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Letter to the Editor

Severe *Toxoplasma gondii* infection in a member of a NFKB2-deficient family with T and B cell dysfunction

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To the Editor,

We report the case of a 9 years old girl (III.1), first referred at the age of 2 years for alopecia totalis, trachonychia and recurrent respiratory infections. Few months later, she was admitted for pneumonia and adrenal insufficiency secondary to ACTH hormone deficiency. The family history identified grandmother (I.2) and mother (II.2) with common variable immunodeficiency (CVID) (Fig. 1A). Her mother (II.2), also affected by ACTH deficiency, had alopecia areata during childhood. The grandmother (I.2) had developed later in life chronic intestinal CMV infection and died at the age of 68. The immune evaluation of the child showed mild decrease of IgG, low IgM and IgA, absence of isohemagglutinins, low specific response against *Tetanus*, *H. influenzae* and *S. pneumoniae*, but normal against measles and rubella. Immunoglobulin replacement therapy (IgRT) led to improvement of recurrent infections. She had persistent lymphocytosis, with normal T, B and NK distribution. Increased naïve T cell frequency with decreased T cell memory subsets were reported including marked reduction of regulatory T cells (Treg), T follicular helper (Tfh) and Th17. Low Treg was confirmed molecularly by measuring demethylation of the Treg-specific-demethylated region (TSDR) of *FOXP3*. However, Treg suppressive activity in vitro resulted normal. Decreased memory B cells were detected (Table 1) with impaired response to TLR9-ligand CpG. Similar perturbations in T and B cells differentiation were detected in her 36 years old mother (II.2) (Table 1, Supplementary Fig. E1, E2). The trend to increased naive versus memory cells becomes more evident with age, as shown by the mother's percentage of naive T cells (Table 1). Unfortunately data on grandmother (I.2) are not available. Despite CMV and EBV persistent viremia the girl had undergone a complete CMV- but incomplete EBV-seroconversion. The endocrinopathy was not associated with autoantibodies (Abs). However, we found high levels of Abs against multiple type I interferons (IFN-αB2, IFN-α2A, and IFN-ω), type III IFNs (IFN-λ1, IFN-λ2), and IL-12 using protein microarrays. Anti-IFN-ω Abs were confirmed in the patient's serum but were absent in the mother's (II.2) and grandmother's sera (I.2). At the age of 6 years, the child experienced severe bilateral visual impairment. Neurophysiology studies showed retinal and bilateral optical nerve damage. The MRI showed bilateral optic neuritis with severe nerve swelling and brain multifocal punctate hyperintense lesions suggesting an inflammatory and infective pathogenesis (Fig. 1D). Cerebrospinal fluid analysis detected *Toxoplasma gondii*, compatible with the recent contact with cats. As CMV and EBV viremia were still detectable, a possible role as triggers for generation of inflammation could not be excluded. Pyrimethamine, sulfadiazine and gancyclovir in combination with long course of systemic and intraocular steroids were started with gradual radiological and clinical improvement, recovering monolateral visual function. Whole exome-sequencing (WES), confirmed by Sanger sequencing, revealed a dominant heterozygous mutation c.2557C>T (p.Arg853*) in the *NFKB2* gene (Fig. 1A). This mutation has already been described [1,2] and causes a premature stop codon that generates a truncated p100 protein and diminished p52 level (Fig. 1B). An heterozygous mutation c.2309G>A (p.R770Q) in the *STAT5A* gene was detected in the child (III·I) and in her mother (II.2) (Fig. 1A).This variant has been described in the EXAC database but the functional significance has never been reported. In addition, an intronic variant of the *AIRE* gene c.1095 + 6G>A (NM_000383) was found in heterozygosity in patient (III.1) and mother (II.2), and in homozygosity in the grandmother (I.2). For this polymorphism splicing effects are not recognized, thus rendering unlikely a genetic *AIRE* contribution to the pathology. The NF-kB signalling pathways play a crucial role in innate and adaptive immune function, conferring resistance to infections in mice and humans. The canonical pathway (NFkB1, p105/p50) primarily mediates T cell differentiation and inflammatory response. The non-canonical pathway (NFkB2, p100/p52) is crucial for lymphoid organogenesis and B-cell differentiation. However, the two pathways show a close cross talk and an overlap in their function. Dominant mutations in *NFKB2* have been described in patients with CVID, autoimmunity, and ACTH deficiency, associated mostly to alterations in the B cell compartment [1–7] although alterations in T cell activation and terminal differentiation of memory T cells were recently reported in mice and humans [2,5,8]. This suggests a role of non-canonical pathway in the late T cell development. In the described family the same *NFKB2* mutation led to heterogeneous clinical phenotypes, ranging from a mild CVID to life threatening opportunistic and viral infections. Initially the child had only mild hypogammaglobulinemia but we observed a marked progressive reduction of B cells over time (Table 1), which moved from normal to barely detectable values. This reduction is probably consequent to the low T_{fh}, crucial for B cell differentiation and survival. Thus the immunological alterations due to NFkB2-deficiency could not always be detected through basic immunologic tests early in life. The severity of immune-dysregulation was associated with the detection of anti-cytokine auto-Abs which may contribute to the inability to clear the parasitic and viral infections. Anti-IFN Abs have been described especially in AIRE deficiency. This could support the notion that mutations in *NFKB2* lead to dysregulation of AIRE expression and subsequent autoimmunity [9]. Therefore, it might be reasonable to systematically check for anti-cytokine auto-Abs in this group of patients, to use an immunotherapy to modulate auto-Abs titer, in addition to the specific anti-microbial therapy. Alternatively, anti-cytokine Abs could be due to *NFKB2* mutation altering

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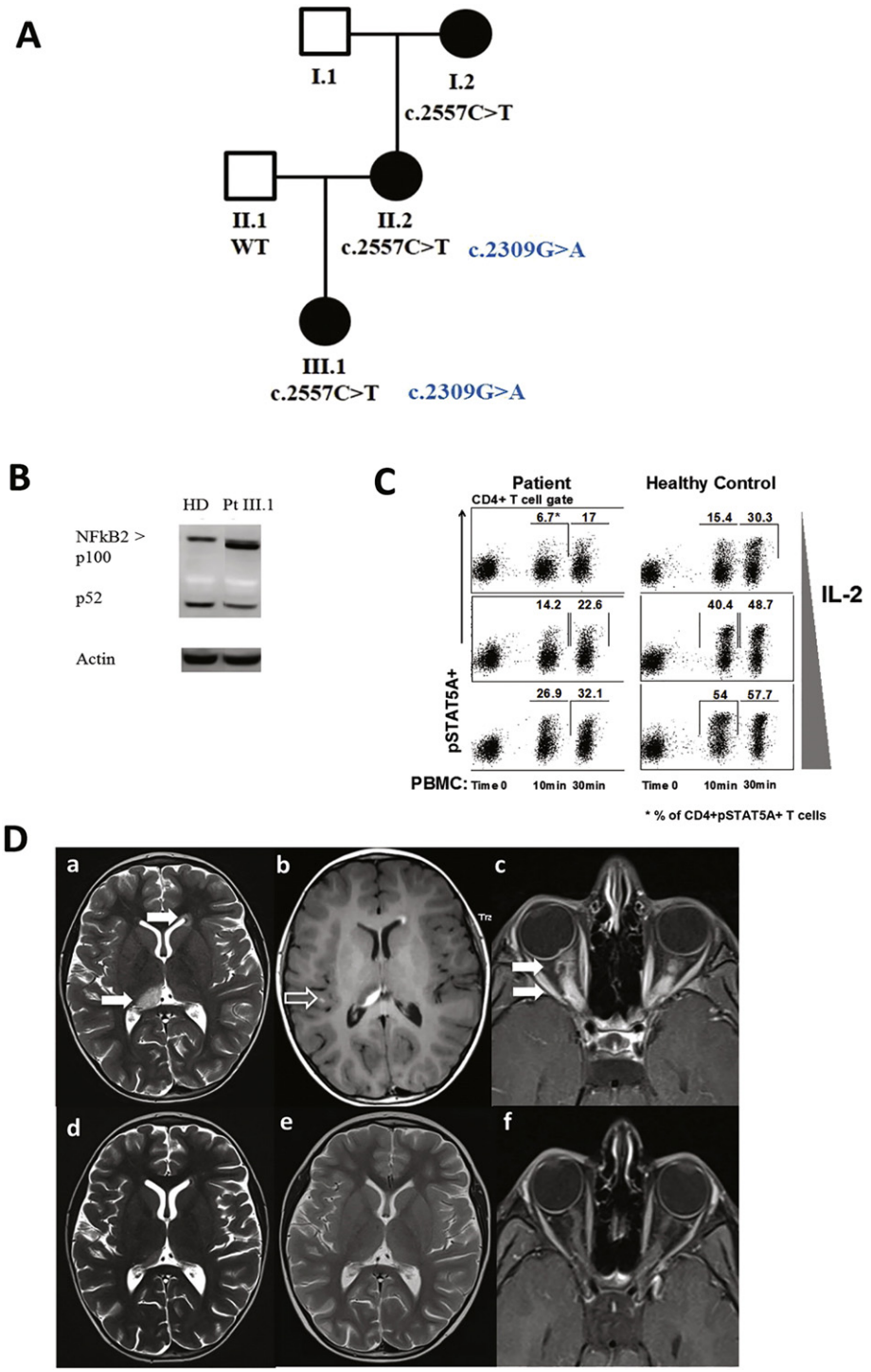

Fig. 1. A) Family pedigree. Mutation status of *NFKB2* and *STAT5A* are indicated for each subject in black and in blue respectively. Individual I.2 was wild type for *STAT5A*. Individual I.1 was unavailable for testing. B) Immunoblot of wild-type and truncated mutant NF-kB2/p100 (arrows) from whole-cell lysates of EBV-B cells derived from a healthy donor (HD) and III.1. C) Freshly isolated PBMC were stimulated in vitro with three different concentrations of IL-2, and pSTAT5A expression was determined in CD4 + T cells at 10 and 30 min of stimulation. Patient's CD4 + T cells expressed consistently lower levels of *STAT5A* phosphorilated compared to healthy control, at any IL-2 concentrations and time points. The result was confirmed in multiple experiments at different time points. D) MRI: T2w (a,d,e), Gd T1w (b) and Gd t1w fat-sat (c,f) axial images. MRI at onset shows thalamic and periventricular hyperintense lesions with intense contrast enhancement (a, b, arrows), punctate contrast enhancement of other subcortical temporal right lesion (b, open arrow) and diffuse thickening and contrast-enhancement of the optic nerves (c). Progressive reduction of the brain lesions and the involvement of the optic nerves after 1 month (d) and 6 months (e,f).

lymphocytes differentiation, triggered by the early encounter with viral infections, as also recently reported in patients with *RAG* hypomorphic mutations [10]. In addition, we detected in our patients a reduction in Treg frequency as reported by Lee et al. [4]. Quantitative defects of Treg have been associated with autoimmunity in immunodeficient patients. Other than a picture of immune-dysregulation, we described for the first time a severe opportunistic infection in a patient with *NFKB2* mutation. A direct role of NFkB2 in maintaining T cell mediated

Table 1
Clinical features and laboratory findings of affected individuals.

Parameter	III.1[a]				II.2[a]	
Sex	Female				Female	
Age	6 yr				36 yr	
Age at onset (infections)	<2 yr				Childhood	
Age at CVID diagnosis	2 yr				16 yr	
Infections	Upper respiratory infections, otitis media, pneumonia, CMV, Toxoplasma gondii				Pericarditis, otitis media, pneumonia, sinusitis	
Other clinical features	ACTH deficiency, alopecia universalis, trachyonychia asthma, bronchiectasis				ACTH deficiency, alopecia areata asthma, bronchiectasis	
Blood tests	**Age 2 yr**	**Age 6 yr**	**Age 8 yr**	**HS (2–6 yr)**	**Age 36 yr**	**HS (>16 yr)**
White blood cells (10^3/μL)	25.33	18.39	15.33	5.2–11.00	9.65	4.0–11.00
Lymphocyte subsets						
Total lymphocyte count (10^3/μL)	15.17	8.74	7.31	2.3–5.4[b]	3.03	1.60–2.40
CD3+ **[%/cell count (10^3/μL)]**	82.4/12.5	63.7/5.57	96/7.02	56–75/1.4–3.7[b]	83.6/2.5	56–84/1.0–2.2[b]
CD3 + CD4+	59.4/9.02	24.7/2.16	64/4.71	28–47/0.7–2.2[b]	70/2.12	31–52/0.5–1.3[b]
CD3+/CD8+	20.3/3.08	32.0/2.8	27.5/2.0	16–30/0.49–1.3[b]	13.3/0.40	18–35/0.33–0.0.9[b]
CD16+CD56+	8.9/1.35	31.3/2.74	3/0,22	04–17/0.13–0.72[b]	13.7/0.41	03–22/0.07–0.48[b]
CD19+	9.4/1.43	3.7/0.33	0.4/0.03	14–33/0.39–1.4[b]	1.8/0.055	06–23/0.11–0.57[b]
CD4+CD45RA+	85/7.67	82/1.8	87/4.1	53–86/0.42–1.5[b]	79.3/1.68	33–66/0.21–0.75[b]
CD4+CD45RO+	15/1.36	17.3/0.38	12/0.57	09–26/0.22–0.66[b]	20/0.42	18–38/0.24–0.7[b]
CD3+CD4+CD27+CD45RA+ Naïve CD4+	NT	66/1.43	81.8/3.86	69 (52–92)/0.2–2.5[c]	73.9/1.57	46(16–100)/0.1–2.3[c]
CD3+CD4+CD27+CD45RA− Central memory CD4+	NT	32.1/0.7	17.7/0.828	28(15–56)/0.037–0.51[c]	20/0.424	42(18–95)/0.18–1.1[c]
CD3+CD4+CD27−CD45RA− Effector memory CD4+	NT	1.8/0.04	0.47/0.03	2(0.26–9)/0.003–0.17[c]	0.67/0.015	5(1−23)/0.013–0.22[c]
CD3+CD4+CD27−CD45RA+ Effector memory CD4+ CD45RA	NT	0.17/0.001	0.09/0.001	0.14(0.006–1.2)/0.000025–0.0251[c]	0,1/0.003	0.35(0.008–6.8)/0.00009–0.068[c]
CD8+CD45RA+	79.8/2.46	60/1.68	74/1.49	69–97/0.2–0.65[b]	35/0.14	61–91/0,17–0.56[b]
CD8+CD45RO+	20/0.62	39/1.1	25/0.51	04–16/0.09–0.44[b]	47/0.2	04–23/0.06–0.31[b]
CD3+CD8+CCR7+CD45RA+ Naive CD8+	NT	35.3/0.99	57.1/1.15	46 (19–100)/0.042–1.3[c]	35,6/0.14	29 (6–100)/0.016–1[c]
CD3+/CD8+/CCR7+/CD45RA− Central Memory CD8+	NT	5.3/0.15	2.03/0.04	3(1–9)/0.0061–0.0043[c]	6/0.024	5(1−10)/0.0047–0.12[c]
CD3+/CD8+/CCR7−CD45RA− Effector memory CD8+	NT	50.4/1.42	33.2/0.67	23(10–55)/0.045–0.41[c]	47,1/0.19	36(14–98)/0.04–0.64[c]
CD3+/CD8+/CCR7−CD45RA+ Effector memory CD8+ CD45RA+	NT	9.2/0.26	7.5/0.15	22(6–83)/0.057–0.34[c]	11,2/0.004	19(7–53)/0.025–0.28[c]
CD27−IgD+naive B cells of CD19+	93.4/1.34	97.6/0.33	98.7/0.027	54–88.4/0.13–0.46[d]	95.7/0.054	48.4–79.7/0.04–0.47[d]
CD27+IgD+marginal zone/non switched memory B cells of CD19+	3.1/0.05	1/0.001	1/0.0003	2.7–19.8/0.02–0.1[d]	2.6/0.002	7.0–23.7/0.01–0.08[d]
CD27+IgD− switched memory B cells of CD19+	0.5/0.01	0.7/0.001	0.25/0,00008	4.7–21.2/0.04–0.14[d]	1.8/0.001	8.3–27.8/0.02–0.09[d]
CD4+CD25+CD127^{low}FOXP3+	1.4/0.13	1.6/0.04	1.2/0.057	4–8[e]	2/0,043	4–8[e]
CD3+CD4+ IL17+ %	NT	NT	0.048	0.6–1.85[e]	0.07	0.6–1.85[e]
CD4+CD45RO+CXCR5+ T_{fh}	NT	NT	0.59	2.6–5.8[e]	0.93	2.6–5.8[e]
Immunoglobulins (g/L)						
IgG	6	16.1[f]	11.9[f]	5–15,5[g]	10.8[f]	6–19[g]
IgM	0.1	0.2	0.15	0.6–2.9[g]	0.2	0.5–2.9[g]
IgA	0.05	0.05	0.05	0.2–1.7[g]	0.3	0.6–3[g]
IgE (kU/L)	<2	<2	<2	<40[g]	NT	
Isohemagglutinins	Absent				NT	
IgG Responses to						
Tetanus (IU/ml)	0.01			>0.6[h]	NT	>0.6[h]
Pneumococcus (mg/L)	3[i]			>35[h]	NT	>35[h]
OKT3-induced lymphoproliferation ($\times 10^3$ cpm)	34	NT	70	>20	NT	
PHA- induced lymphoproliferation	61		33	>30	NT	
TCRVβ Spectratyping	Polyclonal	Polyclonal			NT	
TREC	Normal				NT	
Autoantibodies						
Anti IFN-omega antibodies	Positive	1739.6 pmol/L[j]	NT	≤240 pmol/L[j]	Negative	≤240 pmol/L[j]
Antithyroid Ab (U/mL)	TPO 35.3 Tg < 20	TPO 28.8 Tg < 20	TPO 115 Tg < 20[k]	TPO 0.0–60[h] Tg 0.0–40[h]	TPO 85.3 Tg < 20	TPO 0.0–60[h] Tg 0.0–40[h]
Anti adrenal, pituitary Ab	Negative	Negative	Negative	Negative	Negative	Negative

Yr, years; HS, healthy subjects; Ab, antibody; Tfh, T helper Follicular; NT, not tested; TCR, T cell receptor; TPO Ab, Thyroid peroxidase Ab; Tg Ab, Thyroglobulin Ab.
[a] III.1[a] Parameters at the moment of diagnosis (2 yr) and during the follow up, II.2[a] parameters during therapy (IVIG).
[b] Reference median values from *Shearer et al. Allergy Clin Immunol 2003.*
[c] Reference range values (5th -95th percentile) from *Schatorié E.J.H.* et al. *Scand J Immunol. 2012.*
[d] Reference range values from *Piatosa* et al. *Cytometry B Clin Cytom. 2010.*
[e] Internal reference range from "Tor Vergata University Laboratories".
[f] On IVIG.
[g] Reference range from *Ladomenou F, Gaspar B. Arch Dis Child Educ Pract Ed 2016.*
[h] Reference range from "Bambino Gesù Hospital Laboratories".
[i] Obtained 1 month after immunization.
[j] Rerferences from" FIRS Laboratories".
[k] Reference range values (5th–95th percentile) from *Schatorié E.J.H.* et al. *Scand J Immunol. 2012.*

immunity against *Toxoplasma gondii* has been described in a *Nfkb2* −/− mouse model, which develops a severe toxoplasmic encephalitis [11]. A decrease in T effector cell populations, including the Th17 subset, is reported here as a novel finding in this group of patients. This defect could play a role in diminishing the host defence to fungi, parasites and viruses, possibly explaining susceptibility to opportunistic infections in our child (III.1) and in other two patients reported [1,7]. This hypothesis could provide a rationale for the use of specific antimicrobial prophylaxis in these patients. The girl (III.1) and her mother (II.2) harbour a second mutation in *STAT5A*, never described before. Although the clinical phenotype was consistent with NFkB2 deficiency, we investigated STAT5A function through flow cytometry analysis of STAT5A phosphorylation. The results revealed a reduced phosphorylation in patient (III.1) and in the mother (II.2) in different analyses at different ages (Fig. 1C and Supplementary E3). The particularly reduced STAT5 phosphorylation in memory T cells could play an additional role in the impaired Treg differentiation and in the increased susceptibility to opportunistic infections. Although we cannot draw definitive conclusions on the role of *STAT5A* mutation, we can hypothesise that it could influence the heterogeneous clinical presentation. Additional functional studies are demanded to clarify the role of this rare genetic variant. We described a family affected by *NFKB2* mutation with a great clinical variability and impairment in B and T cell differentiation. The severe clinical manifestations reported in a family member suggest that the use of specific antibiotic prophylaxis in association to immunosuppression should be considered. In some cases, the availability of a suitable donor could even lead to hematopoietic stem cell transplantation as therapeutic option.

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References

[1] K. Chen, E.M. Coonrod, A. Kumanovics, Z.F. Franks, J.D. Durtschi, R.L. Margraf, et al., Germline mutations in NFKB2 implicate the noncanonical NF-kappaB pathway in the pathogenesis of common variable immunodeficiency, Am. J. Hum. Genet. 93 (5) (2013 Nov 7) 812–824.
[2] V. Lougaris, G. Tabellini, M. Vitali, M. Baronio, O. Patrizi, G. Tampella, et al., Defective natural killer-cell cytotoxic activity in NFKB2-mutated CVID-like disease, J. Allergy Clin. Immunol. 135 (6) (2015 Jun) 1641–1643.
[3] T. Brue, M.H. Quentien, K. Khetchoumian, M. Bensa, J.M. Capo-Chichi, B. Delemer, et al., Mutations in NFKB2 and potential genetic heterogeneity in patients with DAVID syndrome, having variable endocrine and immune deficiencies, BMC Med. Genet. 15 (2014 Dec 19) 139.
[4] C.E. Lee, D.A. Fulcher, B. Whittle, R. Chand, N. Fewings, M. Field, et al., Autosomal-dominant B-cell deficiency with alopecia due to a mutation in NFKB2 that results in nonprocessable p100, Blood 124 (19) (2014 Nov 6) 2964–2972.
[5] A.W. Lindsley, Y. Qian, C.A. Valencia, K. Shah, K. Zhang, A. Assa'ad, Combined immune deficiency in a patient with a novel NFKB2 mutation, J. Clin. Immunol. 34 (8) (2014 Nov) 910–915.
[6] Y. Liu, S. Hanson, P. Gurugama, A. Jones, B. Clark, M.A. Ibrahim, Novel NFKB2 mutation in early-onset CVID, J. Clin. Immunol. 34 (6) (2014 Aug) 686–690.
[7] C. Shi, F. Wang, A. Tong, X.Q. Zhang, H.M. Song, Z.Y. Liu, et al., NFKB2 mutation in common variable immunodeficiency and isolated adrenocorticotropic hormone deficiency: a case report and review of literature, Medicine 95 (40) (2016 Oct), e5081.
[8] A.M. Rowe, S.E. Murray, H.P. Raue, Y. Koguchi, M.K. Slifka, Parker DC. A cell-intrinsic requirement for NF-kappaB-inducing kinase in CD4 and CD8 T cell memory, J. Immunol. 191 (7) (2013 Oct 1) 3663–3672.
[9] M. Zhu, R.K. Chin, P.A. Christiansen, J.C. Lo, X. Liu, C. Ware, et al., NF-kappaB2 is required for the establishment of central tolerance through an Aire-dependent pathway, J. Clin. Invest. 116 (11) (2006 Nov) 2964–2971.
[10] J.E. Walter, L.B. Rosen, K. Csomos, J.M. Rosenberg, D. Mathew, M. Keszei, et al., Broad-spectrum antibodies against self-antigens and cytokines in RAG deficiency, J. Clin. Invest. 126 (11) (2016 Nov 1) 4389.
[11] J. Caamaño, C. Tato, G. Cai, E.N. Villegas, K. Speirs, L. Craig, J. Alexander, C.A. Hunter, Identification of a role for NF-kappa B2 in the regulation of apoptosis and in maintenance of T cell-mediated immunity to Toxoplasma gondii, J. Immunol. 165 (10) (2000 Nov 15) 5720–5728.

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