Comprehensive analysis of *ADA2* genetic variants and estimation of carrier frequency driven by a function-based approach

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Table E1. DADA2-associated variants grouped by variant type. [1-37]

Missense variants

M1T	A109D	L188V	L311R	F355L	V414M	V458D
R9W	P106S	P193L	G321A	A357T	N423K	Y482C
G25C	H112Q	F207S	G321E	G358R	M445K	W501R
R34W	H112Y	M232T	G326V	T360A	F446S	
R45W	N127I	D238N	E328D	N370I	G450C	
G47A	T129P	M243R	E328K	N370K	L451F	
G47R	Y130C	L249P	H335P	G383D	L451W	
G47V	R169Q	P251L	P344L	G383S	Y453C	
G47W	F178S	W264S	L351Q	C408Y	D454H	
I93T	L188P	S291L	Y353H	P409S	Y456C	

Single AA deletions

K55Del
F212Del
Y236Del

Frameshift variants

R49Gfs4*	T317Rfs*25
R49A fs*13	M465fsX
R131Sfs*52	K466Tfs*2
I143Sfs*41	S483Pfs*5
I210Tfs*57	K449N fs *2
Y227C fs*27	V325Tfs*7
D261P fs*2	

Nonsense variants

Y220X
S265X
R306X
312X
W399X
M465X
W501X

Table E2. Description of in silico prediction algorithms.

Algorithm (Ref)	Description
mCSM [38]	mCSM (mutation Cutoff Scanning Matrix) utilizes a graph-based approach in characterizing geometric patterns of the wild-type protein and predicting a given variants's impact on the protein's structural and functional stability.
SDM [39]	SDM (Site Directed Mutator) calculates the difference in free energy between wild-type and mutant proteins based on environment-specific amino-acid substitution frequencies within homologous protein families.
DUET [40]	DUET integrates mCSM and SDM to provide consensus predictions.
PoPMuSiC-2.1 [41]	PoPMuSiC-2.1 predicts the thermodynamic stability and solvent accessibility changes caused by amino acid substitutions.
SIFT [42]	SIFT (Sorting Intolerant from Tolerant) analyzes the evolutionary relationships of a target sequence to determines whether a given variant will likely impact protein functionality.
Polyphen2 [43]	Polyphen2 utilizes multiple sequence-based and structure-based features to predict the impact of amino acid substitutions. The HumDiv dataset was derived from all damaging alleles with known effects on the molecular function causing human Mendelian diseases, present in the UniProtKB database, together with differences between human proteins and their closely related mammalian homologs, assumed to be non-damaging. The HumVar dataset was comprised of all human disease-causing variants from UniProtKB, together with common human nsSNPs (MAF>1%) without annotated involvement in disease, which were treated as non-damaging.
REVEL [44]	REVEL (rare exome variant ensemble learner) is an ensemble method for evaluation of missense variants using data from 13 other algorithms.
MutationTaster [45]	Mutation Taster utilizes integrated data from GeneDistiller, Ensembl, SwissProt, UniProt2, and HapMap4 and determines variant deleteriousness using a naïve Bayes classifier.
Mutation Assessor [46]	Mutation Assessor predicts the functional impact of amino-acid substitutions by comparing protein family sequence alignments and effects of residue placement in known or homology-based 3D protein structures.
PROVEAN [47]	PROVEAN (Protein Variation Effect Analyzer) evaluates protein sequence homology, substitution frequency and the biochemical impact of amino acid substitution as well as insertions / deletions.
VEST 3.0 [48]	VEST (Variant Effect Scoring Tool) uses a supervised machine learning algorithm to identify likely functional missense variants based on training dataset of missense variants from the Human Gene Mutation Database and Exome Sequencing Project.
MetaSVM [49]	MetaSVM is an ensemble predictor that utilizes support vector machine (SVM) methodology to integrate data from 18 other prediction algorithms.
MetaLR [49]	MetaLR is an ensemble predictor that utilizes logistic regression (LR) methodology to integrate data from 18 other prediction algorithms.
M-CAP [50]	M-CAP (Mendelian Clinically Applicable Pathogenicity) in an ensemble predictor that incorporates 9 established pathogenicity likelihood scores; 7 seven established measures of base-pair, amino acid, genomic region, and gene conservation; and additional new features to facilitate machine learning.

Algorithm (Ref)	Description
CADD [51]	Combined Annotation Dependent Depletion (CADD) is an ensemble method that integrates diverse genome annotations to evaluate any possible human single nucleotide variant or small insertion/deletion event by machine learning.
DANN [52]	DANN is an ensemble method that uses the same feature set and training data as CADD to train a deep neural network (DNN).
FATHMM-MKL [53]	FATHMM-MKL integrates functional annotations from ENCODE with nucleotide-based sequence conservation measures based on multiple kernel learning (MKL).
Eigen [54]	Eigen is an unsupervised approach that integrates functional annotations of human genetic variations into one measure of functional importance without reliance on labeled training data.
GERP++ [55]	GERP(Genomic Evolutionary Rate Profiling)++ is a prediction tool based on evolutionary conservation that uses maximum likelihood evolutionary rate estimation for position-specific scoring and a novel dynamic programming approach to subsequently define constrained elements

Table E3. Functional analysis of ADA2 variants.[+]

Rank	Protein	Transcript	Chr 22 Position	RefSNP ID	Allele Frequency[+]	ADA2 activity*
1	p.His335Arg	c.1004A>G	17669306	rs2231495	3.44E-01	99.6
2	p.Val349Ile	c.1045G>A	17669265	rs74317375	2.14E-03	100.6
3	p.Met309Ile	c.927G>A	17670877	rs146597836	1.67E-03	34.3
4	p.Arg171Trp	c.511C>T	17687992	rs115986203	1.57E-03	78.9
5	p.Thr65Met	c.194C>T	17690374	rs61747288	6.29E-04	87.8
6	p.Pro435Ala	c.1303C>G	17662849	rs186147069	5.66E-04	32.0
7	p.Arg230Gln	c.689G>A	17684517	rs186116639	4.95E-04	89.3
8	p.Arg169Gln	c.506G>A	17687997	rs77563738	4.74E-04	0.5
9	p.Glu489Asp	c.1467G>C	17662442	rs61738625	4.00E-04	59.1
10	p.Phe355Leu	c.1065C>A	17669245	rs116020027	2.34E-04	58.6
11	p.Ile462Thr	c.1385T>C	17662767	rs61744537	1.45E-04	39.9
12	p.Met121Thr	c.362T>C	17688141	rs189403607	1.41E-04	57.6
13	p.Met84Val	c.250A>G	17690318	rs143853882	1.41E-04	94.2
14	p.Glu489Gln	c.1465G>C	17662444	rs45497794	1.34E-04	92.9
15	p.Arg49Trp	c.145C>T	17690423	rs199614299	1.10E-04	36.1
16	p.Gly47Arg	c.139G>A	17690429	rs202134424	1.06E-04	7.9
17	p.Val458Gly	c.1373T>G	17662779	rs748893301	1.03E-04	100.0
18	p.Tyr453Cys	c.1358A>G	17662794	rs376785840	8.84E-05	3.3
19	p.Phe211Tyr	c.632T>A	17684574	rs373928007	8.84E-05	18.2
20	p.Arg9Gln	c.26G>A	17690542	rs766367978	8.44E-05	30.2
21	p.Met121Ile	c.363G>A	17688140	rs756689332	8.36E-05	42.1
22	p.Arg154Cys	c.460C>T	17688043	rs200153182	8.35E-05	24.1
23	p.Arg45Gln	c.134G>A	17690434	rs571235882	8.13E-05	75.9
24	p.Asp4Asn	c.10G>A	17690558	rs370257828	7.91E-05	100.5
25	p.Arg34Trp	c.100C>T	17690468	rs750955849	7.78E-05	6.1
26	p.Met445Ile	c.1335G>A	17662817	rs1265300292	6.37E-05	75.4
27	p.Ser7Cys	c.20C>G	17690548	rs1157179878	6.37E-05	95.8
28	p.Thr213Ile	c.638C>T	17684568	rs1446203580	6.37E-05	98.4
29	p.Gly47Ala	c.140G>C	17690428	rs200930463	6.01E-05	20.9
30	p.Leu188Val	c.562C>G	17684644	rs765219776	5.98E-05	45.7
31	p.Thr317Met	c.950C>T	17670854	rs146788085	5.66E-05	90.4
32	p.Ala247Val	c.740C>T	17684466	rs750868279	5.31E-05	55.9
33	p.His91Arg	c.272A>G	17690296	rs149466386	5.30E-05	90.5
34	p.Leu311Arg	c.932T>G	17670872	rs780693700	5.17E-05	2.1
35	p.Val508Met	c.1522G>A	17662387	rs767440845	5.17E-05	94.0
36	p.Ala35Val	c.104C>T	17690464	rs151283756	4.95E-05	57.7
37	p.Ala345Thr	c.1033G>A	17669277	rs752798667	4.95E-05	94.3
38	p.Gly48Glu	c.143G>A	17690425	rs140149634	4.60E-05	38.8
39	p.Gly47Val	c.140G>T	17690428	rs200930463	4.60E-05	1.4
40	p.Asp329Asn	c.985G>A	17669325	rs369716341	4.25E-05	67.3
41	p.Gln145His	c.435G>C	17688068	rs752626692	4.24E-05	61.2

Rank	Protein	Transcript	Chr 22 Position	RefSNP ID	Allele Frequency+	ADA2 activity*
42	p.Ser205Leu	c.614C>T	17684592	rs369306297	3.58E-05	18.2
43	p.Gly383Ser	c.1147G>A	17663586	rs770689762	3.58E-05	1.9
44	p.Val418Leu	c.1252G>T	17662900	rs142726959	3.55E-05	30.4
45	p.Pro251Leu	c.752C>T	17684454	rs148936893	3.54E-05	4.0
46	p.Arg45Trp	c.133C>T	17690435	rs777683953	3.54E-05	55.5
47	p.Arg312Gln	c.935G>A	17670869	rs746970158	3.54E-05	65.3
48	p.Gly25Ser	c.73G>A	17690495	rs373732727	3.19E-05	71.1
49	p.Ile367Leu	c.1099A>C	17663634	rs780583418	3.18E-05	62.6
50	p.Asp167Gly	c.500A>G	17688003	rs745559968	3.18E-05	30.7
51	p.Met64Val	c.190A>G	17690378	rs771930342	3.18E-05	45.2
52	p.Arg154His	c.461G>A	17688042	rs545602214	3.18E-05	90.7
53	p.Met445Thr	c.1334T>C	17662818	rs776544525	3.18E-05	27.3
54	p.His391Arg	c.1172A>G	17663561	rs749413678	3.18E-05	84.4
55	p.Thr379Ala	c.1135A>G	17663598	rs561591791	3.18E-05	91.8
56	p.Ala357Thr	c.1069G>A	17669241	rs374974565	2.83E-05	35.5
57	p.Lys338Arg	c.1013A>G	17669297	rs573337330	2.83E-05	85.6
58	p.Arg369Gly	c.1105A>G	17663628	rs750824133	2.83E-05	87.4
59	p.Gly326Arg	c.976G>A	17669334	rs770635459	2.79E-05	0.4
60	p.Ala221Thr	c.661G>A	17684545	rs1417290846	2.78E-05	24.0
61	p.Gly358Arg	c.1072G>A	17669238	rs45511697	2.48E-05	1.7
62	p.Arg126Lys	c.377G>A	17688126	rs765071162	2.48E-05	90.2
63	p.Ala69Thr	c.205G>A	17690363	rs374965869	2.47E-05	81.9
64	p.Met460Ile	c.1380G>C	17662772	rs779529433	2.47E-05	33.8
65	p.Arg9Trp	c.25C>T	17690543	rs753994372	2.42E-05	9.5
66	p.His293Arg	c.878A>G	17672576	rs767494439	2.39E-05	61.1
67	p.Gly48Val	c.143G>T	17690425	rs140149634	2.39E-05	25.7
68	p.Gly47Arg	c.139G>C	17690429	rs202134424	2.39E-05	7.9
69	p.Met121Val	c.361A>G	17688142	rs750075105	2.39E-05	48.6
70	p.Ile93Thr	c.278T>C	17690290	rs767399919	2.39E-05	1.0
71	p.Tyr236Cys	c.707A>G	17684499	rs145966045	2.39E-05	18.8
72	p.Asp373Val	c.1118A>T	17663615	rs375443506	2.39E-05	99.6
73	p.Met243Ile	c.729G>A	17684477	rs151014930	2.12E-05	56.7
74	p.Thr492Ala	c.1474A>G	17662435	rs780459163	2.12E-05	93.9
75	p.Ala11Val	c.32C>T	17690536	rs147655483	2.01E-05	93.1
76	p.Pro106Ser	c.316C>T	17690252	rs747107966	1.99E-05	4.0
77	p.Pro193Leu	c.578C>T	17684628	rs199567025	1.99E-05	17.7
78	p.Leu28Pro	c.83T>C	17690485	rs777404100	1.99E-05	18.0
79	p.Gln97Glu	c.289C>G	17690279	rs370709874	1.99E-05	95.7
80	p.Ile196Thr	c.587T>C	17684619	rs538708563	1.99E-05	99.7
81	p.Met71Ile	c.213G>A	17690355	rs770042365	1.99E-05	0.4
82	p.His391Gln	c.1173C>G	17663560	rs780182069	1.99E-05	97.2
83	p.Arg306Gln	c.917G>A	17670887	rs768927379	1.77E-05	76.4
84	p.Arg230Trp	c.688C>T	17684518	rs777662147	1.77E-05	12.1

Rank	Protein	Transcript	Chr 22 Position	RefSNP ID	Allele Frequency[+]	ADA2 activity*
85	p.Gly25Cys	c.73G>T	17690495	rs373732727	1.59E-05	64.0
86	p.Lys481Asn	c.1443G>T	17662466	rs774795047	1.59E-05	51.8
87	p.His112Gln	c.336C>G	17688167	rs587777241	1.59E-05	1.1
88	p.Ile30Thr	c.89T>C	17690479	rs1350001862	1.59E-05	100.6
89	p.Thr120Ile	c.359C>T	17688144	rs755786336	1.59E-05	12.8
90	p.Glu94Asp	c.282G>C	17690286	rs761776202	1.59E-05	85.3
91	p.Asp297Tyr	c.889G>T	17670915	rs766661729	1.59E-05	23.7
92	p.Ala302Thr	c.904G>A	17670900	rs1333898789	1.59E-05	70.5
93	p.Ser452Cys	c.1355C>G	17662797	rs747458336	1.59E-05	10.5
94	p.Gly450Ala	c.1349G>C	17662803	rs771622323	1.59E-05	49.4
95	p.Ala393Thr	c.1177G>A	17663556	rs746332176	1.59E-05	94.4
96	p.His335Pro	c.1004A>C	17669306	rs2231495	1.42E-05	86.4
97	p.Phe354Leu	c.1060T>C	17669250	rs373498904	1.42E-05	34.1
98	p.Arg126Gly	c.376A>G	17688127	rs762848398	1.20E-05	9.8
99	p.Thr33Ala	c.97A>G	17690471	rs558427920	1.19E-05	91.3
100	p.Leu52Val	c.154C>G	17690414	rs776351526	1.19E-05	26.3
101	p.Met1?	c.1A>C	17690441	rs771035807	1.19E-05	2.5

[+] Data are ranked by allelic frequency in all populations from the Genome Aggregation Database (gnomAD).

* Enzymatic activity of each variant is expressed as % activity relative to wild-type ADA2.

Red text indicates DADA2-associated variant.

Abbreviations: RefSNP: reference single nucleotide polymorphism ID number

Table E4. Analysis of aggregate minor allelic frequency and most common deleterious ADA2 variants at different cut-offs of residual ADA2 activity.

	Cut-off = 10%		Cut-off = 25%		Cut-off = 35%	
All populations	1.637E-03	R169Q	2.122E-03	R169Q	4.618E-03	M309I
African	9.158E-04	R126G	1.098E-03	R126G	1.418E-03	M309I
Latino	1.483E-03	R34W	2.199E-03	R34W	7.901E-03	P435A
Ashkenazi Jewish	9.664E-05	R49Afs*13	9.664E-05	R49Afs*13	5.695E-03	M309I
East Asian	3.806E-04	I93T	8.657E-04	R154C	1.326E-03	M460I
Finnish European	2.652E-03	R169Q	3.112E-03	R169Q	3.152E-03	R169Q
Non-Finnish European	1.760E-03	R169Q	2.227E-03	R169Q	4.487E-03	M309I
South Asian	1.797E-03	G47R	2.483E-03	G47R	5.684E-03	M309I

Figure E1.

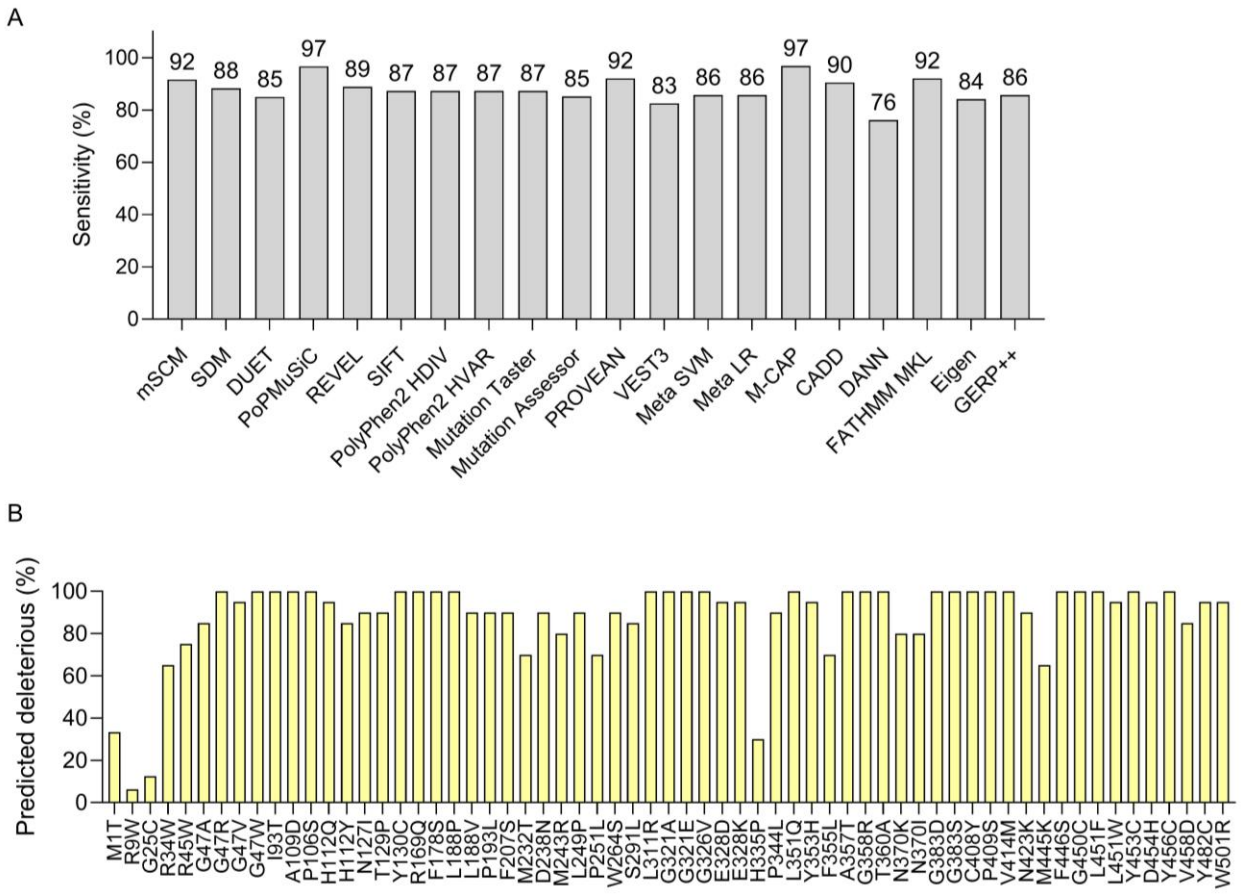

Figure E1. Evaluation of missense variants found in DADA2 patients by silico prediction algorithms. A) Sensitivity of various algorithms in predicting the pathogenicity of DADA2-associated variants. B) Proportion of algorithms predicting each variant as deleterious.

Figure E2.

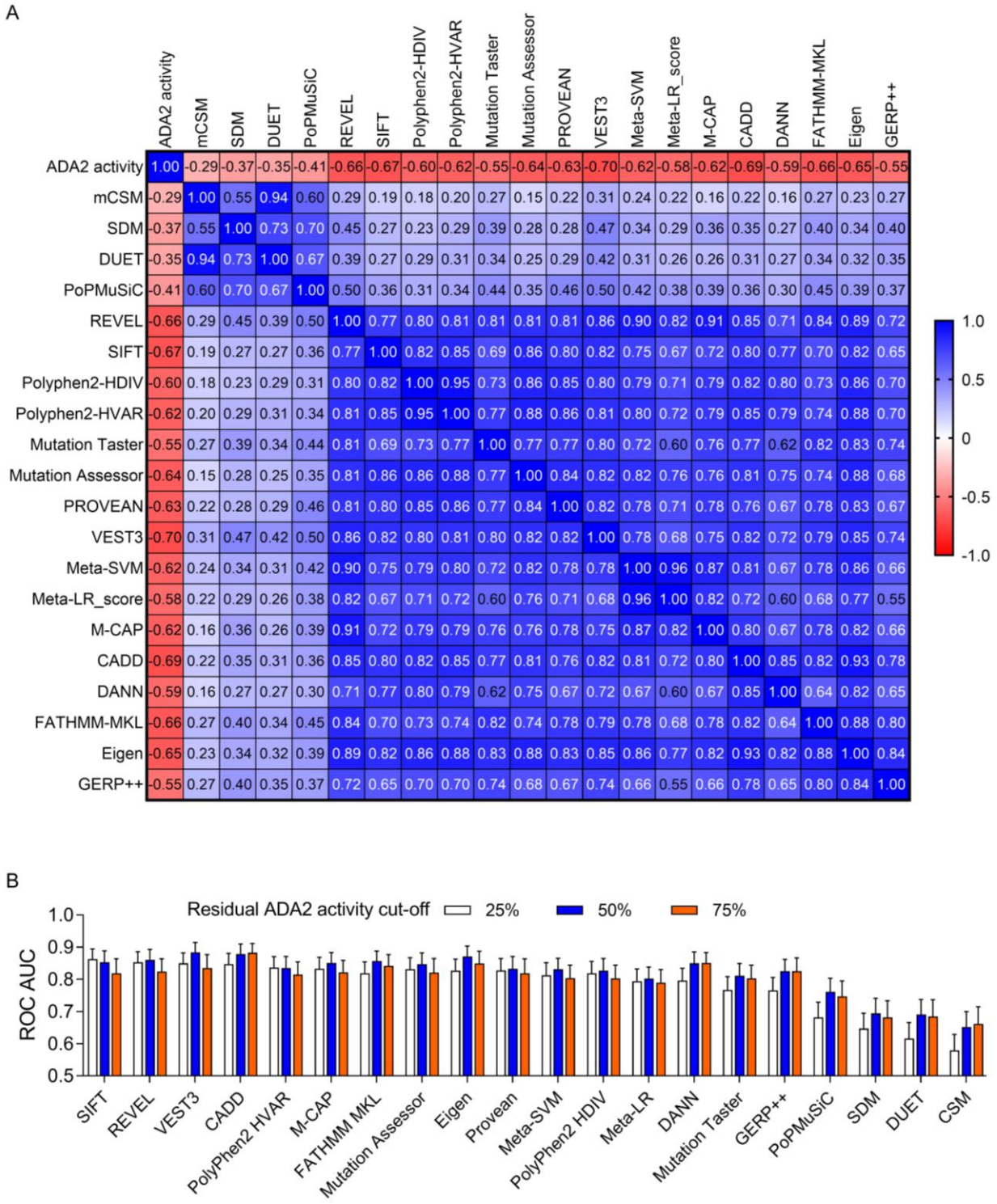

Figure E2. Evaluation of 100 ADA2 variants in gnomAD by in silico prediction algorithms. A) Correlation matrix of residual ADA2 activity and raw scores generated from prediction algorithms. B) Performance of prediction algorithms at different cut-offs of residual ADA2 activity used to determine deleterious variants.

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