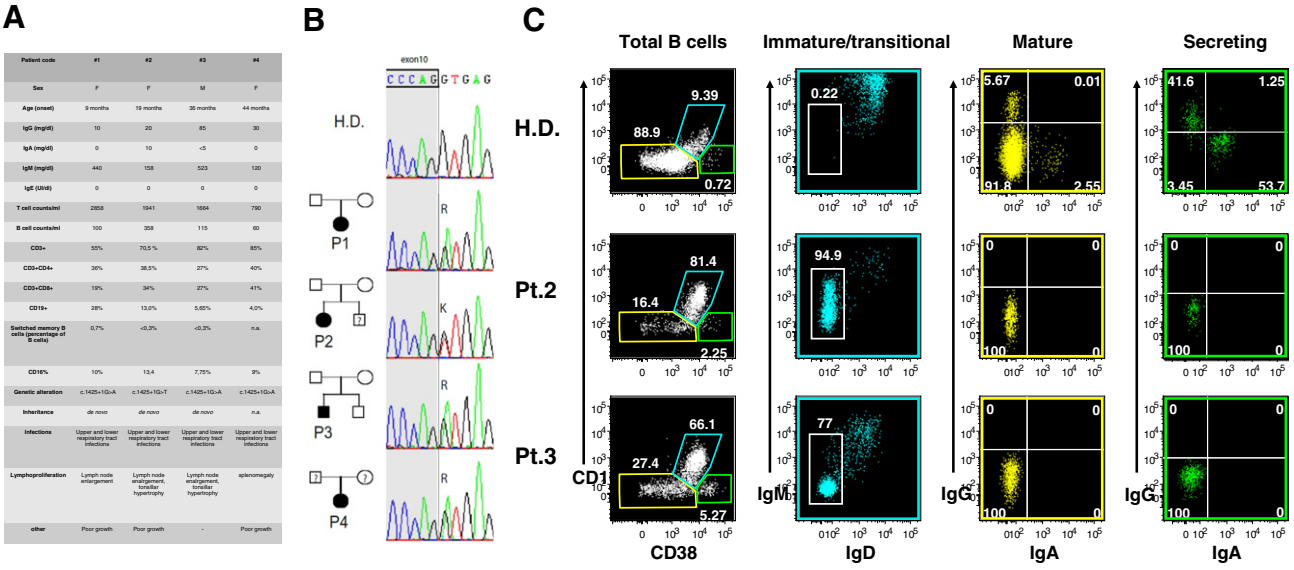


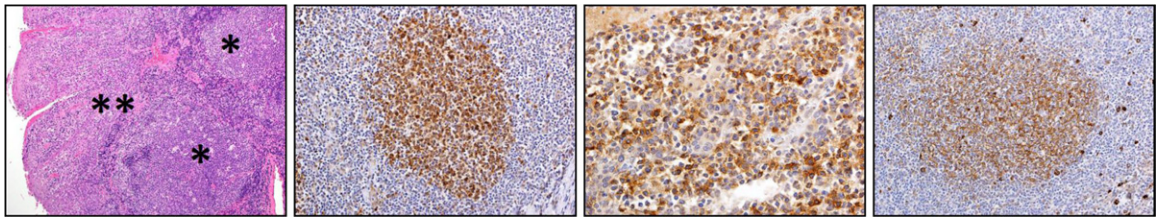


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| Patient code | #1 | #2 | #3 | #4 | §5 | §6 | §7 | §8 | *9 | *10 | *11 | *12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex | f | f | m | f | f | m | f | f | f | f | m | m |
| Age (onset) | 10 months | 19 months | 36 months | 44 months | 24 months | 6 months | 6 months | 13 years | 6 years | 9 years | 17 years | 5 years |
| Respiratory infections | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymh node enlargement | + | + | + | - | - | - | - | - | + | + | + | + |
| Tonsillar hypertrophy | + | + | + | - | + | - | - | - | n.a. | n.a. | n.a. | n.a. |
| Lymphoma | - | - | - | - | - | - | - | - | - | + | - | - |
| Autoimmunity | - | - | - | - | - | - | - | - | + | n.a. | - | + |
| Allergy | - | - | + | - | - | - | - | - | - | - | - | - |
| Viral infections | - | - | - | - | - | + (CMV, EBV viremia) | - | - | - | n.a. | + (CMV lymphoadenitis) | - |
| Poor Growth | + | + | - | + | n.a. | n.a. | n.a. | n.a. | + | - | + | + |

**Figure 1** Clinical, molecular and immunological findings from the index *PIK3R1* mutated patients. **A.** Clinical and immunological presentation at onset for index patients. **B.** Electropherograms showing the mutations in *PIK3R1* identified in the index patients. **C.** Peripheral B cell maturation evaluated by means of flow cytometry. The dot plots depict the gating strategy applied for the definition of immature/transitional (light blue), mature (yellow) and secreting B cells (green) in a healthy donor (HD) and two index patients (Pt.2 and Pt.3) (first column). The second column shows the identification of immature cells (second column), while the third and fourth column shows the IgG+ or IgA+ switched cells (third and fourth column) as defined by the use of monoclonal anti-surface immunoglobulin antibodies within the B cell subsets gated in the first column. **D.** Histological evaluation of tonsillar sections from a *PIK3R1*-mutated patient (Patient 2). The tonsillar sections show follicular (*) and marginal zone-B cell (**) hyperplasia (far left panel). Germinal center (mid left panel) as well as marginal zone B cells (mid right panel) express IgD. IgM staining in the germinal center labels the follicular dendritic cell network and scattered intrafollicular and extrafollicular plasma cells (far right panel). **E.** Summary of clinical features among reported *PIK3R1* mutated patients. n.a.: not available; # index patients; § patients reported by Deau et al [6]; * patients reported by Lucas et al [7].