ADA2 Deficiency Complicated By EBV-driven Lymphoproliferative Disease

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Introduction

ADA2 deficiency was first reported in 2 parallel publications in 2014 in association with polyarteritis nodosa, vasculopathy and early-onset stroke [1,2]. The clinical phenotype has since expanded to include immune deficiency without vasculopathy, cytopaenias, enteropathy, red cell aplasia, lymphadenopathy, splenomegaly and hepatomegaly. The range of severity and heterogeneity of presentation is highly variable, even with in patients with the same variant or members of the same family. Schepp *et al*. screened 181 antibody deficient patients and found ADA2 deficiency in 9 [3]. This is the first reported case of ADA2 deficiency complicated by EBV-driven lymphoproliferative disease.

Patient/Pedigree

We report a 29-year old male, third child of non-consanguineous parents, with microcytic anaemia, neutropaenia and recurrent infections in infancy including skin infections (*Staphylococcus aureus* and *Streptococcus pneumoniae*), respiratory tract infection (RSV, *Haemophilus influenzae* and *Streptococcus pneumoniae*) and herpetic mouth ulcer. Age 6 his immunoglobulins, B-cells and CD4 T-cells were low and immunoglobulin replacement was commenced. He remained well until he presented age 29 with a two-week history of fatigue, sore throat and fever. EBV-driven lymphoproliferative disease was diagnosed and treated with chemotherapy and allograft. EBV became undetectable. Complications included CMV reactivation, severe hepatic veno-occlusive disease and failure to engraft. A repeat allograft engrafted successfully but he had multiple infections and died.

Table 1: Results of standard immunological investigations at different ages.
Immunoglobulins (g/L), Lymphocyte count (x10^6/L), Lymphocyte subsets (x10^9/L), (R) on immunoglobulin replacement.

	Age 2	Range		Age 6	Range	Age 28	Range
IgG	3.51	3-10.9	**IgG**	2.03	6.0-16.0	9.6 (R)	6.0-16.0
IgA	0.13	0.2-0.7	**IgA**	0.10	0.08-2.8	0.11	0.8-4.0
IgM	0.29	0.6-2.1	**IgM**	0.22	0.5-1.9	<0.2	0.5-2.0
IgE	56	3-40	**Lymphocyte count**	1.65	1.1-5.9	1.43	1.0-2.8
			CD3	1.44	0.7-4.2	1.16	0.7-2.1
IgG1	208	330-729	**CD19**	0.03	0.2-1.6	0	0.1-0.5
IgG2	<3	40-188	**$CD16^+CD56^+$**	0.15	0.09-0.9	0.26	0.09-0.6
IgG3	30	14.5-36.5	**$CD3^+CD4^+$**	0.46	0.3-2.0	0.31	0.3-1.4
IgG4	30	5.5-31.5	**$CD3^+CD8^+$**	0.92	0.3-1.8	0.83	0.2-0.9

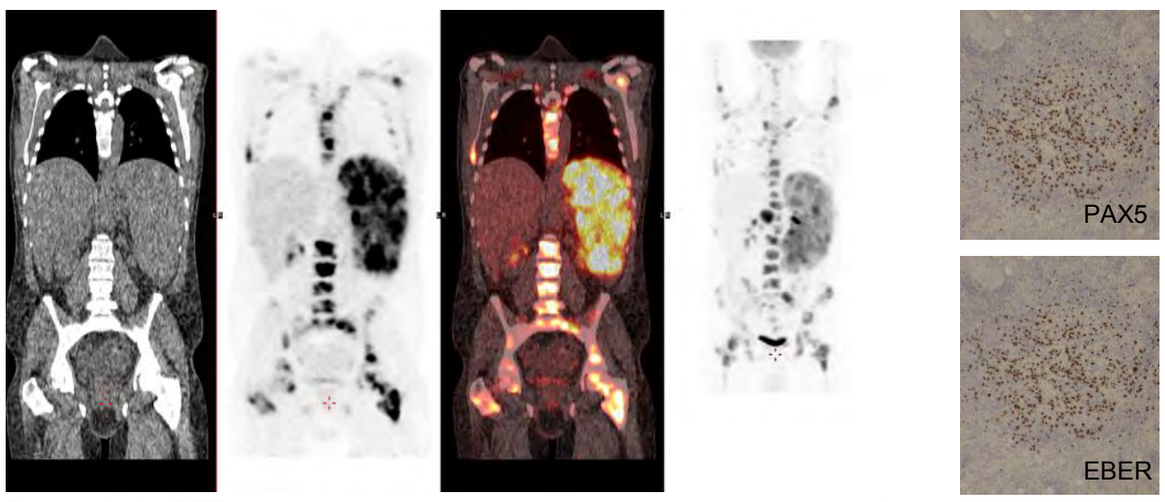

Figure 1. A: PET scan demonstrating active disease on both sides of the diaphragm and marrow involvement. **B: Bone marrow trephine biopsy immunostains.**

His older brother had neutropaenia, required transfusions for red cell aplasia and had recurrent HSV and warts. He died age 5 from septicaemia secondary to neutropaenic enterocolitis (*Clostridium septicum* identified post-mortem by specific immunofluorescence). His sister is healthy and well. His younger brother had neutropaenia and recurrent infections. Abdominal vasculitis was diagnosed aged 11, IvIg commenced age 12 and he received a bone marrow transplant (matched unrelated donor) age 14, with good immune reconstitution.

Methods and Results

The proband's DNA was submitted to GRID which identified variants in *CR2*, *ADA2* and *STXBP2*. These regions were Sanger sequenced in the proband, parents and sister. Only ADA2 co-segregated appropriately, the proband compound heterozygote for Asn370Lys (also occurring in his father) and Arg169Gln (also occurring in his mother).
ADA2 enzyme activity was absent in the proband and reduced in the heterozygous parents, consistent with a diagnosis of ADA2 deficiency.

Table 2: ADA2 variants and frequencies

Variant		ExAC frequency	gnomAD
c.1110 C>A	p.Asn370Lys (N370K)	0	0
c.506 G>A	p.Arg169Gln (R169Q)	0.0004862	0.000505

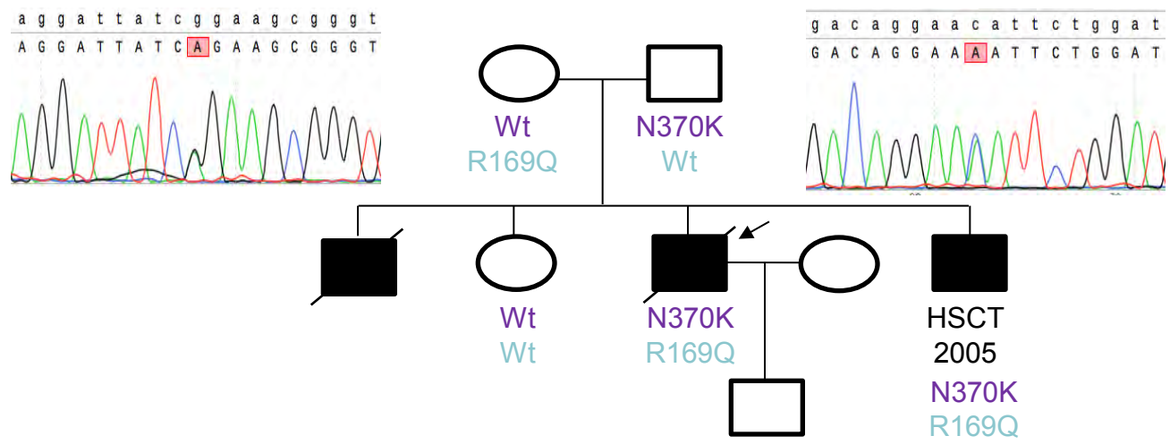

Figure 2. Sanger confirmation and cosegregation of ADA2 variants.

Table 3: ADA2 activity **(Normal range in adult plasma** 8.7 to 30.0 IU/L)

	Serum ADA activity(IU/L)	Date sample taken
Proband	0	11/7/2011
Freeze control	13.3	14/7/2011
Father	7	6/11/17
Mother	7.6	6/11/17
Tranplanted brother	13.8	6/11/17
Healthy sister	17.7	6/11/17

Discussion

ADA2 is predominantly expressed by monocytes and other cells of the myeloid lineage. It converts adenosine to inosine and deoxyadenosine to deoxyinosine. It has approximately 100-fold lower affinity for its substrates than ADA1 (mutations in which cause a SCID phenotype). It also differs from ADA1 in being predominantly secreted rather than intracellular and in the cell types it binds. ADA2 is most active at acid pH and is therefore thought to have a role in sites of inflammation and tissue hypoxia [4].
This is the first description of EBV-driven lymphoproliferative disease in ADA2 deficiency. Primary immune deficiencies manifesting as severe EBV-induced disease are associated with mutations in genes including *SH2D1A, XIAP*, *ITK*, *MAGT1*, *CORO1A*, *CD27*, *CD70*, *NFKB1* and *RASGRP1* [5]. These genes all code for proteins that are involved in the interaction between $CD8^+$ T cells and B cells and/or intrinsic T cell signalling pathways. EBV susceptibility is also a feature of monogenic NK cell disorders including CD16 deficiency, MCM4 deficiency and GATA2 deficiency.
High serum ADA2 activity has been detected in a number of infectious, inflammatory and malignant diseases but was particularly increased in infectious mononucleosis [6]. In addition to its enzymatic role in purine metabolism ADA2 can act as a growth factor. ADA2 is reported to increase $CD4^+$ T cell proliferation, with ADA2 having increased effect in the context of low monocyte concentration [7]. Deficiency of ADA2 may also have indirect effects as ADA2R receptors are present on most immune cells. Adenosine binding at ADA2R G_s protein-coupled leads to reduced T cell activation, TCR-signalling and reduced cytotoxic activity of $CD8^+$ T cells and NK cells [8]. A2aR small molecule inhibitors are being investigated for cancer immunotherapy [9]. Other environmental or genetic factors may have played a role in the development of EBV-driven lymphoproliferative disease in this patient but this case raises the possibility that ADA2 deficiency is another primary immunodeficiency that causes susceptibility to severe EBV-induced disease.

Conclusions

ADA2 deficiency has highly variable severity and a broad ranging clinical phenotype including EBV-driven lymphoproliferative disease. Monitoring of EBV viral load should be considered in this condition.

References **1.** Zhou *et al.* NEJM 2014; 370:911-920 **2.** Navon Elkan *et al.* NEJM 2014; 370: 921-931 **3.** Schepp *et al.* Arthritis Rheumatol. 2017; 69:1689–1700 **4.** Zavialov *et al.* Biochem. J. 2005; 391:51–57 **5.** Tangye *et al.* JEM 2017; 214:269–283 **6.** Ungerer *et al.* Clin. Chem. 1992; 38:1322-6 **7.** Zavialov *et al.* J. Leukocyte Biol. 2010; 88:279–290 **8.** Cekic *et al.* Nat. Rev. Imm. 2016; 16:177–192 **9.** Leone and Emens J. for ImmiunoTherapy of Cancer 2018; 6:57