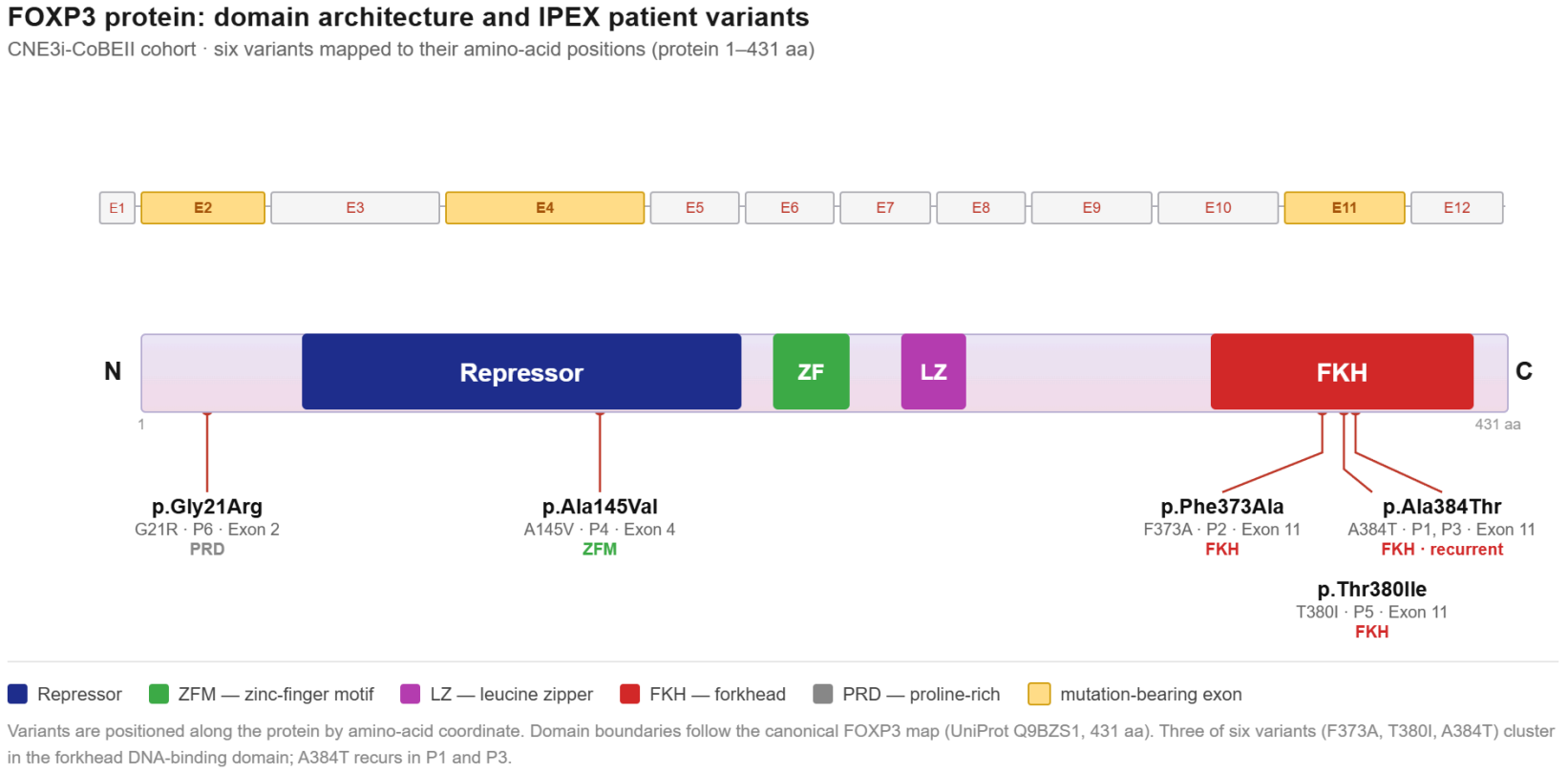
Table 1. Integrated clinical, molecular, immunological, and therapeutic summary of the Brazilian CNE3i-CoBEII IPEX cohort (n = 6). For each patient, the affected FOXP3 domain and variant are shown alongside disease burden, immunological findings, cytokine profile, and treatment. Autoimmune burden is the number of six autoimmune features (arthritis, enteropathy, vitiligo, thrombocytopenia, haemolytic anaemia, type 1 diabetes) present per patient, and allergy burden the number of five allergic features (rhinitis, conjunctivitis, asthma, eczema, food allergy), as detailed in Supplementary Table 1. Serum IgE (IU/mL) and IgG (mg/dL) are categorized relative to age-matched reference ranges (Supplementary File Table 4). Cytokine changes are reported relative to healthy controls (Supplementary Table 3); ↑ denotes elevation above the control level. *Abbreviations:* FKH, forkhead domain; ZFM, zinc-finger motif; PRD, proline-rich domain; BMT, bone marrow transplantation; GT, gene therapy.

Patient	Domain	Variant (protein)	Autoimmune burden	Allergy burden	IgE, IU/mL	IgG, mg/dL	Cytokine profile	Treatment escalation
P1	FKH	*p.Ala384Thr*	3/6	4/5	Low (4.9)	Normal (1057)	IL-17A↑	Sirolimus + BMT
P2	FKH	*p.Phe373Ala*	2/6	3/5	High (13,704)	Normal (1048)	IL-17A↑	Sirolimus + BMT
P3	FKH	*p.Ala384Thr*	4/6	3/5	Low (15.7)	Elevated (3334)	IL-17A↑	Sirolimus + biologics + GT
P4	ZFM	*p.Ala145Val*	1/6	5/5	High (3,094)	Low (414)	Not available	Sirolimus + BMT
P5	FKH	*p.Thr380Ile*	1/6	3/5	Low (<1)	Low (75)	IL-17A↑, IL-6↑	Sirolimus + biologics + BMT
P6	PRD	*p.Gly21Arg*	2/6	2/5	Low (15.7)	Normal (944)	Not available	Sirolimus + biologics + GT

Figure 1 — FOXP3 variants in the CNE3i-CoBEII IPEX cohort segregate into two mechanistic classes. Five distinct pathogenic variants across six patients mapped onto the FOXP3 protein (431 aa; NM_014009.4). Three cluster within aa 373–384 of the forkhead DNA-binding domain (p.Phe373Ala, p.Thr380Ile, p.Ala384Thr; the latter recurrent in P1 and P3) and were modelled structurally; two lie in the intrinsically disordered N-terminus (p.Gly21Arg, exon 1; p.Ala145Val, proline-rich repressor region), where no experimental structure exists for residues 1–190 and interpretation relied on disorder- and annotation-based analysis. Upper track, exon structure; lower track, domain architecture.



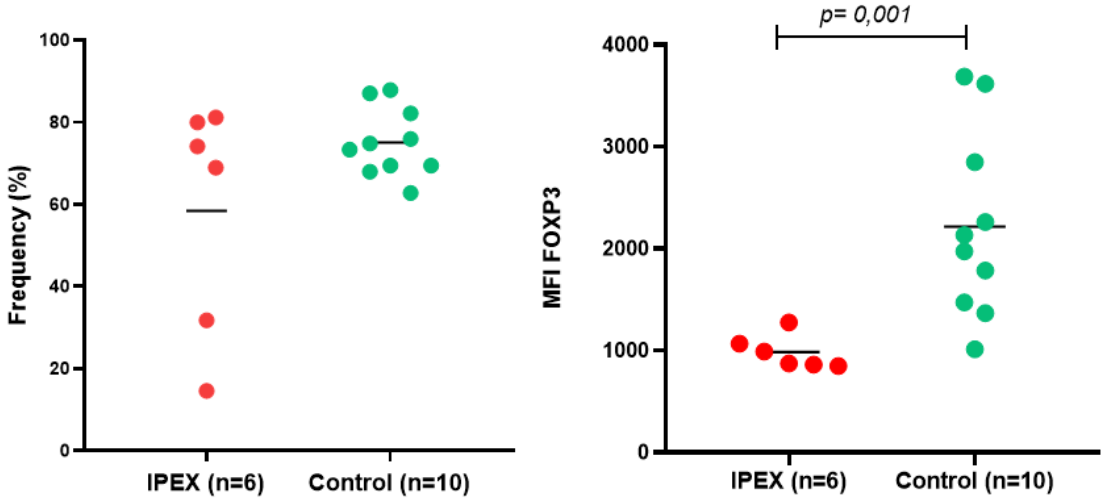
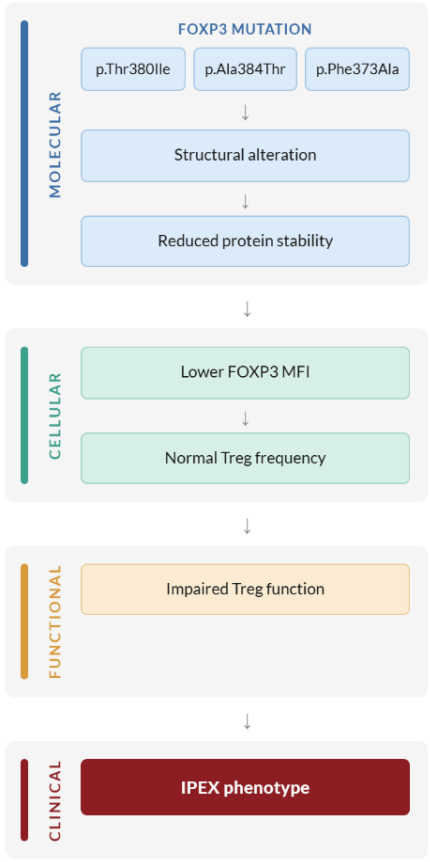
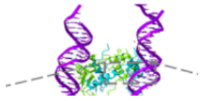


Figure 2. FOXP3 Treg frequency (%) and expression intensity (MFI) in IPEX patients versus controls. Left panel: Treg frequency ($CD4^+CD25^+CD127^-FOXP3^+$ %) was highly variable in IPEX patients and overlapped with healthy controls (p = ns). Right panel: FOXP3 expression intensity (MFI) was consistently and significantly reduced in all IPEX patients compared to controls ($p<0.001$). Each dot represents one individual; horizontal bars indicate medians. ns: not significant; Mann-Whitney test. Red dots: IPEX patients; green dots: healthy controls.

Figure 3. Structural basis of FOXP3 dysfunction in IPEX: FKH-domain variants perturb the DNA-bound dimer and link molecular defects to the clinical phenotype. *(Left)* Mechanistic cascade connecting FOXP3 pathogenic variants to the IPEX phenotype across the molecular, cellular, functional and clinical levels. The three variants modelled here (p.Thr380Ile, p.Ala384Thr, p.Phe373Ala) converge on structural alteration of the forkhead (FKH) domain and reduced protein stability, translating into lower FOXP3 mean fluorescence intensity (MFI) despite preserved Treg frequency, impaired Treg function and, ultimately, the IPEX phenotype. *(Right)* Integrated structural analysis of the FOXP3 FKH-domain variants in the DNA-bound dimer. The inset shows the complete FKH-domain dimer from PDB structure 3QRF, with chains F and G displayed in lawn green and dark turquoise, respectively, and the DNA chains in dark violet; the dashed line indicates the DNA-binding interface enlarged in panels A–F. In all panels, wild-type residues are shown in green and mutant residues in magenta, each indicated by a black arrowhead. Cyan dotted lines represent hydrogen bonds, numbered consistently throughout the figure: bond 1, His387–thymine 4015; bonds 2 and 3, Arg386–thymine 4014; bond 4, Ser390–deoxyribose moiety of nucleotide T4014; bond 5, Thr380–thymine 5008. Yellow dotted lines represent steric clashes and are marked by open arrowheads. **(A, B) p.Thr380Ile.** (A) Wild-type Thr380. (B) In the p.Thr380Ile model, hydrogen bond 5 is lost while bonds 1–4 remain preserved, and steric clashes arise between Ile380 in chain G and the DNA molecule. **(C, D) p.Ala384Thr.** (C) Wild-type Ala384, located adjacent to the DNA-contacting α-helix containing Arg386, His387 and Ser390. (D) In the p.Ala384Thr model, hydrogen bonds 1–4 are preserved, but Thr384 in chain G introduces steric clashes with Tyr342 of the adjacent chain F, perturbing the dimer interface. **(E, F) p.Phe373Ala.** (E) Wild-type Phe373. (F) In the p.Phe373Ala model, replacement of the bulky aromatic side chain by alanine occurs adjacent to the α-helices that establish direct hydrogen bonds with DNA, without loss of hydrogen bonds or steric clashes; open arrowheads indicate the position of residue 373 relative to those helices. Together, the three variants illustrate distinct routes to the same cascade: direct disruption of a DNA contact (p.Thr380Ile), perturbation of the dimer interface (p.Ala384Thr), and loss of core packing adjacent to the DNA-binding helices without contact loss (p.Phe373Ala).

Mutation to phenotype



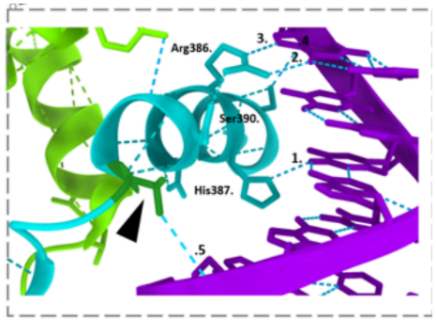


FOXP3 forkhead (FKH) domain – DNA-binding interface

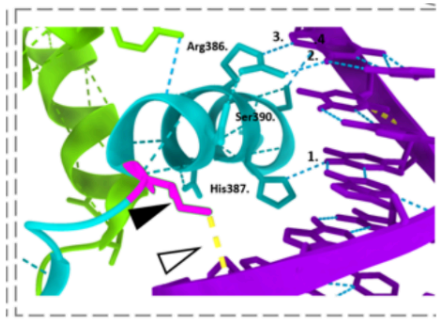
FOXP3 FKH domain of variants p.Thr380Ile, p.Ala384Thr and p.Phe373Ala – DNA-binding interface details.

p.Thr380Ile

A Wild type p.Thr380

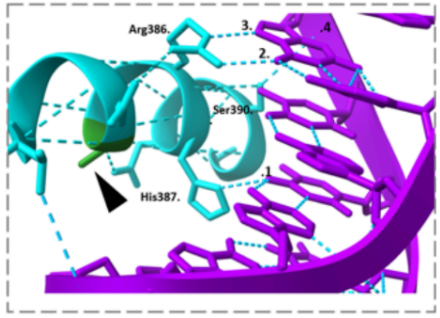


B Mutated p.Thr380Ile

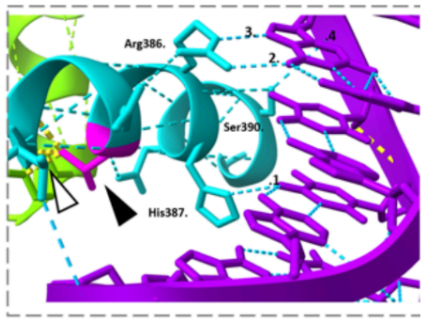


p.Ala384Thr

C Wild type p.Ala384

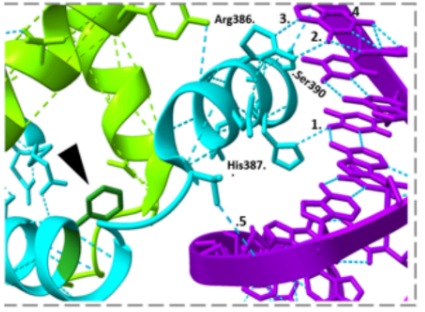


D Mutated p.Ala384Thr



p.Phe373Ala

E Wild type p.Phe373



F Mutated p.Phe373Ala