



The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

203.LYMPHOCYTES AND ACQUIRED OR CONGENITAL IMMUNODEFICIENCY DISORDERS

Biallelic PI4KA mutations Disrupt B Cell Mitochondrial Metabolism and Cause Hypogammaglobulinemia

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Introduction

Biallelic mutations in the *PI4KA* gene cause PI4KA-related disorder, a novel condition with neurological (limb spasticity, developmental delay, intellectual disability, seizures, ataxia, nystagmus), gastrointestinal (GI; inflammatory bowel disease [IBD] and multiple intestinal atresia [MIA]) and immunological manifestations. *PI4KA* encodes phosphatidylinositol (PI) 4-kinase alpha, which catalyzes the first step of phosphoinositide metabolism, phosphorylating PI to PI4P (phosphatidylinositol 4-phosphate). Phosphoinositides collectively have fundamental signalling roles in the plasma membrane and other organelles. We collected comprehensive clinical and laboratory data on 13 patients and performed a multiomic strategy, combining transcriptome, proteome, lipidome and metabolome analyses of three patients' EBV-transformed cell lines to unravel the burden of B cell deficiency in PI4KA-related disorder and define the role of PI4KA in B cells.

Results

Recurrent or severe infections (8/13) and GI (either gastroesophageal reflux disease, MIA or IBD; 6/13) manifestations were frequent. Thyroid disease (either hypo- or hyperthyroidism; 2/13), juvenile idiopathic arthritis (JIA; 1/13) and non-Hodgkin lymphoma (1/13) were reported. At last follow up 11 patients were alive. Ten had either hypogammaglobulinemia, decreased absolute numbers of B cells or decreased B cell subsets. B cell subsets showed increased transitional (4/7), decreased naive (2/7) and decreased switched memory (4/7) B cells. B cell proliferation to T independent mitogens, specifically IgM + CpG or pokeweed, was impaired in 2/2. T-cell (either CD3+, CD4+ or CD8+) lymphopenia was detected in 4/13. T-cell proliferation was maintained in 5/5. NK cells were decreased in 6/13. Ig replacement therapy (4/13) and immunosuppressive treatment (due to JIA [1/13] or IBD [2/13]) were given.

Lipidome analysis showed that different glycerophospholipids profile (i.e., phosphatidylcholine [PC], phosphatidylethanolamines [PE] and phosphatidylserine [PS]) was altered. Metabolome analysis and pathway enrichment analysis confirmed impaired phosphatidylethanolamine biosynthesis and additionally showed altered PIP metabolism and mitochondrial dysfunction (i.e., beta-oxidation of short and long fatty acids). Although the total number of mitochondria was not affected, the mitochondrial activity was significantly reduced. Integration analyses of proteomics, metabolomic and lipidomic data reveals biochemical mechanisms underlying biallelic *PI4KA* mutation. In particular, dysregulation of metabolic enzymes and pathways involved in glutathione metabolism and tricarboxylic acid cycle was found (Fig. 1). These metabolic impairments ultimately impacted on oxidative phosphorylation, thus disrupting mitochondrial integrity.

Gene set enrichment analysis of differentially expressed genes suggested a skewing towards naive B cell gene sets. Pathways enrichment analysis of transcriptome and proteome showed enrichment in B cell receptor (BCR) pathway and complex, PI3K/mTOR pathway and phospholipids metabolism. PI4P and its metabolite PI(4,5)P2 are determinants of PM identity, specifically controlling cytoskeletal dynamics. Deranged basal actin organization was reverted after anti-IgM stimulation. Hyperactivation of PI3K targets (pAKT473, p4EBP1 and pS6) was observed. Dysregulation of *RABGAP1*, *RAB8B* and *LAMP3* mRNA prompted us to investigate autophagy. Indeed, LC3 was increased either in the presence or absence of chloroquine, showing that *PI4KA* mutations enhanced autophagosome formation and inhibited its degradation.

Conclusion

Overall, by altering the production of several key lipids, biallelic *PI4KA* mutations disrupt B cell metabolism causing mitochondrial dysfunction, mTOR hyperactivation, increased autophagy and deranged cytoskeleton organization. *PI4KA*-related disorder is a novel syndromic inborn error of immunity causing B cell deficiency and hypogammaglobulinemia.

Figure 1. Integration of proteomics, metabolomics and lipidomics reveals biochemical mechanisms underlying biallelic *PI4KA* mutations.

Disclosures Biondi: Agmen: Speakers Bureau; Novartis: Speakers Bureau; ColImmune: Membership on an entity's Board of Directors or advisory committees, Research Funding; Galapagos: Membership on an entity's Board of Directors or advisory committees; BMS: Membership on an entity's Board of Directors or advisory committees.

<https://doi.org/10.1182/blood-2023-185551>

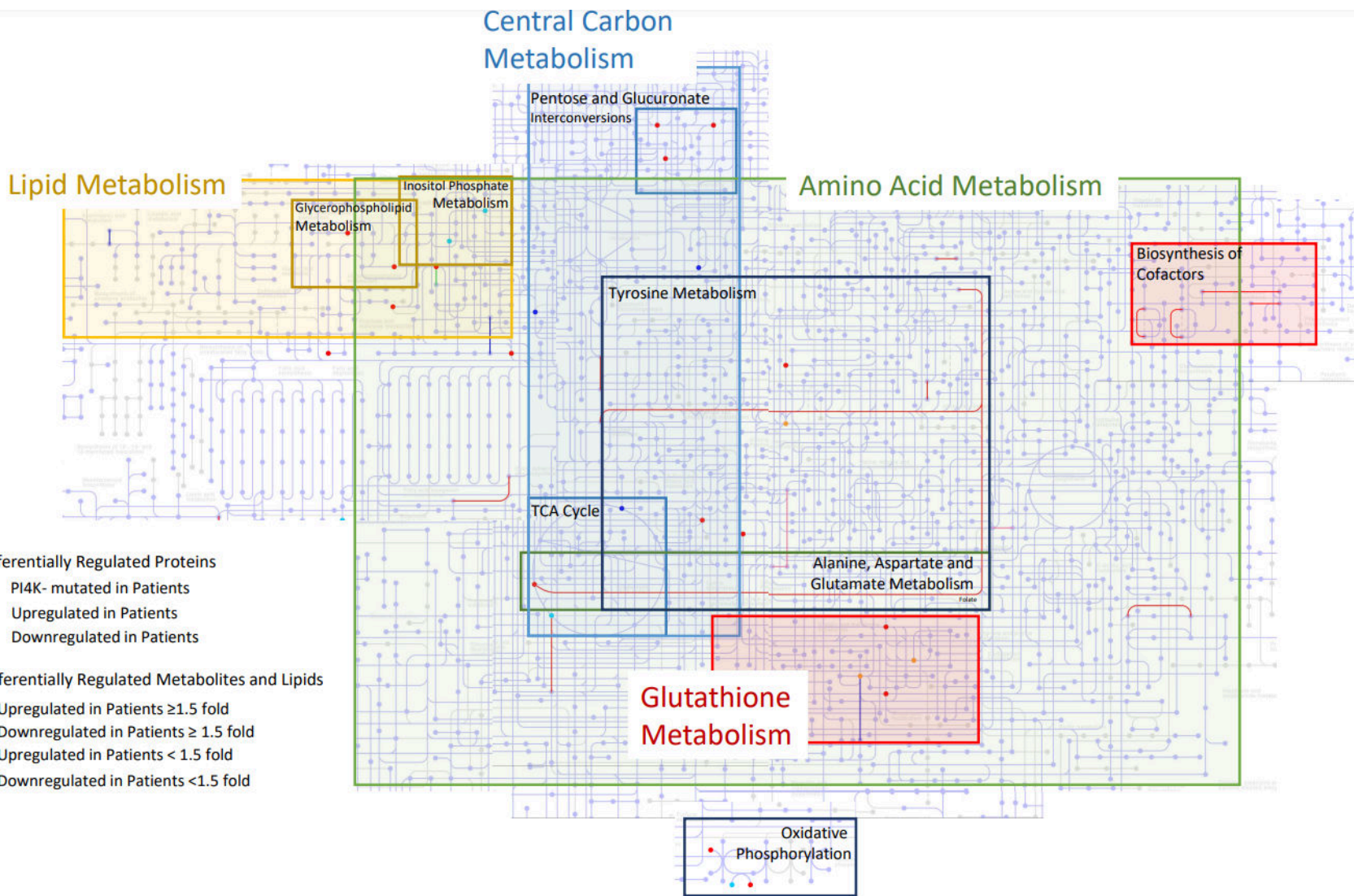


Figure 1