

Table S1 – Missense *RAG1* and *RAG2* mutations: protein expression, recombination activity, structural effects and in vivo clinical phenotype

Gene	Mutation	Domain	Protein Expression	Recombination Activity	Function affected (based on structural modeling)	Clinical Phenotype	References
<i>RAG1</i>	R314W	RING	↓↓	↓↓	Unknown	CID-G/AI	1, 2
	C328Y	RING	↓↓	↓↓	Unknown	OS	3
	C358Y	RING	nd	↓	Unknown	CVID	4
	K391E	RING	nd	↓↓↓	Unknown	OS	2
	R394Q	NBD	↓↓↓	↓↓↓	Nonamer binding	SCID	2, 5
	R394W	NBD	N	↓↓↓	Nonamer binding	OS	2, 6
	R396C	NBD	↓	↓↓↓	Nonamer binding	OS	2, 7
	R396H	NBD	↓↓↓	↓↓↓	Nonamer binding	OS	2, 7
	R396L	NBD	↓	↓↓↓	Nonamer binding	OS	2, 3
	S401P	NBD	↓↓↓	↓↓↓	Nonamer binding	OS	2, 3
	T403P	NBD	↓↓↓	↓↓↓	Nonamer binding	SCID	2
	R404Q	NBD	N	↓↓↓	Nonamer binding	SCID, OS	2, 8
	R410Q	NBD	↓↓↓	↓↓↓	Nonamer binding	OS, Leaky SCID	2, 3
	R410W	NBD	↓↓↓	↓↓↓	Nonamer binding	OS	2
	L411P	NBD	nd	↓↓↓	Structural integrity of NBD	OS	2, 9
	D429G	NBD	↓↓	↓↓↓	Structural integrity of NBD	SCID	2, 7
	V433M	NBD	↓↓	↓↓↓	Structural integrity of NBD	OS, Leaky SCID	2, 3
	M435V	NBD	↓↓	↓	Structural integrity of NBD	OS, CID-G/AI	2, 3
	A444V	NBD	nd	↓↓↓	Structural integrity of NBD	SCID, OS, Leaky SCID	2, 3
	L454Q	NBD	↓↓↓	↓↓↓	Structural integrity of RAG1 dimer	OS	2, 10
	R474C	DDBD	nd	N	Structure and DNA binding of DDBD	OS, CID-G/AI, ICL	2, 11, 12
	L506F	DDBD	nd	↓↓↓	Structural integrity of DDBD	ICL	2, 11
	L514R *	DDBD	nd	nd	Structure may be tolerated	CID-G/AI	-
G516A	DDBD	N	↓	Structural integrity of RAG1	CID-G/AI	2	
W522C	PreR	nd	↓	Structural integrity of PreR	CID-G/AI	2, 3, 12, 13	
D539V	PreR	nd	↓↓↓	Exposed, near RAG1/2 interface	OS	2	
R559S	PreR	↓↓↓	↓↓↓	At the edge of RAG1/2 interface	OS	2	

	R561H	PreR	↓↓↓	↓↓↓	RAG1/2 interface with T169 of RAG2	OS, $\gamma\delta$ T ⁺ SCID	2, 7
	H612R	RNH	N	N	Disordered structural integrity of RAG2 binding region	CID-G/AI	2, 14, 15
	R624H	RNH	nd	↓↓↓	Active site, adjacent to D600	SCID	2, 16
	R699Q/W	RNH	N	↓	Structural stability of RNH	CID-G/AI, CMO	2, 17
	E722K	ZnC2	nd	↓↓↓	RAG1/2 interface	SCID	2, 16
	Y728H *	ZnC2	nd	nd	Structural stability, may affect zinc finger and RAG2 interface	CID-G/AI	-
	C730F	ZnC2	↓↓↓	↓↓↓	Structural integrity of ZnC2	OS	2, 18
	L732P	ZnC2	N	↓↓↓	Structural integrity of ZnC2	SCID	2, 19
	R737H	ZnC2	↓	↓↓↓	Possibly DNA/coding end binding	OS	2, 7
	R764P	ZnC2	↓	↓↓↓	At the edge of RAG1/2 interface	SCID	2
	R764H	ZnC2	nd	↓↓	At the edge of RAG1/2 interface	IgAD	20
	E770K	ZnC2	nd	↓↓	RAG1/2 interface	SCID	2, 19
	R778Q	ZnC2	N	↓↓↓	Structural integrity of RAG1/2	SCID	1, 2
	R778W	ZnC2	↓	↓↓↓	Structural integrity of RAG1/2	SCID	2, 6, 21
	P786L	ZnC2	N	↓↓↓	RAG1/2 interface	OS	2
	R841W	ZnH2	↓↓↓	↓↓↓	Structural integrity of DNA binding near heptamer	$\gamma\delta$ T ⁺ SCID, Leaky SCID	2, 7, 22
	W896R	ZnH2	N	↓↓↓	Structural integrity of ZnH2	SCID	2, 23
	Y912C	ZnH2	N	↓↓↓	Structural integrity of ZnH2	OS	2, 7
	I956T	ZnH2	nd	↓↓↓	Structural integrity of ZnH2	OS	2, 6
	F974L	CTD	nd	↓	Structural integrity of CTD	CID-G/AI	2, 24
	R975Q	CTD	N	↓	Structural integrity of CTD	Leaky SCID, OS	2, 25
	R975W	CTD	nd	↓	Structural integrity of CTD	CID-G/AI	1, 2
	Q981P	CTD	↓↓↓	↓↓↓	Heptamer binding of inter-subunit	$\gamma\delta$ T ⁺ SCID	2, 22
	K992E	CTD	nd	↓↓↓	Probable DNA binding	OS	2, 18
RAG2	V8I ⁺ *	Core	nd	nd	Conserved structural change, may be tolerated	CID-G/AI	-
	G35A	Core	nd	↓↓	Structural integrity of RAG1/RAG2 interface (with E669 of RAG1)	CID-G/AI, HIGHM	26, 27
	G35V	Core	nd	↓↓↓**	Structural integrity of RAG1/RAG2 interface (with E669 of RAG1)	SCID	18
	R39G	Core	nd	↓↓↓**	Structural integrity of RAG1/RAG2 interface (with E719 and R773 of RAG1)	SCID	18

C41W	Core	nd	↓↓↓**	Structural integrity of RAG1/RAG2 interface	OS, CID-G/AI	7
F62L	Core	nd	↓↓	Structural integrity destabilizing the RAG complex	CID-G/AI	27
T77N	Core	nd	↓**	Moderately destabilizing the RAG complex	CID-G/AI	1
G95R	Core	nd	↓↓↓**	Structural integrity of RAG1/RAG2 interface	SCID	28
R229E	Core	nd	↓↓↓**	Structural integrity of RAG1/RAG2 interface (D546 of RAG1)	SCID	18
M285R	Core	nd	↓↓↓**	Partially exposed, maybe structural integrity	OS	7
M322T *	Core	nd	nd	Structure may be tolerated	CID-G/AI	-
D400H + *	Hinge	nd	nd	Unknown structural modeling	CID-G/AI	-
G451A	PHD	nd	↓/N**	Unknown structural modeling	CID-G/AI	1
M459L	PHD	nd	↓	Unknown structural modeling	OS, HIGM	27, 29

This Table includes a list of missense mutations in *RAG1* and *RAG2* genes for which information on protein expression, recombination activity, and/or effects on structure or function are known. Correlation with the clinical phenotype of affected patients is shown. The mutations reported were either present in homozygous state or, when compound heterozygous, corresponded to the allele with the higher recombination activity. For patients who were compound heterozygous for missense mutations whose recombination activity has not been tested, both alleles are reported and marked with the symbol ⁺. Novel mutations are marked with *, and recombination activity determined by other methods than the *Rag*^{-/-} Abelson virus-transformed pro-B cell platform is marked by **.

Semiquantitative description of protein expression (as % of wild-type) is as follows: ↓↓↓, 0-<20; ↓↓, 20-<50; ↓, 50-<80; N, ≥80.

Recombination activity (as % of the activity of wild-type) is expressed as follows: ↓↓↓, 0-<10; ↓↓, 10-<30; ↓, 30-<60; ↓/N, 60-<75; N, >75.

CID-G/AI: combined immune deficiency with granulomas and/or autoimmunity; CMO: chronic multifocal osteomyelitis; CTD: carboxy-terminal domain; CVID: common variable immune deficiency; DDBD: DNA binding and dimerization domain; $\gamma\delta$ T⁺ SCID: severe combined immunodeficiency with expansion of $\gamma\delta$ ⁺ T cells; HIGM: hyper-IgM; ICL: idiopathic CD4⁺ T cell lymphopenia; IgAD: IgA deficiency; NBD: nonamer-binding domain; OS: Omenn syndrome; PHD: plant homology domain; PreR: pre-RNase H-like domain; RNH: RNase H-like domain; SCID: severe combined immune deficiency; ZnC2: zinc-binding domain with two cysteine residues; ZnH2: ZnC2: zinc-binding domain with two histidine residues.

nd: not done

References to Supplementary Information Table S1

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