Table S1. Anti-D typing results for the 3 novel DAU samples

Patient		Reaction strength (0 to 4+)							
		Gel matrix test (anti-D clones)					Tube test		
DAU variant	Location	D7B8*	MAD2*	MS-201†	LDM1, RUM-1 TH28 ‡	Microtiter test § MS26, TH28	Immediate spin ‖	Antiglobulin phase ‖	Incubation 15 min at room temperature ¶
DAU-5.1	Linz	4+	1+	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
DAU-11	Salzburg	n.t.	n.t.	n.t.	4+	(+)	n.t.	n.t.	1+
DAU-11	NIH	n.t.	n.t.	3+	n.t.	n.t.	0	3+	n.t.

***** ORTHO Clinical Diagnostics, BioVue System ABO-Rh Grouping Cassette: D7B8 and MAD2 (both IgM)
† ORTHO Clinical Diagnostics, ID-Micro Typing System Gel Test Cards: MS-201 (IgM)
‡ Bio-Rad, ID-Card: LDM1, RUM-1 and TH28 (all IgM); oligoclonal anti-D
§ Immucor, ImmuClone Anti-D duo Galileo IgM + IgG: TH28 (IgM) and MS26 (IgG); oligoclonal anti-D
‖ Bio-Rad, Anti-D (RH1) Blend: BS232 (IgM), BS221 (IgG) and H41 11B7 (IgG); oligoclonal anti-D
¶ Bio-Rad, Anti-CDE (RH2,1,3): P3x61 (IgM) as the only anti-D in the oligoclonal antiserum
n.t. – not tested

Table S2. *DAU* variants and D antigen expression

Samples tested						
	Molecular substitution				Anti-D clone (titer)	
D variant	Nucleotide	Amino acid	Phenotype	D antigen density	HM16 (IgG)	BS226 (IgM)
Weak D type 25	341G>A	R114Q	CcDee	604	256	1
Weak D type 3	8C>G 254C>T	S3C A85V	CcDee	1117	1024	1024
DAU-11	835G>A 1136C>T	V279M T379M	ccDee	2483	2048	256
DVII	329T>C 667T>G	L110P F223V	CCDee	2528	1024	256
DAU-5.1	697G>C 1122C>T 1136C>T	E233Q I374I T379M	ccDee	6236	n.t.	n.t.
DVII	329T>C 667T>G	L110P F223V	CcDee	7936	4096	256
DAU-5	697G>C 1136C>T 667T>G	E233Q T379M F223V	ccDee	9849	1024	512
DAU-5	697G>C 1136C>T	E233Q T379M	ccDee	10,131	2048	512
Controls (n=3)*	No mutation		ccDee	22,105	4096	2048

* 3 different ccDee samples showed 22,105 ± 2210 D antigens per RBC (mean ± SD).
n.t. – not tested (sample not available)

Table S3. Serologic reactivity with panels of anti-D

Monoclonal anti-D			Reaction strength (0 to 4+)*			
Clone	Isotype	epD	DAU-11‖ ccDee	DAU-5.0‖ ccDee	Positive control ccDee	Negative control ccdee
LHM76/58†	$IgG_{1\lambda}$	ND	4+	2+	4+	0
LHM76/59†	IgG_1	ND	4+	4+	4+	0
LHM174/102†	$IgG_{3\kappa}$	1.2	3+	1+	4+	0
LHM50/2B†	$IgG_{1\lambda}$	6.3	4+	4+	4+	0
LHM169/81†	$IgG_{3\kappa}$	1.1	4+	2+	4+	0
ESD1†	$IgG_{1\kappa}$	ND	4+	4+	4+	0
LHM76/55†	$IgG_{1\kappa}$	3.1	3+	4+	4+	0
LHM77/64†	$IgG_{1\kappa}$	9.1	4+	4+	4+	0
LHM70/45†	$IgG_{1\lambda}$	1.2	1+	0	4+	0
LHM59/19†	$IgG_{3\kappa}$	8.1	3+	4+	4+	0
LHM169/80†	$IgG_{3\lambda}$	6.3	4+	4+	4+	0
LHM57/17†	$IgG_{1\lambda}$	6.3	3+	4+	4+	0
HM10‡	IgM	6.6	4+	4+	4+	0
HM16‡	IgG	6.4	4+	4+	4+	0
P3X61‡	IgM	6.1	4+	4+	4+	0
P3X35‡	IgG	5.4	4+	1+	4+	0
P3X212 11 F1‡	IgM	8.2	2+	2+	4+	0
P3X212 23 B10‡	IgM	9.1	2+	4+	4+	0
P3X241‡	IgG	5.4	4+	2+	4+	0
P3X249‡	IgG	2.1	4+	4+	4+	0
P3X290‡	IgG	3.1	4+	4+	4+	0
BS221§	IgG	6.3	3+	3+	3+	0
H41§	IgG	3.1	3+	4+	4+	0
BS226§	IgM	6.4	4+	4+	4+	0
BS232§	IgM	6.4	3+	4+	4+	0

* Gel matrix tests with antiglobulin; Anti-Human Globulin Anti-IgG (Rabbit) MTS Anti-IgG Card, Ortho

† ALBAclone Advanced Partial RhD typing kit, Alba Bioscience

‡ D-Screen, Diagast

§ Seraclone Anti-D (RH1) panel, Bio-Rad

‖ Testing for DAU-5.1 and DAU-11 (Salzburg sample) was not done because of lack of RBCs.

¶ DAU-3 sample for comparison with DAU-11 was not done because of lack of RBCs.

Table S4. The 18 known *DAU* alleles

	Nucleotide positions in *RHD* exons 1 to 10 (rs numbers)*																				
	1	2					3	4			5				6	7	8		9	10	
Allele	48	150	201	203	209	254	340	535	542	579	667	689	697	739	835	998	1122	1136	n.a.	n.a.	GenBank number
Reference*	G	T	G	G	G	C	C	T	T	G	T	G	G	G	G	G	C	C	-	-	NM_016124.4
DAU-0	─	─	─	─	─	─	─	─	─	─	─	─	─	─	─	─	─	T	─	─	n.a.
DAU-0.1	─	─	─	─	─	─	─	─	─	A	─	─	─	─	─	─	─	─	─	─	n.a.
DAU-0.2	─	C	─	─	─	─	─	─	─	─	─	─	─	─	─	─	─	─	─	─	n.a.
DAU-1	─	─	─	─	─	─	─	─	─	─	─	T	─	─	─	─	─	─	─	─	n.a.
DAU-2	─	─	─	─	A	─	─	─	─	─	─	─	─	─	─	A	─	─	─	─	n.a.
DAU-3	─	─	─	─	─	─	─	─	─	─	─	─	─	─	A	─	─	─	─	─	n.a.
DAU-4	─	─	─	─	─	─	─	─	─	─	─	─	A	─	─	─	─	─	─	─	n.a.
DAU-5	─	─	─	─	─	─	─	─	─	─	G	─	C	─	─	─	─	─	─	─	n.a.
DAU-5.1	─	─	─	─	─	─	─	─	─	─	G	─	C	─	─	─	T	─	─	─	HG918112.1
DAU-6	─	─	─	─	─	─	─	─	─	─	─	─	─	─	─	A	─	─	─	─	n.a.
DAU-7	─	─	─	─	─	─	─	─	─	─	─	─	─	─	A	A	─	─	─	─	n.a.
DAU-8	─	─	─	─	─	─	T	─	─	A	─	─	─	─	─	─	─	─	─	─	n.a.
DAU-9	─	─	─	─	─	─	─	C	─	─	─	─	─	─	─	─	─	─	─	─	n.a.
DAU10	─	─	─	─	─	─	─	─	─	A	─	─	─	C	─	─	─	─	─	─	n.a.
DAU-11	─	─	─	─	─	T	─	─	─	─	─	─	─	─	A	─	─	─	─	─	KU248927.1‡
DAU-12	─	─	─	─	─	─	─	─	C	─	─	─	─	─	─	─	─	─	─	─	JX193761.1
DAU-13	C	─	─	─	─	─	─	─	─	─	─	─	─	─	─	─	─	─	─	─	HG423861.1
DAU-14	─	─	A	A	─	─	─	─	─	─	─	─	─	─	─	─	─	─	─	─	KF861938.1
rs number†	772865539	rs1132758	41302032	62621068	142925159	139501061	novel	novel	149700508	77813628	1053356	374920252	1053359	novel	139704879	144996388	novel	61740966	n.a.	n.a.	

* The *RHD* mRNA RefSeqGene (GenBank accession number NM_016124.4) was used as the reference sequence. All variations are described according to current mutation nomenclature guidelines,[26] ascribing the A of the first ATG translational initiation codon as nucleotide +1 in the mRNA's coding region. The numbers on the top refer to the nucleotide positions of the exon polymorphisms of the *RHD* gene. No polymorphisms have been observed in exons 9 and 10 in any of the *DAU* alleles.

† rs number ─ reference SNP number

‡ Three non-coding variations, -368A>G, IVS3+117T>C and IVS3+124G>A, have previously been observed by us in other *RHD* alleles (data not shown) such as weak D type 1 and weak D type 4.0 and hence did not represent a prevalent allele used for the R*H*D reference sequence (GenBank accession number NG_007494.1).

n.a. – not applicable

Table S5. DAU variants and D antigen densities

	Molecular substitution				Immunohematology				
						Anti-D reactivity			
D variant	Nucleotide	Amino acid	Phenotype	Ethnicity	D antigen density	Weak	Variable	Anti-D in carriers	Reference
DAU-0	1136C>T	T379M	ccDee	European, African	15,285	No	No	n.r.	7,13
DAU-0.1	579G>A 1136C>T	E193E T379M	ccDee	African	n.r.	n.r.	n.r.	n.r.	13
DAU-0.2	150T>C 1136C>T	V50V T379M	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	*
DAU-1	689G>T 1136C>T	S230I T379M	ccDee	African	2,113	Yes	Yes	n.r.	7
DAU-2	209G>A 998G>A 1136C>T	R70Q S333N T379M	ccDee	African	373	Yes	Yes	n.r.	7
DAU-3	835G>A 1136C>T	V279M T379M	ccDee	African	10,879	n.r.	n.r.	Yes	7
DAU-4	697G>A 1136C>T	E233K T379M	ccDee	African	1,909	Yes	Yes	Yes	7,16
DAU-5	667T>G 697G>C 1136C>T	F223V E233Q T379M	ccDee	European, African	10,131	No	Yes	n.r.	10,14, Present study
DAU-5.1	667T>G 697G>C 1122C>T 1136C>T	F223V E233Q I374I T379M	ccDee	African	6236	No	Yes	n.r.	Present study
DAU-6	998G>A 1136C>T	S333N T379M	ccDee	African	n.r.	n.r.	n.r.	n.r.	14
DAU-7	835G>A 998G>A 1136C>T	V279M S333N T379M	ccDee	African	n.r.	Yes	n.r.	n.r.	15
DAU-8	340C>T 579G>A 1136C>T	R114W E193E T379M	ccDee	n.r.	n.r.	n.r.	n.r.	n.r.	17
DAU-9	535T>C 1136C>T	F179L T379M	ccDee	n.r.	n.r.	n.r.	n.r.	n.r.	17
DAU-10	579G>A 739G>C 1136C>T	E193E V247L T379M	ccDee	n.r.	n.r.	n.r.	n.r.	n.r.	17
DAU-11	254C>T 835G>A 1136C>T	A85V V279M T379M	ccDee	African, African American	2483	Yes	Yes	n.r.	Present study
DAU-12	542T>C 1136C>T	L181P T379M	ccDee	n.r.	n.r.	n.r.	n.r.	n.r.	JX193761.1
DAU-13	48G>C 1136C>T	W16C T379M	ccDee	African	n.r.	n.r.	n.r.	n.r.	HG423861.1
DAU-14	201G>A 203G>A 1136C>T	S67S S68N T379M	ccDee	Arabian	n.r.	No	n.r.	n.r.	18

* ISBT website (see Web resources)
n.r. ─ not reported

Table S6. The *RHCE* alleles associated with the 18 known *DAU* alleles

	Associated *RHCE* allele							
	Most likely allele				Supporting observations			
DAU allele	Allele	Trivial name	Evidence	Other possible allele	Reference	Observed at NIH (n)	Observed at Linz (n)	GenBank number
DAU-0	*RHCE*ce48C, 667T*	*RHCE*ceMO*	CWD	n.a.	51	n.a.	n.t.	n.a.
	*RHCE*ce48C, 662G*	n.a.	CWD *	n.a.	52	n.a.	n.t.	n.a.
	*RHCE*ce48C*	n.a.	CWD	*RHCE*ce*	53,54	1	n.t.	n.a.
	*RHCE*ce254G*	*RHCE*ceAG*	CWD	*RHCE*ce*	53,54	n.a.	n.t.	n.a.
	*RHCE*733G**	n.a.		*RHCE*ce*	53,54	n.a.	n.t.	n.a.
	*RHCE*ce48C,712G,787G,800A*	*RHCE*ceEK*	CWD *	*RHCE*ce*	n.a.	1	n.t.	KU556685
DAU-0.1	n.t.	n.a.		n.a.	n.a.	n.t.	n.t.	n.a.
DAU-0.2	n.t.	n.a.		n.a.	n.a.	n.t.	n.t.	n.a.
DAU-1	n.t.	n.a.		n.a.	n.a.	n.t.	n.t.	n.a.
DAU-2	n.t.	n.a.		n.a.	n.a.	n.t.	n.t.	n.a.
DAU-3	*RHCE*ce48C*	n.a.	CWD	*RHCE*cE*	53,54	1	n.t.	n.a.
DAU-4	*RHCE*ce48C*	n.a.		n.a.	53,54	n.t.	n.t.	n.a.
DAU-5	*RHCE*ce48C*	n.a.	CWD	*RHCE*ce48C, 254G*	53,54	3	n.t.	n.a.
DAU-5.1	*RHCE*ce48C, 105T*	n.a.		*RHCE*ce*	n.a.	n.t.	1	n.a.
DAU-6	n.t.	n.a.		n.a.	n.a.	n.t.	n.t.	n.a.
DAU-7	n.t.	n.a.		n.a.	n.a.	n.t.	n.t.	n.a.
DAU-8	n.t.	n.a.		n.a.	n.a.	n.t.	n.t.	n.a.
DAU-9	n.t.	n.a.		n.a.	n.a.	n.t.	n.t.	n.a.
DAU-10	n.t.	n.a.		n.a.	n.a.	n.t.	n.t.	n.a.
DAU-11 (NIH)	*RHCE*ce254G*	*RHCE*ceAG*		*RHCE*733G* †	n.a.	1	n.t.	n.a.
DAU-12	n.t.	n.a.		n.a.	n.a.	n.t.	n.t.	n.a.
DAU-13	n.t.	n.a.		n.a.	n.a.	n.t.	n.t.	n.a.
DAU-14	n.t.	n.a.		n.a.	n.a.	n.t.	n.t.	n.a.

* 1 case observed, confirmed by family study
† Often linked to conventional *RHD*[55]
CWD ─ common and well-documented haplotype
n.a. ─ not applicable
n.t. ─ not tested

Table S7. The *RHCE* alleles associated with *DAU* alleles*

RHCE genotype		*DAU* evidence (n)			
Allele 1	Allele 2	*DAU*	*DAU-0, -1, -2, -3*	*DAU-4, -5*	Total
*RHCE*ce48C, 733G*	*RHCE*ce*	11	1	0	12
*RHCE*ce48C*	*RHCE*ce*	1	4	1	6
*RHCE*ce48C*	*RHCE*ce48C*	2	1	1 †	4
*RHCE*ce48C, 667T*	*RHCE*ce*	3	0	0	3
*RHCE*ce48C, 1025T*	*RHCE*ce48C*	0	3	0	3
*RHCE*ce48C, 667T*	*RHCE*Ce*	1	2	0	3
*RHCE*ce48C*	*RHCE*Ce*	0	2	0	2
*RHCE*ce733G*	*RHCE*ce*	2	0	0	2
*RHCE*ce254G*	*RHCE*ce*	1	1	0	2
*RHCE*ce48C, 733G*	*RHCE*ce48C*	1	1	0	2
*RHCE*ce*	*RHCE*ce*	0	1	0	1
*RHCE*ce254G*	*RHCE*ce48C*	1 ‡	0	0	1
*RHCE*ce48C, 667T*	*RHCE*ce48C*	0	1	0	1
*RHCE*ce254G*	*RHCE*ce48C*	1	0	0	1
*RHCE*ce48C, 667T*	*RHCE*ce48C*	1	0	0	1
*RHCE*ce1025T*	*RHCE*ce733G*	0	1	0	1
*RHCE*ce48C, 1025T*	*RHCE*ce254G*	0	1	0	1
*RHCE*ce254G*	*RHCE*ce48C, 667T*	1	0	0	1
*RHCE*ce254G*	*RHCE*ceS*	1	0	0	1
*RHCE*ce48C, 733G*	*RHCE*ceS*	1	0	0	1
*RHCE*ce48C*	*RHCE*cE*	1	0	0	1
*RHCE*ce48C, 667T*	*RHCE*cE*	0	1	0	1
*RHCE*ce48C, 667T*	*RHCE*CE*	1	0	0	1

* Observations at BloodCenter of Wisconsin, where further molecular differentiation of "*DAU-0, -1, -2, -3*" and "*DAU-4, -5*" was not performed at this time.

† *DAU-0*/*DAU-5*

‡ *DAU*/*weak D type 4.2*

Table S8. *DAU* mutations and predicted effect on protein structure

DAU mutation			C-alpha atom distance from central pore (Å)	Bioinformatics program and computational analysis results					
Nucleotide position*	dbSNP reference no.	Amino acid substitution*		PolyPhen-2 †	SIFT‡	PROVEAN§	SNAP2‖	INPS (DDG)¶	Prediction **
48G>C	rs772865539	Trp16Cys	25.559	0.000	0.16	-2.476	-75	-0.738	Neutral
203G>A	rs62621068	Ser68Asn	6.782	0.012	0.19	-1.098	-79	-0.069	Neutral
209G>A	rs142925159	Arg70Gln	9.384	0.747	0.37	-2.292	-26	-0.529	Neutral
254C>T	rs139501061	Ala85Val	15.95	0.584	0.05	-2.483	-51	-0.057	Neutral
340C>T	n.a.	Arg114Trp	6.827	0.999	0.07	-2.610	37	0.028	Deleterious
535T>C	n.a.	Phe179Leu	12.858	1.000	0.05	-5.335	70	-1.323	Deleterious
542T>C	rs149700508	Leu181Pro	12.285	0.997	0.05	-5.647	83	-3.142	Deleterious
667T>G	rs1053356	Phe223Val	6.145	0.025	0.10	-5.062	93	-2.614	Neutral
689G>T	rs374920252	Ser230Ile	9.753	0.005	0.19	-1.117	-69	-0.502	Neutral
697G>A	rs1053359	Glu233Gln	12.654	0.062	0.47	-1.678	53	-0.153	Neutral
739G>C	n.a.	Val247Leu	18.408	0.990	0.05	-2.384	19	-0.376	Deleterious
835G>A	rs139704879	Val279Met	7.398	1.000	0.01	-2.749	61	-1.749	Deleterious
998G>A	rs144996388	Ser333Asn	9.508	0.254	0.00	-0.349	-4	-0.303	Neutral
1136C>T	rs61740966	Thr379Met	20.602	0.162	0.34	-0.162	-52	0.532	Neutral

* relative to NCBI Reference Sequence NM_016124.4 and NP_057208.2

† score 0.000–0.452 = benign, 0.453–0.956 = possibly damaging, 0.957–1.000 = probably damaging

‡ score ≤0.05 = damaging, >0.05 = tolerated; MIC = median sequence information (range 0 to 4.32)

§ score >−2.5 = neutral, ≤−2.5 = deleterious

‖ score<0 = no effect, >0 = effect, >50 = strong signal for effect

¶ DDG ≥ -0.5 and ≤ 0.5 = neutral; DDG ≥ -1.0 and < -0.5 = slightly destabilizing; DDG ≤1.0 and > 0.5 = slightly stabilizing; DDG ≥ -2.0 and < -1.0 = destabilizing; DDG ≤2.0 and > 1.0 = stabilizing; DDG < -2.0 = highly destabilizing; DDG > 2.0 = highly stabilizing

** To qualify as deleterious, the mutation had to be predicted as damaging by at least 3 of the 5 bioinformatics programs used.

n.a. not available

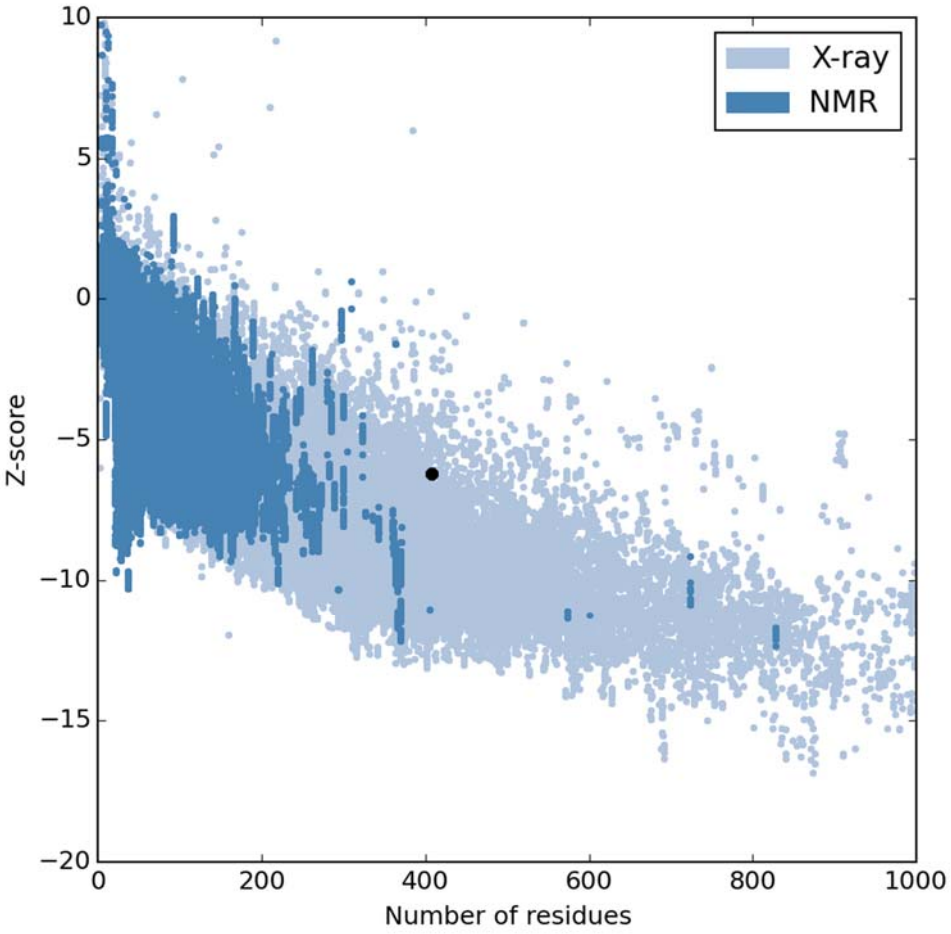

Figure S1

Fig. S1. ProSA-web z-score of RhD protein model. ProSA-web *z*-score of all protein chains in PDB are shown (dots) as determined by X-ray crystallography (light blue dots) or NMR-spectroscopy (dark blue dots) with respect to their length (number of residues). The *z*-score of RhD protein model is highlighted (black dot).

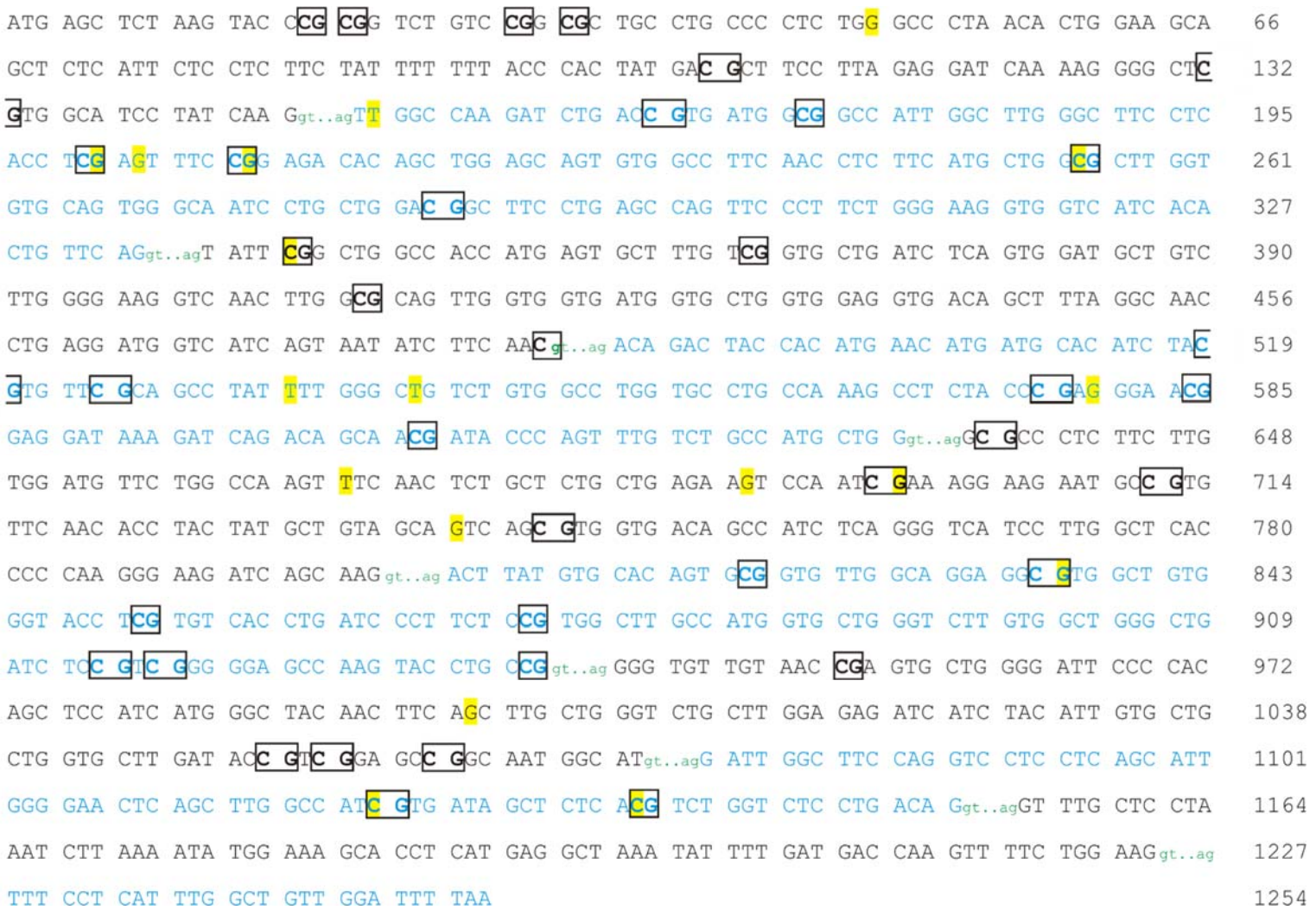

Figure S2

Fig. S2. Distribution of CpG dinucleotides in the *RHD* coding sequence (CDS). The nucleotides of the 10 exons (alternately black and blue) and the splice site nucleotides of the introns (green) are listed. The RhD CDS has 38 CpG sites (boxes). There are 18 nucleotide positions mutated in *DAU* alleles (yellow).