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Tregs and Th17 lymphocytes in human DYRK1A haploinsufficiency

The *DYRK1A* gene encodes for a dual-specific tyrosine phosphorylation-regulated kinase 1A involved in different cellular processes such as cell differentiation, proliferation and survival. This tyrosine kinase plays a key role in brain growth and function. Indeed, haploinsufficiency of DYRK1A results in defective kinase activity and is responsible for mental retardation autosomal dominant 7 (MRD7). This syndrome is characterized by microcephaly, autistic behaviors, intellectual disability, speech delay, epileptic seizures, and facial dysmorphisms [1]. There is also evidence that DYRK1A is also expressed in lymphocytes where it can have a regulatory role of T cell differentiation. Khor et al. [2] showed that chemical inhibition of DYRK1A with harmine enhances differentiation of regulatory T cells (Tregs) from murine CD4+ naive T cells. Newly generated Tregs were able to suppress the proliferation of CD4+ effector cells stimulated with anti CD3/CD28. Moreover, administration of harmine in animal models of autoimmune diseases and mucosal inflammation improved such clinical conditions similarly as infusion of purified Tregs, while lymphocyte treatment with harmine resulted in reduction of absolute numbers of T helper 17 cells (Th17). Furthermore, Th17 tended to have higher expression of *DYRK1A* than Tregs.

Tregs play a key role in maintaining immune tolerance, both against autologous and allogeneic antigens. Indeed, mutations in FOXP3, a transcription factor indispensable for generation and survival of Tregs, lead to an immune dysregulation disorder characterized by different autoimmune conditions [3,4].

Th17 cells are effector T cells that express the transcription factor RORC and constitutively produce IL-17. Th17 cells are involved in the protection against bacterial and mycotic pathogens [5] and a complete loss of this population leads to immune dysregulation disorders.

A fine balance between these two populations is essential for immune homeostasis: they are involved in opposite conditions, even if their development is regulated by the same cytokine milieu, but an increase of Tregs inhibits the production of Th17.

We describe two patients with MRD7: patient 1 (Pt1) is a 5 years old boy with psychomotor and growth delay, hypotonia, microcephaly and dysmorphic features (downslanting palpebral fissures, micrognathia). Whole Genome Sequencing revealed a *de novo* stop codon mutation c.787C > T (p.Arg263Ter) in *DYRK1*A (NM_130436).

Patient 2 (Pt2) is a 50 years old man, presenting with microcephaly, epileptic seizures, intellectual disability and self-injurious behaviors. NGS of a panel genes including *DYRK1A* (NM_130436) revealed the loss-of-function c.269_270 deletion.

Pt2 suffered from recurrent bacterial and fungal infections, leading us to hypothesize that he could have an immune imbalance with reduced Th17 and increased Tregs, due to defective DYRK1A activity, as supported by the findings from Khor et al.

Thus, immunological evaluation was performed in both patients to assess the distribution of Tregs and Th17 cells.

Tregs were evaluated by FOXP3 staining: peripheral blood mononuclear cells were incubated with anti-CD3, anti-CD45, anti-CD25, anti-CD127 and anti-CD4 antibodies and, after fixation and permeabilization steps, with anti-FOXP3 or isotype control antibodies.

To identify and measure Th17, CD4 T cells were selected by microbeads isolation and stimulated with anti-CD3/anti-CD28 antibodies in the presence of human recombinant IL-2. After 5 days of incubation at 37 °C, 5% CO2, cells were stimulated for 6 h with Phorbol Myristate Acetate, Ionomycin and Brefeldin A.

Activated cells were finally incubated with anti-IFN-γ/PE and anti-IL17A/FITC (intracellular staining) and with anti-CD45R0, anti-CCR6 and anti-CCR4 antibodies (extracellular staining).

Our data showed a similar percentage of Treg and Th17 in two patients when compared to healthy controls, thus not supporting a role for reduced Th17 in recurrent infections in Pt2 (Fig. 1a and b). These findings are in contrast with the observations obtained by Khor et al. [2]. This discrepancy could be related to a different extent of residual DYRK1A activity in patients with MRD7 as compared to chemically treated murine cells. In fact, *in-vitro* harmine treatment leads to a near-complete chemical inhibition of DYRK1A, while MRD7 is characterized by haploinsufficiency, i.e. halved activity, of the kinase. Alternatively, the interplay between Treg and Th17 seems to be distinctly regulated in humans compared with mice. In both cases, the induction of Tregs may reflect on a decrease of Th17, since FOXP3 inhibits the differentiation of naive CD4+ T cells towards Th17. Inhibition of Th17 generation from Tregs is thought to be mediated by FOXP3-dependent inhibition of RORC, which is due to a repressor domain coded by the exon 2 of *FOXP3*. Since in humans, but not in mice, there is an isoform of FOXP3 that lacks exon 2, it may be possible that the inhibition of Th17 generation is less profound in humans than in mice [6].

The recurrence of bacterial and fungal infections in our patient remains thus unexplained. To exclude main immunological anomalies we performed further analysis, including recent thymic emigrants, memory T and B lymphocytes, markers of activation on T cells and Th1 cells and no significant defects were found (Table 1). Even if we cannot exclude a role for DYRK1A, this was not likely to depend on a Tregs/Th17 imbalance. Thus, we hypothesize that haploinsufficiency of DYRK1A is sufficient to lead to neurological phenotype but not to cause the immunological defects.

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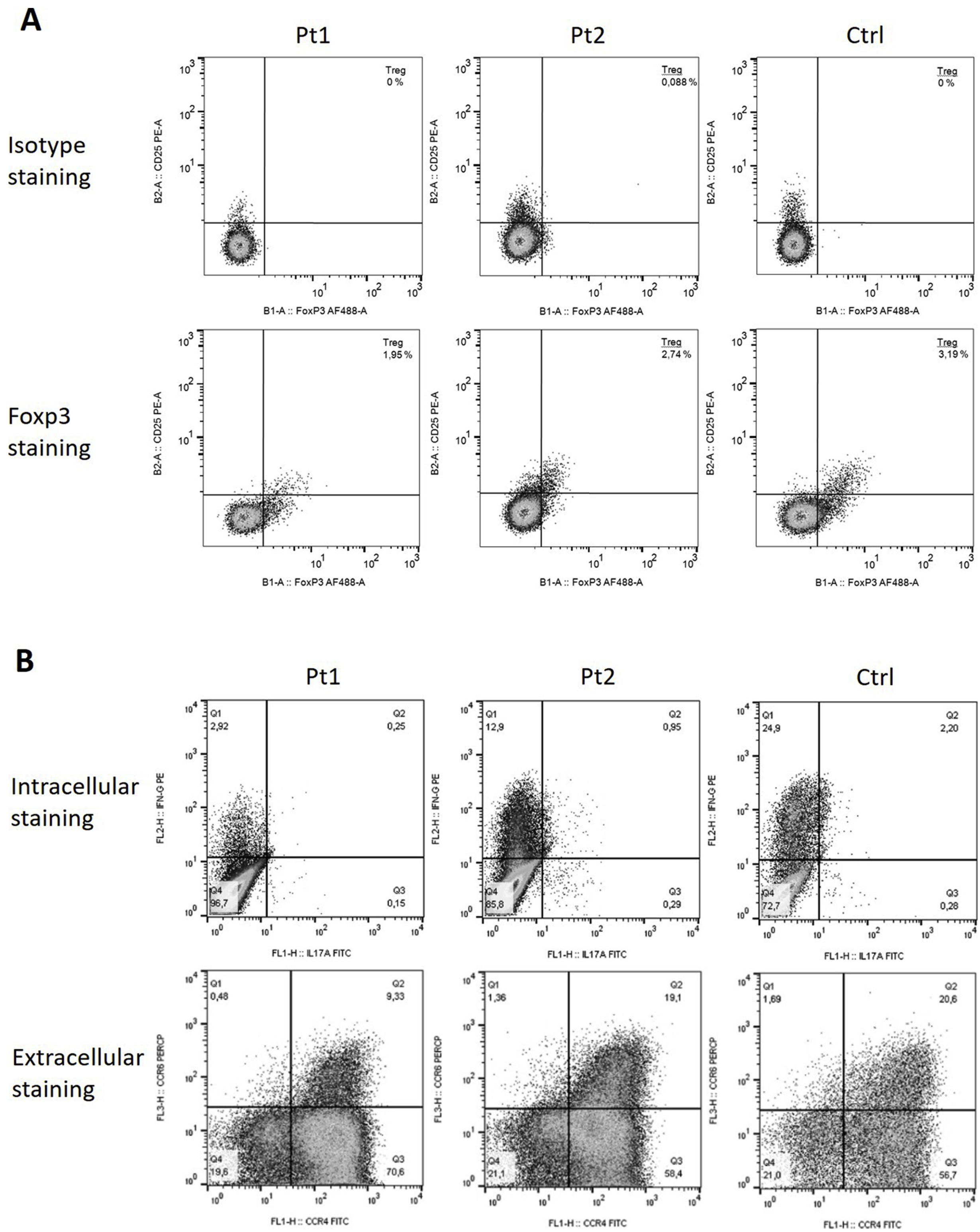

Fig. 1. Cytometric analysis of Tregs and Th17 cells.
A. Staining for Tregs cells: Tregs are identified as Foxp3+ and CD25+ cells (upper right quadrant). Gated on CD45++ CD3+ CD4+ cells. B. Staining for Th17 cells: Th17 are identified as IL17A + cells in intracellular staining gated on CD4+ (lower right quadrant) and as CCR6+ and CCR4+ cells in extracellular staining, gated on CD45R0+ cells (upper right quadrant).

Table 1
Flow cytometric immunophenotyping.

Lymphocyte Subsets	Patient 1 [ref. ranges]	Patient 2 [ref. ranges]
T cells (% of lymphocytes) CD45 + CD3 +	71.6 [43.1-67.1][a]	52.0 [59.0-83.0][a]
Helper T cells (% of T cells) CD45 + CD3 + CD4 +	40.4 [26.8-42.3][a]	31.3 [31.0-59.0][a]
Suppressor T cells (% of T cells) CD45 + CD3 + CD8 +	23.7 [17.5-31.1][a]	13.7 [12.0-38.0][a]
Naïve helper T cells (% of helper T cells) CD45 + CD3 + CD4 + CD45RA + CCR7 +	66.1 [20.5-54.8]^	52.1 [20.5-54.8]^
Central memory helper T cells (% of helper T cells) CD45 + CD3 + CD4 + CD45RA-CCR7 +	18.2 [8.4-32.8][b]	31.5 [8.4-32.8][b]
Effector memory helper T cells (% of helper T cells) CD45 + CD3 + CD4 + CD45RA-CCR7-	14.2 [19.9-52.4][b]	15.2 [19.9-52.4][b]
Effector helper T cells (% of helper T cells) CD45 + CD3 + CD4 + CD45RA + CCR7-	1.6 [1.4-17.0][b]	1.2 [1.4-17.0][b]
Naïve suppressor T cells (% of suppressor T cells) CD45 + CD3 + CD8 + CD45RA + CCR7 +	58.6 [18.8-71.0][b]	40.3 [18.8-71.0][b]
Central memory suppressor T cells (% of suppressor T cells) CD45 + CD3 + CD8 + CD45RA-CCR7 +	1.5 [1.2-7.3][b]	11.1 [1.2-7.3][b]
Effector memory suppressor T cells (% of suppressor T cells) CD45 + CD3 + CD8 + CD45RA-CCR7-	10.5 [14.6-63.0] [b]	40.0 [14.6-63.0] [b]
Effector suppressor T cells (% of suppressor T cells) CD45 + CD3 + CD8 + CD45RA + CCR7-	29.4 [4.5-33.7][b]	8.7 [4.5-33.7][b]
Activated T cells (% of helper T cells) CD45 + CD3 + CD4 + CD25 + HLA-DR +	0.7	1.8
Th1 cells (% of memory T cells) CD45 + CD3 + CD4 + CD45RO + CCR6 + CXCR3 +	4.4	5.5
Recent thymic emigrants (% of helper T cells) CD45 + CD3 + CD4 + CD45RA + CD31 +	47.1 [56.8-72.8][a]	21.3 [6.4-51.0][a]
DNT cells (% of T cells) CD45 + CD3 + CD4-CD8-TCRa/b +	1.4 [< 2.5][c]	1.1 [< 2.5][c]
NK cells (% of lymphocytes) CD45 + CD3-CD16/56 +	14.2 [4.0-17.0][a]	31.7 [6.0-27.0][a]
B cells (% of lymphocytes) CD45 + CD3-CD19 +	12.0 [7.9-22.5][a]	8.0 [2.8-17.4][a]
Naïve follicular B cells (% of B cells) CD45 + CD19 + CD38-IgD/M + CD27-CD10-	71.0 [52.3-72.1] [a]	36.4 [42.0-82.0] [a]
Marginal zone B cells (% of B cells) CD45 + CD19 + CD38-IgD/M + CD27 + CD10-	8.4 [6.7-18.1][a]	24.4 [1.7-29.3][a]
Class switched B cells (% of B cells) CD45 + CD19 + CD38-IgD/M-CD27 + CD10-	4.9 [1.8-14.2][a]	24.6 [2.3-26.5][a]

Ref. ranges (reference ranges) were adapted from the following publications:
[a] van Gent R, et al. Clin Immunol. 2009 Oct;133(1):95-107.
[b] Boldt A, et al. Cytometry B Clin Cytom. 2014 May;86(3):191-206.
[c] Oliveira JB, et al. Blood. 2010 Oct 7;116(14):e35-40.

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Declaration of Competing Interest

None.

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