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by David J Swan, Dominik Aschenbrenner, Christopher A Lamb, Krishnendu Chakraborty, Jonathan Clark, Sumeet Pandey, Karin R Engelhardt, Rui Chen, Athena Cavounidis, Yuchun Ding, Natalio Krasnogor, Christopher D Carey, Meghan Acres, Stephanie Needham, Andrew J Cant, Peter D Arkwright, Anita Chandra, Klaus Okkenhaug, Holm H Uhlig, and Sophie Hambleton

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Immunodeficiency, autoimmune thrombocytopenia and enterocolitis caused by autosomal recessive deficiency of *PIK3CD*-encoded phosphoinositide 3-kinase δ

David J. Swan[1]*, Dominik Aschenbrenner[2]*, Christopher A. Lamb[1,3]*, Krishnendu Chakraborty[4], Jonathan Clark[4], Sumeet Pandey[2], Karin R. Engelhardt[1], Rui Chen[1], Athena Cavounidis[2], Yuchun Ding[5], Natalio Krasnogor[5],Christopher D. Carey[3], Meghan Acres[1,3], Stephanie Needham[3], Andrew J. Cant[3], Peter D. Arkwright[6], Anita Chandra[4,7], Klaus Okkenhaug[4,8], Holm H. Uhlig[2,9,10]**, Sophie Hambleton[1,3]**

* and ** joint authorship

1 Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK.
2 Translational Gastroenterology Unit, John Radcliffe Hospital, University of Oxford, Oxford, UK.
3 Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK.
4 Babraham Institute, Cambridge, UK
5 School of Computing Science, Newcastle University, Newcastle upon Tyne, UK
6 University of Manchester & Department of Paediatric Allergy & Immunology, Royal Manchester Children's Hospital, Manchester, UK
7 Department of Medicine, University of Cambridge, Cambridge, UK.
8 Division of Immunology, Department of Pathology, University of Cambridge, Cambridge, UK.
9 Department of Paediatrics, University of Oxford, Oxford, UK.
10 Oxford NIHR Biomedical Research Centre, Oxford, UK

Corresponding authors:

Sophie Hambleton (sophie.hambleton@newcastle.ac.uk), *Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne NE2 4HH, United Kingdom*

and

Holm H. Uhlig (holm.uhlig@ndm.ox.ac.uk), *Translational Gastroenterology Unit, Experimental Medicine, University of Oxford, John Radcliffe Hospital Oxford, OX3 9DU, United Kingdom*

tial diarrhoea (3L/day) until the addition of immunosuppression (corticosteroid, cyclosporine, infliximab). Clinical improvement was accompanied by amelioration of inflammatory changes on repeat endoscopic examination. However, weaning of immunosuppressive treatment led to relapse of his gut disease, indicating chronic immune-mediated inflammation.

$CD4^+$ and $CD8^+$ T lymphoblasts, as was IL-2-induced phosphorylation of the mTOR target S6 (fig.2A and supplementary fig.2). Glycolysis stress test showed impaired IL-2-stimulated glycolysis and glycolytic reserve in patient cells, similar to the behaviour of $CD4^+$ and $CD8^+$ T cells treated with Idelalisib (fig.2C-D). These findings show that germline p110δ deficiency impairs lymphocyte metabolism, which we hypothesised might contribute to immunodysregulation through altered T cell polarization and behaviour.

To investigate the cellular immunophenotype within the patient's inflamed gut, we performed immunohistochemistry on colonic biopsies taken prior to HSCT. Relative to healthy age-matched control tissue, there was a modest expansion of $CD8^+$ T cells in the lamin.3 (a)g (a)oth n stress

suspicion for an underlying monogenic cause is required in this setting.

In summary, we report a child with homozygous germline loss-of-function mutation in *PIK3CD*, who developed refractory immune thrombocytopenia, inflammatory bowel disease and susceptibility to infection, cured by HSCT. The immune defect was characterized by defective PI3Kδ signaling, al-

References:

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expression leads to combined immunodeficiency and multisystem syndromic features. J Allergy Clin Immunol. 2018;142(2):618-629.

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Figure Legends

Figure 1: *PIK3CD* mutation in a patient with immunodeficiency and immune dysregulation.

(**A**) Pedigree.

(**B**) Haematoxylin & eosin staining showing colitis and crypt abscess formation.

(**C**) Immunostaining for CD3 (gold) and CD20 (purple); P1, patient; HD, healthy donor.

(**D**) CCR7 and CD45RA staining and quantification of memory CD8+ cells among CD25-CD8+ cells.

(**E**) Expression of perforin and transcription factor TBET in naïve and memory CD8+ T cells.

(**F**) Sanger sequencing confirming frameshift deletion plus 2bp insertion.

(**G**) p110δ schematic showing p.Q170Vfs*41 and previously reported mutations.

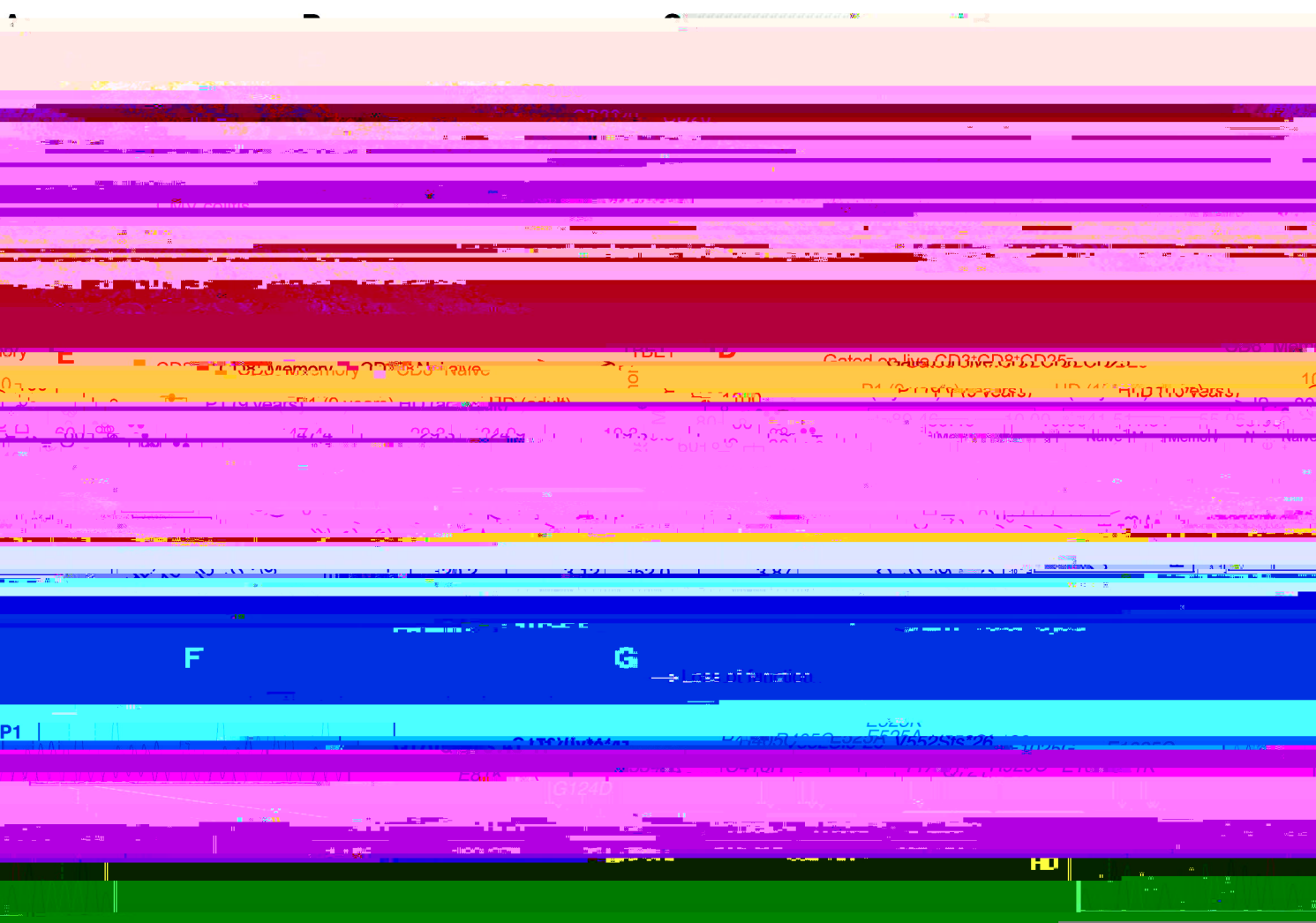
Figure 2: Functional impact of *PIK3CD* mutation.

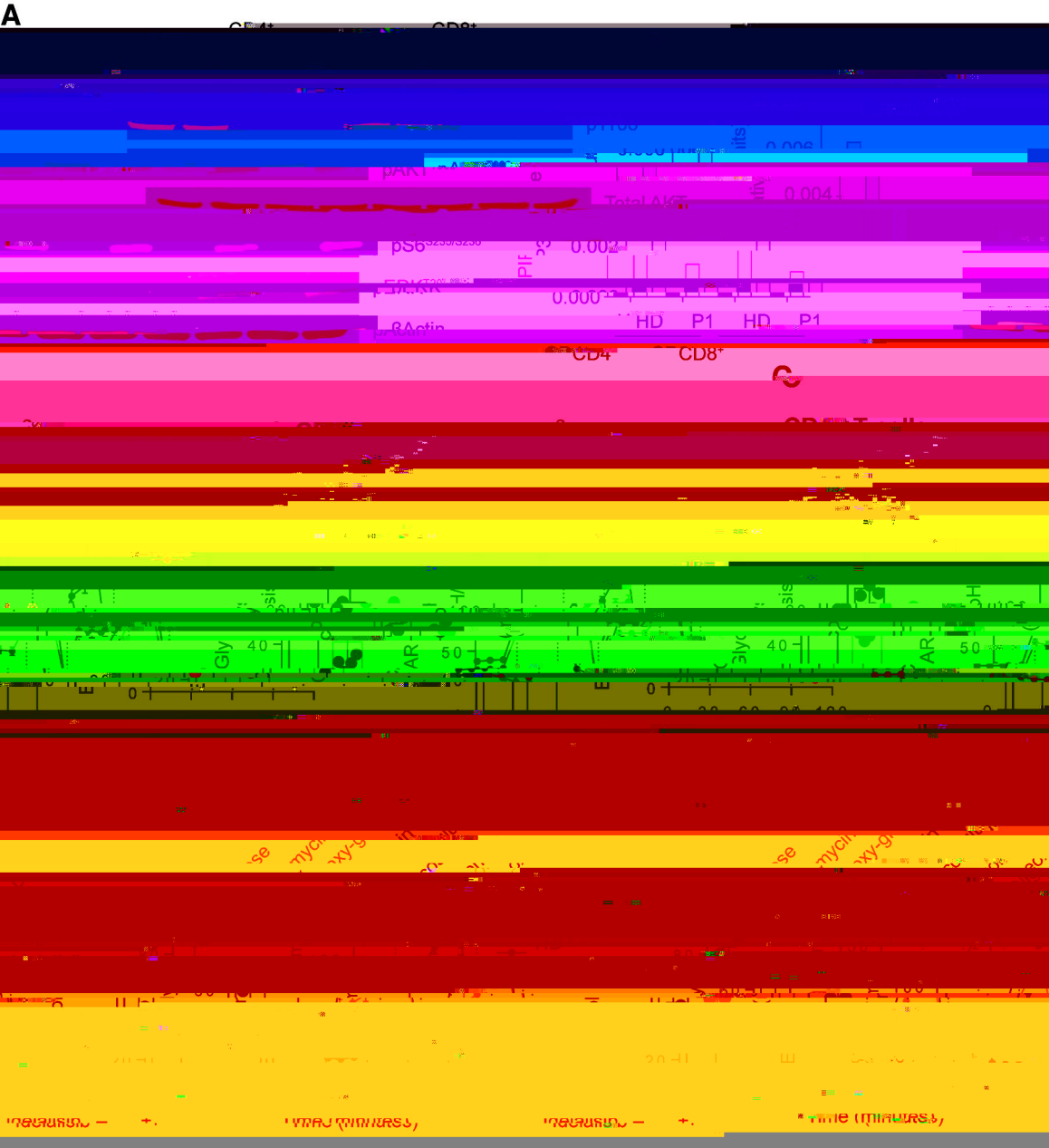
(A) Immunoblotting of p110δ, AKT, pAKT^{T308}, pERK$^{T202/Y204}$, pS6$^{S235/236}$ and beta-actin in control (HD) and patient (P1) CD4+ and CD8+ T lymphoblasts with and without CD3 stimulation.

(B) PIP_3 quantification before/after TCR stimulation.

(C) 4.5 m (3.6 (quantification) 12.3 (ion of TKV) 2.1 lTf 0.462 (s) -2.9 (Htt) 10.3 0 464 0 Td[(8)-17.4 EA)6.4 (x)13.8 (ra

m ulat(by)-20.7 (sg of)-17.5 HtP 20.4 1g761re/f(in 3.6


F
G
P1


A
CD4+
CD8+
pAKT
Total AKT
pS6
GAPDH
βActin
HD
P1
HD
P1
CD4
CD8+
Gly
AR
Gly
AR
40
50
40
50
0
0
0.006
0.004
0.002
0.000

SUPPLEMENTARY INFORMATION

Me d

Pa e

W e e e e e c

Me ab c a a

S a c

S e e a Refe e ce :

S e e a F e :

S e e a F 1

N a f e e c f FOXP3 e e a e CD4+ T ce .

S e e a F 2

Defec e IL-2 a AKT 110 -def c e CD4+ a d CD8+ T ce e .

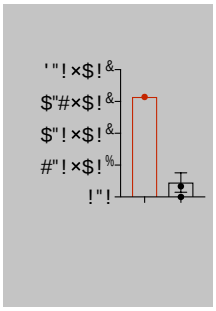
(A)

B)

C

S e e a F 4

l c ea ed e a TBET a d e f a a a e 110 -def c e c .



! "#$%& .Q170Vf *41 a a .

Va a	Ge - e	E Ac	I ac ed c-	Ge e a e	A c a ed d ea e (OMIM)

Supplementary Table 3. Comparison of current and previously described patients with AR *!"#$%&* deficiency.

Patient		current	P2	P3	P4	P5	P6
!"#$%& gene variant(s)		h m. c.703_723de-lin GT	c m nd he mi en e & ema e	h m c.2161C>T (l h m a ian in *KNSTRN*)		h m c.1653_1653delG	
Effect on protein	a ian	.Q170Vf *41		.Q721*		.V552Sf *26	
	110 e e - i n	ab en	ed ced	ab en		ND	
Immune disease phenotype		9	childh d	5m	<1m	2	2m

Abbreviations (Supplementary Table 3):