Supplemental Data

Meta analysis of variant predictions in congenital adrenal hyperplasia caused by mutations in CYP21A2

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Table S1. Single predictors selected for performance analysis with *CYP21A2* variants.

Single Predictors	Description	Website	Ref.
CADD	Integrative annotation built based on diverse genomic feature derived from surrounding sequence context, gene model **annotation**, **evolutionary** constraint, **epigenetic** measurements, and **functional** predictions.	https://cadd.gs.washington.edu/	[1]
ConSurf	Algorithm uses phylogenetic relationships among homologous sequences and the specific dynamics of the analyzed sequence with **evolutionary** models to estimate the evolutionary rates of the amino acid of the macromolecules and to map them onto the structure and/or sequence.	https://consurf.tau.ac.il/	[2]
DANN	Deep neural network which takes non-linear relationships among features based on diverse genomic derived from surrounding sequence context, gene model **annotation**, **evolutionary** constraint, **epigenetic** measurements, and **functional** predictions.	https://cbcl.ics.uci.edu/public_data/DANN/	[3]
FATHMM	**Evolutionary** conservation algorithm which uses homologous sequences with species-specific weighting to predict the protein's tolerance to missense variants.	http://fathmm.biocompute.org.uk/	[4]
MAPP	A statistical framework predictor which uses protein **physicochemical** characteristics of each amino acid position on the **evolutionary** variation.	http://mendel.stanford.edu/sidowlab/downloads/MAPP/index.html	[5]
MutPred2	Machine learning-based to predict amino acid substitution through **evolutionary**, **structural,** and **functional** proprieties.	http://mutpred.mutdb.org/	[6]
PANTHER-PSEP	Predict using **evolutionary** preservation data, measuring though the length of time estimation that a site has been preserved.	http://www.pantherdb.org/tools/csnpScoreForm.jsp	[7]
PhD-SNP^{g}	Machine learning algorithm for predicting SNVs in both non-coding and coding regions through **evolutionary data**.	https://snps.biofold.org/phd-snpg/	[8]
PolyPhen-2	It uses human protein **evolutionary** and **structural** data to predict amino acid substitution effect on the protein stability and functionality.	http://genetics.bwh.harvard.edu/pph2/	[9]
PROVEN	Predict the functional effect through amino acid exchange **evolutionary** data and quality of the neighborhood sequence alignment rather than the target position.	http://provean.jcvi.org/genome_submit_2.php?species=human	[10]
SIFT	Predicts through sequence homology algorithm assuming **evolutionary** conserved regions tend to be less tolerant.	https://sift.bii.a-star.edu.sg/www/SIFT4G_vcf_submit.html	[11]
SNAP2	A neural network method based on machine learning to predict the variant effect in the molecular function through **evolutionary** and **structural** protein data with an amino acid substitution matrix of effect probabilities.	https://rostlab.org/services/snap2web/	[12]
SNPs&GO	Predict using **evolutionary** data, profile and gene ontology (biological process, cellular component and molecular function). When protein function is not available, it run PANTHER and PhD-SNP.	https://snps.biofold.org/snps-and-go/snps-and-go.html	[13]

Table S2. Performance of predictor tools.

Predictor	PPV	NPV	Se	Sp	Ac	MCC	AUC-ROC	Dataset	Ref.
CADD			93.6	57.1	0.85			ClinVar (2015)	[1]
DANN							0.95	ClinVar (2014)	[3]
FATHMM (weighted)	0.85	0.8	0.78	0.87	0.82	0.65		SwissVar (2012)	[4]
MAPP					0.626-0.767			Experimental studies	[5]
Meta-SNP	0.79	0.8	0.8	0.79	0.79	0.59	0.86	SwissVar (2009-2012)	[14]
MutPred2	96		42.3	95.6			84.9	ClinVar32 (2015) and UniProt80 (2015)	[6]
PANTHER-PSEP							0.721	Derived from SwissVar	[7]
PhD-SNPg	0.85	0.85	0.94	0.67	0.85	0.65	0.91	NewClinvar (2016)	[8]
PolyPhen-2*			0.85	0.6015			0.79	Mutations on the genes BRCA1, MSH2, MLH1 and TP53	[15]
PredictSNP					0.642	0.281	0.7	Protein Mutant Database (07Mar26)	[16]
PredictSNP2					0.773	0.55	0.804	Mendelian diseases (multiple databases)	[17]
PROVEAN			0.78	0.79				UniProt human protein	[10]
SNPs&GO	0.83	0.8	0.78	0.85	0.82	0.63		Derived from Swiss-Prot (2008)	[13]
SIFT 4G			0.8	0.735	0.7732	0.53		UniRef90 (2011)	[11]
SNAP2					0.688	0.24		Data set consisting of 9,657 variants from 678 human proteins	[12]
SNP&GO3d	0.84	0.86	0.87	0.83	0.85	0.7	0.92	Derived from Swiss-Prot (2009)	[18]

*Data from an article recommended on the original developer article. PPV, positive predictive value; NPV, negative predictive value; Se, sensibility; Sp, specificity; Ac, accuracy; MCC, Matthews' correlation coefficient test.

Table S3..List of the 103 single nucleotide variants (SNVs) on *CYP21A2* gene selected to test the performance of predictor tools. SNVs are grouped into classical (enzyme activity < 10%), non-classical (between 10 and 78 %) and neutral (> 78 %) groups. The enzyme activity levels of both 21-hydroxylase substrates - 17-hydroxyprogesterone and progesterone - were obtained from the original paper of the functional characterization. The phenotype was obtained from either the same paper or the original description of the new SNV. [a] Shows the percentage of enzyme activity measured for the conversion of both 21-hydroxylase substrates, considering as 100 % the 21-hydroxylase wild type activity. 17OHP: 17-hydroxyprogesterone. SW: salt wasting. SV: simple-virilizing. NC: non-classical. ND: non-determinate.

		CYP21A2 Activity *in vitro*					
Group	**NP_000491.4**	**17OHP[a]**	**SD (±)**	**Progesterone**	**SD (±)**	**Phenotype**	**Publication**
CL	p.P31Q	0.2	0.2	0	0	SW	[19]
	p.G57R	0.7	ND	1.4	ND	SV	[20]
	p.G65E	0	ND	0	ND	SW	[21]
	p.I78T	3	2	5	3	SV	[22]
	p.G91V	0	ND	0	ND	SW	[23]
	p.L108R	0.4	ND	0.3	ND	SW	[20]
	p.S114F	4	1	4	2	SV	[24]
	p.L123P	1.42	2.13	-1.86	5.19	SW	[25]
	p.V140E	0.7	1.3	0.5	0.6	SW	[26]
	p.L143P	0.4		0.4		SW	[20]
	p.C148R	4.3	0.9	3.6ny	1.8	SV-NC	[26]
	p.L167P	0.3	0.06	0.4	0.6	SW	[27]
	p.L168P	0.7	ND	0.4	ND	SW	[28]
	p.C170R	0.1	0.02	0	2	SW	[29]
	p.I172N	0.7	0.3	0.6	0.03	SV	[30]
	p.I173N	4.3	1.7	4.4	1.8	SV	[28]
	p.G179R	0.4	0.5	0	0.6	SW	[29]
	p.R234G	8	2	2	1	SV-NC	[31]
	p.I237N	1	0.7	2.4	1.4	SV	[32]
	p.V238E	0	0	0.1	0.3	SW	[32]
	p.V282G	3.9	1.7	3.9	2	SV	[33]
	p.H283N	1.6	6	2.7	5	SW	[34]
	p.G292C	0	ND	0	ND	SW	[23]
	p.G292R	0.5	0.7	0.7	0.2	SW	[26]
	p.G292S	0.8	0.4	0.8	0.4	SW	[35]
	p.G293D	0.5	0.2	0.7	0.4	SW	[28]
	p.L301F	9.5	6.4	4.4	2.5	SV	[33]
	p.W303S	3	0.3	3	0.5	SV-NC	[36]
	p.W303R	0.1	0.2	0	0.5	SW	[29]
	p.L309F	0.2	0.3	0.1	0.3	SW	[26]
	p.E321K	4.6	1.8	4.5	2.6	SV	[28]
	p.R342P	0.7	0.3	0.7	0.2	SV	[30]
	p.R342W	5	0.4	4	3	SV-NC	[31]
	p.E352K	1.1	0.5	1.2	0.3	SV	[37]
	p.R355H	0	ND	0	ND	SW	[23]
	p.R357P	0.15	0.3	0.15	0.3	SW	[38]
	p.R357Q	0.65	0.44	1.1	0.94	SV	[38]
	p.R357W	0	ND	0	ND	SW	[39]
	p.A363V	0	ND	0	ND	SW	[21]
	p.G376S	1.6	0.8	0.7	0.7	SW	[40]
	p.L389R	1.1	0.6	ND	ND	SW	[41]

Continuation (Table S3)

CL	p.H393Q	2.5	0.6	2.2	0.6	SW	[42]
	p.R409C	1.3	0.5			SW	[20]
	p.G425S	1.6	0.4	2	0.6	SV	[28]
	p.R427C	0	0.5	0	0.6	SW	[29]
	p.R427H	0.5	0.6	0.4	0.2	SW-SV	[30]
	p.L447P	0.5	0.6	0	0.1	SW-SV	[30]
	p.T451P	0.9	ND	0.9	ND	SW	[24]
	p.P464L	2.6	0.8	3	0.5	SV	[43]
	p.R484P	1	0.07	2.2	0.9	SV	[35]
	p.R484Q	1.1	0.7	3.8	1.9	SV	[27]
	Mean	**1.52**		**1.32**			
	SD	**2.00**		**1.61**			
NC	p.P31L	13	0.2	2	0.6	NC	[31]
	p.H63L	44.5	ND	20.7	ND	NC	[20]
	p.P106L	62	9	64	12	NC	[44]
	p.H120R	31.6	8	32.5	7	NC	[45]
	p.K122Q	14	5	19.5	4	NC	[46]
	p.R133C	35.4	7.4	15.5	2.7	NC	[47]
	p.E141K	11.3	2.4	ND	ND	SW	[41]
	p.R150C	35.8	14.6	47.3	12.9	NC	[47]
	p.R150P	23.4	1.7	16.9	2	NC	[48]
	p.M151R	17.66	1.87	4.57	1.96	NC	[25]
	p.G179A	19	ND	ND	ND	NC	[23]
	p.Y192H	37.1	7	25.8	9	NC	[34]
	p.I195N	33.2	9	46.7	10	NC	[45]
	p.R225W	51.9	9	45.6	8	NC	[49]
	p.I231T	63.1	22.3	70.6	17	NC	[28]
	p.R234K	15	ND	8.1	ND	SV-NC	[28]
	p.V282L	18	3	18	5	NC	[31]
	p.M284V	16.2	9.3	19	6.8	NC	[47]
	p.V305M	46	18	26	10	NC	[50]
	p.F307V	63.23	5.5	64.17	7.98	SV-NC	[51]
	p.D323G	18	1.2	27	4.7	NC	[36]
	p.R340H	67.1	2.4	45.8	3.7	NC	[52]
	p.V359I	72	7	34	3	NC	[53]
	p.H366N	46.13	4.8	57.77	3.69	NC	[51]
	p.R367C	37	7	28	4	NC	[31]
	p.R370Q	82	6	63	4	NC	[53]
	p.R370W	45.8	1.8	48.5	17.1	NC	[28]
	p.D378Y	81	6	58	4	NC	[53]
	p.E381D	30	ND	ND	ND	SW	[54]
	p.A392T	38.7	9.5	22.9	4.7	NC	[55]
	p.D408N	72.7	7	73.6	10	NC	[49]
	p.E432K	26.2	3.8	24.2	7.4	NC	[47]
	p.A435V	14	2	12	6	SV	[22]
	p.T451M	78	6	43	5	NC	[24]
	p.P454S	38	ND	22.4	3	NC	[31]
	p.L462P	55	8	40	2	NC	[53]
	p.M474I	85	7	66	12	NC	[31]
	p.R480L	75.5	15.7	79.6	12	NC-Normal	[55]
	p.P483S	61	6	54	2	NC	[31]
	Mean	**42.94**		**37.41**			
	SD	**22.59**		**20.98**			

Continuation (Table S3)

Neutral	p.L13M	99	1	100	1	Normal	[24]
	p.A16T	100	0	96	6	Normal- very mildNC	[24]
	p.R17C	95	3	81	3	Normal- very mildNC	[24]
	p.R103K	119.7	22.5	ND	ND	Normal	[41]
	p.A160T	126.6	29.9	ND	ND	Normal	[41]
	p.D184E	100	ND	100	ND	Normal	[56]
	p.S203G	85	2	81	3	Very mild NC	[24]
	p.V212M	99.5	32.4	ND	ND	Normal	[41]
	p.M240K	95.4	24.7	97.7	7.7	Normal	[32]
	p.A266S	90	9	104	15	Normal	[31]
	p.A266V	92	1.4	100	4.3	Normal	[36]
	p.P268L	97	1	87	7	Normal	[24]
	p.S269T	103	15	ND	ND	Normal	[57]
	Average	**100.17**		**94.08**			
	SD	**10.92**		**8.25**			

Table S4. Result of 17 predictors for 51 classical single nucleotide variants (SNVs) on the CYP21A2 gene. The classical group has an enzyme activity of < 10% of the wild-type activity. The genomic SNV nomenclature is based on the human chromatin remodeling 38 (Chr38). Del: deleterious; N: Neutral; Pby: Probably; Psb: Possible; B: Benign; Dse: Disease; Efc: Effect; P-Del: Proxy-deleterious; P-N: Proxy-neutral; Dmg: Damaging; T: Tolerated; Ptg: Pathogenic; Csv: Conserved; V: Variable; NR: no result.

Chr38	SNP	Meta-SNP	PredictSNP	PredictSNP2	S3Ds&GO	CADD	ConSurf	DANN	FATHMM	MAPP	MutPred2	PANTHER	PhD-SNPg	PolyPhen2	PROVEAN	SIFT	SNAP2	SNPs&GO
g.32038514C>A	p.P31Q	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	N;
g.32038591G>A	p.G57R	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32038616G>A	p.G65E	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32038752T>C	p.I78T	N	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	N	N	N	N
g.32038791G>T	p.G91V	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039124T>G	p.L108R	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039142C>T	p.S114F	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	B	Pby	Del	Del	Efc	Dse
g.32039169T>C	p.L123P	Dse	Del	N	Dse	P-Del	Csv	Del	T	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039220T>A	p.V140E	Dse	Del	N	Dse	P-Del	Csv	Del	T	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039229T>C	p.L143P	Dse	Del	N	Dse	P-Del	Csv	Del	T	Del	Del	Pby	Ptg	Pby	N	N	N	Dse
g.32039243T>C	p.C148R	N	N	N	N	P-Del	Csv	N	Dmg	Del	Del	Pby	Ptg	Psb	Del	N	N	N
g.32039408T>C	p.L167P	Dse	Del	N	Dse	P-Del	V	Del	T	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039411T>C	p.L168P	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	B	Pby	N	N	N	Dse
g.32039416T>C	p.C170R	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	B	Pby	Del	Del	Efc	Dse
g.32039423T>A	p.I172N	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039426T>A	p.I173N	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse

Continuation (Table S4)

Chr38	SNP	Meta-SNP	PredictSNP	PredictSNP2	S3Ds&GO	CADD	ConSurf	DANN	FATHMM	MAPP	MutPred2	PANTHER	PhD-SNPg	PolyPhen2	PROVEAN	SIFT	SNAP2	SNPs&GO
g.32039443G>A	p.G179R	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039797A>G	p.R234G	Dse	N	N	Dse	P-Del	Csv	Del	T	N	Del	Pby	B	Pby	Del	Del	Efc	Dse
g.32039807T>A	p.I237N	Dse	Del	N	N	P-Del	V	Del	T	Del	Del	Pby	Ptg	Psb	Del	Del	Efc	Dse
g.32039810T>A	p.V238E	Dse	Del	N	Dse	P-Del	Csv	Del	T	Del	Del	Pby	Ptg	Psb	Del	Del	Efc	Dse
g.32040111T>G	p.V282G	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040113C>A	p.H283N	Dse	N	N	Dse	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Pby	Del	Del	N	Dse
g.32040140G>A	p.G292S	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040140G>C	p.G292R	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040140G>T	p.G292C	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040144G>A	p.G293D	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040167C>T	p.L301F	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	N	Del	Pby	B	Pby	Del	Del	Efc	Dse
g.32040173T>C	p.W303R	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040174G>C	p.W303S	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040191C>T	p.L309F	N	N	Del	N	P-Del	Csv	Del	Dmg	N	N	Pby	B	Pby	Del	Del	N	N
g.32040427G>A	p.E321K	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040490C>T	p.R342W	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	N	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040491G>C	p.R342P	Dse	Del	N	Dse	P-Del	Csv	Del	T	Del	Del	Pby	B	Pby	Del	Del	Efc	Dse
g.32040520G>A	p.E352K	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040530G>A	p.R355H	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Psb	Del	Del	Efc	Dse

Continuation (Table S4)

Chr38	SNP	Meta-SNP	PredictSNP	PredictSNP2	S3Ds&GO	CADD	ConSurf	DANN	FATHMM	MAPP	MutPred2	PANTHER	PhD-SNPg	PolyPhen2	PROVEAN	SIFT	SNAP2	SNPs&GO
g.32040535C>T	p.R357W	Dse	Del	N	Dse	P-Del	Csv	Del	T	N	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040536G>A	p.R357Q	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040536G>C	p.R357P	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040554C>T	p.A363V	N	N	N	Dse	P-Del	Csv	Del	T	N	N	Pby	Ptg	Pby	N	N	N	N
g.32040675G>A	p.G376S	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	N	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040715T>G	p.L389R	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040728C>G	p.H393Q	N	N	N	Dse	P-Del	Csv	Del	T	N	Del	Pby	B	B	Del	Del	Efc	N
g.32040871C>T	p.R409C	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Psb	Del	Del	Efc	Dse
g.32040919G>A	p.G425S	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040925C>T	p.R427C	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040926G>A	p.R427H	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040986T>C	p.L447P	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	B	Pby	Del	Del	Efc	Dse
g.32040997A>C	p.T451P	Dse	Del	N	N	P-Del	Csv	Del	T	Del	Del	Pby	Ptg	Psb	Del	Del	N	N
g.32041037C>T	p.P464L	N	N	Del	N	P-Del	Csv	Del	Dmg	Del	N	Pby	Ptg	Pby	Del	Del	N	N
g.32041097G>A	p.R484Q	Dse	Del	Del	N	P-Del	Csv	Del	Dmg	N	Del	Pby	Ptg	Pby	Del	Del	Efc	N
g.32041097G>C	p.R484P	Dse	Del	N	N	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse

Table S5. Result of 17 predictors for 39 non-classical single nucleotide variants (SNVs) on the CYP21A2. The non-classical group has an enzyme activity between >10% and < 78% of the wild-type activity. The genomic SNV nomenclature is based on the human chromatin remodeling 38 (Chr38). Del: deleterious; N: Neutral; Pby: Probably; Psb: Possible; B: Benign; Dse: Disease; Efc: Effect; P-Del: Proxy-deleterious; P-N: Proxy-neutral; Dmg: Damaging; T: Tolerated; Ptg: Pathogenic; Csv: Conserved; V: Variable; NR: no result.

Chr38	SNP	Meta-SNP	PredictSNP	PredictSNP2	S3Ds&GO	CADD	ConSurf	DANN	FATHMM	MAPP	MutPred2	PANTHER	PhD-SNPg	PolyPhen2	PROVEAN	SIFT	SNP2	SNPs&GO
g.32038514C>T	p.P31L	N	N	N	N	P-Del	Csv	N	Dmg	Del	Del	Pby	Ptg	B	N	N	N	N
g.32038610A>T	p.H63L	Dse	N	N	N	P-Del	V	N	T	Del	Del	Pby	B	B	N	N	Efc	N
g.32039118C>T	p.P106L	N	N	N	N	P-N	V	N	T	N	N	Pby	B	B	N	N	Efc	N
g.32039160A>G	p.H120R	N	N	N	N	P-Del	Csv	Del	Dmg	Del	N	Pby	Ptg	Pby	Del	Del	Efc	N
g.32039165A>C	p.K122Q	Dse	Del	N	N	P-Del	Csv	Del	Dmg	Del	N	Pby	Ptg	Pby	Del	Del	Efc	N
g.32039198C>T	p.R133C	Dse	Del	N	Dse	P-Del	V	Del	T	N	N	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039222G>A	p.E141K	N	N	N	N	P-Del	V	Del	T	N	Del	Pby	Ptg	Psb	N	N	N	N
g.32039356C>T	p.R150C	N	N	Del	Dse	P-Del	Csv	Del	Dmg	N	N	Pby	B	Pby	N	N	N	N
g.32039357G>C	p.R150P	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	N	N	N	Dse
g.32039360T>G	p.M151R	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Psb	Del	Del	Efc	Dse
g.32039444G>C	p.G179A	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	N	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32039570T>C	p.Y192H	N	N	N	N	P-N	Csv	N	T	N	N	Pby	B	B	N	N	N	N
g.32039580T>A	p.I195N	N	N	N	Dse	P-Del	Csv	Del	T	N	Del	Pby	Ptg	Pby	N	Del	Efc	Dse
g.32039770C>T	p.R225W	Dse	N	N	N	P-N	V	N	T	N	Del	Pby	B	B	Del	N	N	N
g.32039789T>C	p.I231T	N	N	N	N	P-Del	V	Del	T	N	N	Pby	B	B	N	Del	N	N
g.32039798G>A	p.R234K	N	N	N	Dse	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Pby	N	Del	Efc	N

Continuation (Table S5)

Chr38	SNP	Meta-SNP	PredictSNP	PredictSNP2	S3Ds&GO	CADD	ConSurf	DANN	FATHMM	MAPP	MutPred2	PANTHER	PhD-SNPg	PolyPhen2	PROVEAN	SIFT	SNP2	SNPs&GO
g.32040110G>T	p.V282L	N	N	N	N	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Psb	N	N	N	N
g.32040116A>G	p.M284V	N	N	Del	Dse	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040179G>A	p.V305M	Dse	N	Del	N	P-Del	Csv	Del	Dmg	N	N	Pby	B	Pby	N	Del	N	N
g.32040185T>G	p.F307V	N	N	N	Dse	P-Del	Csv	Del	Dmg	N	Del	Pby	B	Pby	Del	Del	N	Dse
g.32040434A>G	p.D323G	Dse	Del	N	N	P-Del	V	Del	T	Del	Del	Pby	B	Pby	Del	Del	N	N
g.32040485G>A	p.R340H	Dse	Del	Del	N	P-Del	Csv	Del	Dmg	N	Del	Pby	B	Pby	Del	Del	Efc	Dse
g.32040541G>A	p.V359I	N	N	N	N	P-Del	Csv	Del	Dmg	N	N	Pby	B	Psb	N	Del	N	N
g.32040562C>A	p.H366N	Dse	Del	N	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	N
g.32040565C>T	p.R367C	N	N	N	N	P-Del	Csv	Del	T	N	Del	Pby	B	B	N	N	N	N
g.32040574C>T	p.R370W	Dse	Del	N	Dse	P-Del	Csv	Del	T	Del	N	Pby	B	Pby	Del	Del	Efc	Dse
g.32040575G>A	p.R370Q	N	N	N	N	P-Del	Csv	Del	T	N	N	Pby	B	Psb	N	N	N	N
g.32040681G>T	p.D378Y	N	N	N	N	P-Del	Csv	Del	T	N	Del	Pby	B	Psb	Del	N	N	N
g.32040692G>C	p.E381D	N	N	Del	N	P-Del	Csv	Del	Dmg	N	N	Pby	B	B	N	Del	Efc	N
g.32040723G>A	p.A392T	N	N	Del	Dse	P-Del	Csv	Del	Dmg	Del	N	Pby	B	Pby	N	N	N	Dse
g.32040771G>A	p.D408N	N	N	Del	N	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Pby	N	Del	N	N
g.32040940G>A	p.E432K	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	N	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040950C>T	p.A435V	Dse	Del	Del	Dse	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	Efc	Dse
g.32040998C>T	p.T451M	N	Del	N	N	P-Del	Csv	Del	T	Del	N	Pby	Ptg	Psb	Del	Del	N	N
g.32041006C>T	p.P454S	N	Del	Del	N	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Pby	Del	Del	N	N

Continuation (Table S5)

Chr38	SNP	Meta-SNP	PredictSNP	PredictSNP2	S3Ds&GO	CADD	ConSurf	DANN	FATHMM	MAPP	MutPred2	PANTHER	PhD-SNPg	PolyPhen2	PROVEAN	SIFT	SNP2	SNPs&GO
g.32041031T>C	p.L462P	Dse	Del	Del	N	P-Del	Csv	Del	Dmg	Del	Del	Pby	Ptg	Pby	Del	Del	N	N
g.32041068G>T	p.M474I	N	N	N	N	P-N	V	N	T	N	N	Pby	B	B	Del	N	N	N
g.32041085G>T	p.R480L	N	N	N	N	P-Del	V	Del	T	N	N	Pby	B	B	N	N	N	N
g.32041093C>T	p.P483S	N	N	Del	N	P-Del	Csv	Del	Dmg	N	N	Pby	Ptg	Pby	N	Del	Efc	N

43

44

Table S6. Result of 17 predictors for 13 neutral single nucleotide variants (SNVs) on the CYP21A2 gene. The neutral group has the enzyme activity known as > 78% of the wild-type activity. The genomic SNV nomenclature is based on the human chromatin remodeling 38 (Chr38). Del: deleterious; N: Neutral; Pby: Probably; Psb: Possible; Dse: Disease; Efc: Effect; P-Del: Proxy-deleterious; P-N: Proxy-neutral; Dmg: Damaging; T: Tolerated; Ptg: Pathogenic; B: Benign; Csv: Conserved; V: Variable; NR: no result.

Chr38	SNP	Meta-SNP	PredictSNP	PredictSNP2	S3Ds&GO	CADD	ConSurf	DANN	FATHMM	MAPP	MutPred2	PANTHER	PhD-SNPg	PolyPhen2	PROVEAN	SIFT	SNP2	SNPs&GO
g.32038459C>A	p.L13M	N	N	N	NR	P-Del	NR	Del	T	Del	N	NR	B	Pby	N	Del	N	N
g.32038468G>A	p.A16T	N	N	N	NR	P-N	NR	N	T	N	N	NR	B	B	N	N	N	N
g.32038471C>T	p.R17C	N	N	N	NR	P-N	NR	Del	T	N	N	NR	B	B	N	Del	N	N
g.32039109G>A	p.R103K	N	N	N	N	P-N	V	N	T	N	N	NR	B	B	N	N	N	N
g.32039386G>A	p.A160T	N	N	N	N	P-N	Csv	N	T	N	N	Pby	B	B	N	N	N	N
g.32039548C>G	p.D184E	N	N	N	N	P-N	V	N	T	N	N	Pby	B	B	N	N	N	N
g.32039603A>G	p.S203G	N	N	N	N	P-N	V	N	T	N	N	Pby	B	B	N	N	N	N
g.32039630G>A	p.V212M	N	N	N	N	P-N	V	Del	T	N	N	Pby	B	B	N	N	N	N
g.32039816T>A	p.M240K	N	N	N	N	P-Del	V	N	T	N	N	Pby	Ptg	B	N	N	N	N
g.32040062G>T	p.A266S	N	N	N	N	P-N	V	N	T	N	N	Pby	B	B	N	N	N	N
g.32040063C>T	p.A266V	N	N	N	N	P-N	V	Del	T	Del	N	Pby	B	B	N	N	N	N
g.32040069C>T	p.P268L	N	N	N	NR	P-N	V	N	T	N	N	Pby	B	B	N	N	N	N
g.32040072G>C	p.S269T	N	N	N	NR	P-N	V	N	T	NR	N	Pby	B	B	N	N	N	N

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