

SUPPLEMENTARY INFORMATION:

Immunological investigations on three individuals with *FOSL2* pathogenic variants:

METHODS:

Immunophenotypic characterization of circulating lymphocytes was performed on FACSLyric or LSR II cytometers (BD Biosciences) after dyeing with the follow antibodies either on whole EDTA blood or on PBMC isolated from heparinated blood: BD Multitest™ CD3/CD8/CD45/CD4 (BD Biosciences), BD Multitest™ CD3/CD16+CD56/CD45/19 (BD Biosciences), CD21 FITC (BD Biosciences), CD24 PE (BD Biosciences), CD19 PE-Cy7 (BD Biosciences), IgM APC (Beckman), CD38 APC-H7 (BD Biosciences), CD27 BV421 (BD Biosciences), CD45 V500-C (BD Biosciences), IgD BV605 (BD Biosciences), CD45RA PE-Cy7 (BD Biosciences), CCR7 BV421 (BD Biosciences), CD31 BV605 (BD Biosciences), CD127 AF647 (BD Biosciences), HLA-DR APC R700 (BD Biosciences), FVS780 (BD Biosciences), CD39 BV421, CD45RA BV605 (Biolegend), CD25 BV786 (BD Biosciences), Ki67 FITC (Dako), Foxp3 PE-CF594 (BD Biosciences), CCR7 PC7 (BD Biosciences), CD3 BUV395 (BD Biosciences), CD4 BUV496 (BD Biosciences), CD8 BUV805 (BD Biosciences). Intracellular staining was performed following the manufacture instruction with the Foxp3/Transcription Factor Staining Buffer Set (ebiosciences). Cell counts were obtained using BD tubes TruCount (BD Biosciences).

The reactivity of lymphocytes was studied by measuring the proliferative response when stimulated in vitro by non-specific activators: phytohemagglutinin (10 µg/mL, SigmaAldrich) and pokeweed (0,1 µg/mL, SigmaAldrich) or reacted by specific antigens: Candidin (10 µg/mL, Serion Immunologic), Tetanus toxoid (50µg/mL; Statens) and Varicella-Zoster Virus (0.5µg/mL, Serion Immunologic). Briefly Peripheral Blood Mononuclear Cells (PBMC) were isolated from Heparinated whole blood by centrifugation on a ficoll density gradient. Cells (0.4×10^6) were cultured in a 96-well round-bottom plate in RPMI supplemented by 100 IU/mL penicillin, 100µg/mL streptomycin (SigmaAldrich) and 10% human serum AB (Jaques Boy) for 3 days for mitogens and 6 days for booster antigens. Finally H3T

(1 μ Ci6) was added 6 hours before the end of culture and terminal proliferation was assessed by measuring this incorporation of thymidine using a β counter (Top Count).

The presence of autoreactive antibodies in serum was screened by indirect immunofluorescence in accordance with the supplier's instruction on HEp2 cells (Werfen Inova) for anti-nuclear antibodies, on rat liver, kidney and stomach tissue (Werfen Inova) for hepatic antibodies and on tissue from monkey cerebellum (Werfen Inova) for neural paraneoplastic antibodies. Moreover, anti CCP and anti-extractable nuclear antigen SSA, SSB, Sm, RNP, Scl70 and Centromere B were tested in fluorescence enzyme immunoassay (Thermo Fisher Scientific). An automatized turbidimetric test was used to measure the presence of rheumatoid factor (The Binding Site).

RESULTS:

Individual #1 was reported to have an isolated specific polysaccharide antibody deficiency (impaired antibody response to polysaccharides antigens in the presence of normal serum IgG, IgA and IgM level rates, and normal IgG subclass levels and T-cell subpopulation). She was treated for recurrent infections by immunoglobulins (mainly recurrent otitis media). Three patients (#7, #9 and #11) were assessed for autoimmune disorders (Table S1). None of the three displayed antinuclear antibody, anti-dsDNA, anti-CCP, rheumatoid factor nor hepatic antibodies. As some patients exhibited neurological features, we tested for the presence of so-called paraneoplastic autoantibodies directed against central nervous system, but they were all negative. This absence of biological markers of autoimmunity is consistent with the normality of the blood Treg count found in these patients. All the blood counts for T, B and NK cells were in the normal range for the three patients, although two of them displayed a trend towards a rise in memory B cells. More peculiarly there was an increased frequency of the CD19posIgDposCD27pos unswitched memory B cell. These patients had normal T lymphocyte proliferation assays in response to the different mitogens and antigens.

Table S1: Immunological investigations on three individuals with *FOSL2* pathogenic variants.

Immunophenotype	Individual #7	Individual #9	Individual #11	Normal values
Lymphocytes (/μl)	1998	2,625	2,995	1,400-3,300
T cells (/μL) : CD3 ⁺	1,212	2,11	2,001	1,000-2,200
CD4 ⁺	625	1,202	1,238	530-1,300
CD8 ⁺	510	995	671	330-920
Naïve/memory (% T cells)				
Among CD4 T cells				
CD4 ⁺ CD45RA ⁺ CD31+CCR7 ⁺ (naive)	33	59	56	7-100%
CD4 ⁺ CD45RA ⁺ CCR7 ⁺ (naive)	50	76	74	34-61 %
CD4 ⁺ CD45RA ⁻ CCR7 ⁺ (central memory)	36	17	16	20-33 %
CD4 ⁺ CD45RA ⁻ CCR7 ⁻ (effector memory)	11	5	9	13-32 %
CD4 ⁺ CD45RA ⁺ CCR7 ⁻ (TEMRA)	4	2	2	1-9 %
Among CD8 T cells				
CD8 ⁺ CD45RA ⁺ CCR7 ⁺ (naive)	76	74	68	35-68 %
CD8 ⁺ CD45RA ⁻ CCR7 ⁺ (central memory)	6	5	1	3-11 %
CD8 ⁺ CD45RA ⁻ CCR7 ⁻ (effector memory)	6	3	2	9-26 %
CD8 ⁺ CD45RA ⁺ CCR7 ⁻ (TEMRA)	12	17	29	12-37 %
Regulatory T cells among T CD4				
	5	6	7	2-10 %
B cells (/μL) : CD19⁺				
	276	311	665	110-570
Naïve/memory (% B cells)				
CD27-IgD+ (naive)	63	64	74	62-87 %
CD27-IgD+IgM+ (naive)	63	63	72	
CD27+ (memory)	35	36	23	7-29 %
CD27+IgD- (switched memory)	21	11	14	4-21 %
CD27+IgD+ (unswitched memory)	14	25	9	3-13 %
CD27+IgD+IgM+ (marginal zone)	14	24	9	
CD24HiCD38Hi (transitional)	3	3	3	<10%
NK cells (/μL) : CD16⁺CD56⁺				
	472	159	314	70-480
T-cell proliferation (Stimulation index)				
PHA	177	426	458	>25
Pokeweed	71	43	51	>22
Candidin	18	94	30	>6
Tetanus toxoid	10	25	19	>11
VZV	100	48	40	>10
Specific antibodies				
Tetanus	Low Positive	Positive	Low Positive	
<i>S. pneumoniae</i>	NA	Low Positive	NA	
<i>H. influenzae</i> type b	Positive	Negative	Positive	
Diphtheria toxoid	Low Positive	Low Positive	Low Positive	
Auto-immunity				
Anti-nuclear antibodies (FAN)	Negative	Negative	Negative	
anti-DNA	<1	<1	<1	<15
anti-Extractable Nuclear Antigen (SSA,SSB,Sm,RNP,Sci70,JO1,CenB)	Negative	Negative	Negative	
anti-CCP	1	<1	<1	<10
rheumatoid factor	<8	<8	<8	<12,5
Anti-Smooth Muscle Actine	Negative	Negative	Negative	
Anti-LKM1	Negative	Negative	Negative	
anti-LC1	Negative	Negative	Negative	
Anti-Mitochondrial M2	Negative	Negative	Negative	
Paraneoplastic antibodies (HuD,Ri,Yo,CV2,Amphiphysine,Ma1,Ma2,Sox1, GAD65)	Negative	Negative	Negative	

Neuropsychological assessment and psychomotor development:

Patient #1 had a total intelligence quotient at 90 (WISC test assessed at 6-year-old) but was diagnosed with an ASD.

Patient #2 presented delayed speech development acquisition. She assessed a Kaufman assessment battery for children II at 4-year-old and her global cognitive ability was 60.

Her sister, **patient #3** had a normal psychomotor development, and no ASD.

Patient #4 had good speech abilities, and presented only a selective mutism.

Patient #5 had a normal psychomotor development at 3-month-old.

Patient #7 was schooled in a medical-educational institute at the age of 11 and presented with behavioral disorders, major anxiety and stereotypies treated by cyamemazine and risperidone. Autistic features were present since the age of 8 months. She was initially able to speak with short sentences but had a regression at puberty and was then able to speak with two words. She undergone a neuropsychological assessment aged 20 which revealed a severe ID. The WAIS-IV test was not feasible. The Vineland II scale found out an adaptive profile significantly lower than expected for the age. Communication and social skills were consistent with those expected for a child of 3 years and 5 months. Autonomy was very limited (needing helps with eating, dressing, toileting).

Patient #8 was able to speak with sentences, but also had difficulties in receptive language, and present ASD. Writing and reading abilities were limited by dyslexia, dyscalculia, and dysgraphia.

Patient #9 was able to speak, read and write during childhood but had a regression of autonomy in adulthood and failed employment in an inclusive workplace. The WAIS-IV test aged 20 revealed good verbal comprehension, but altered perceptual reasoning with difficulties in visual-motor coordination and nonverbal reasoning, short working memory, and slow processing speed.

Patient #11 was delayed in speech acquisition but was able to speak with sentences at 10-year-old. He presented ASD with aggressive behavior and ADHD treated with pipamperone.

Longer exposures of α -FLAG blots from figure 4A and 4B for better visualization of FLAG-tagged FOSL2 WT proteins in whole cell extracts (input 5%)

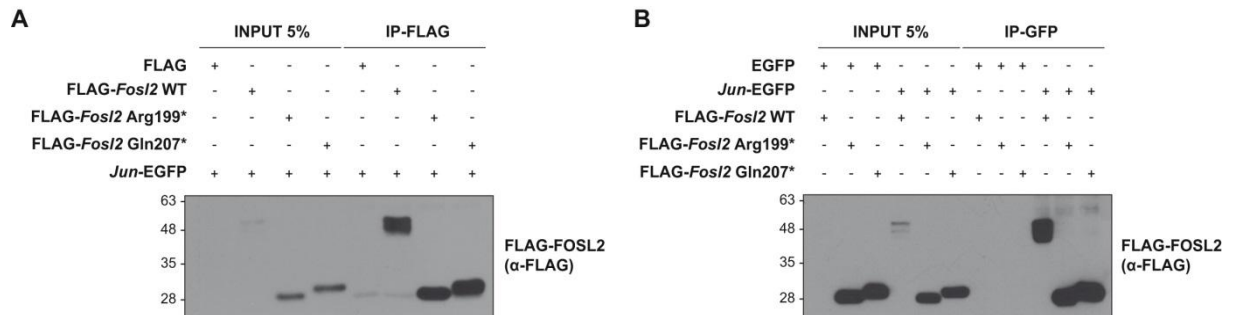


Figure S1: FRA2 Arg199* and Gln207* variants co-precipitate with c-JUN. A and B are longer exposure images of the same α -FLAG blots shown in Figure 4A and B, respectively. Note that in whole cell extracts (input 5%), the signal of FOSL2 WT protein can be better detected in the presence of c-JUN.