Supplementary Table S1. Molecular screening of serologic D-negative donors for the *RHD* gene in 58 studies

Population	Year	Screening method	*RHD* sequence tested	Serologic D-negative donors						Comments and Reference
				All (n)	*RHD* sequence positive (n)	%	C+/E+ (n)	*RHD* sequence positive (n)	%	
Japan	1997	PCR-SSP	intron 4, exon 10	130	38	29.2	103	38	36.9	1
Japan	1997	PCR-SSP	exons 4, 7, 10	306	102	33.3	247	102	41.3	2
Taiwan	1998	PCR-SSP	intron 4, exons 7, 10	230	85	37.0	na	na	na	3
China	2000	PCR-SSP	intron 4, exons 3 to 7, 9, 10	87	35	40.2	44	35	79.5	4
Germany	2001	PCR-SSP	5′-UTR, intron 4, exons 7, 10	8,442	50	0.6	754	48	6.4	South-West region[5]
Brazil	2002	PCR-SSP	intron 4, exon 10	206	27	13.1	na	na	na	6
China	2002	PCR-SSP	exons 1 to 10	102	38	37.3	na	na	na	7
China	2003	PCR-SSP	intron 4, exons 2 to 7, 9, 10	131	48	36.6	71	48	67.6	8
Taiwan	2003	PCR-RFLP	introns 1, 2, 4, 8, exons 1, 3 to 7, 9, 10	204	54	26.5	56	54	96.4	9
Taiwan	2003	PCR-SSP	exons 3, 4, 5, 7, 9	156	48	30.8	79	47	59.5	10
Taiwan	2004	PCR-SSP	exons 4, 5, 7, 9, 10	294	109	37.1	132	109	82.6	11
Europe[a]	2005	PCR-SSP	5′-UTR, exons 3, 10	na	na	na	1,700	89	5.2	12
China	2005	real-time PCR	exon 7	74	28	37.8	na	na	na	13
Korea	2005	PCR-SSP	intron 4, exons 3, 5, 7, 10	264	68	25.8	107	68	63.5	14
China	2006	PCR-SSP	exons 3 to 7, 9	515	79	15.3	235	79	33.6	15
Korea	2006	PCR-SSP	promoter, intron 4, exons 7 and 10	126	32	25.4	54	32	59.2	16
Austria	2007	real-time PCR	exons 4, 7, 10	2,427	3	0.1	na	na	na	17
Germany	2009	PCR-SSP	intron 4	46,133	96	0.2	3,080	81	2.6	18
China	2009	PCR-SSP	exons 3 to 7, 9	1,585	348	22.0	769	347	45.1	19
Austria	2009	real-time PCR	exons 4, 7, 10	23,330	94	0.4	1,934	62	3.2	20
Congo	2009	PCR-SSP	exons 4, 5, 7, 10	110	51	46.4	na	na	na	21
Denmark	2010	PCR-SSP	exon 10	na	na	na	233	7	4.4	22
Canada	2010	PCR-SSP	exons 4, 5	3,980	13	0.3	na	na	na	23
Denmark	2011	real-time PCR	exons 5, 7, 10	5,058	13	0.3	na	na	na	24
Italy	2011	PCR-SSP	introns 4 and 7, exon 7	na	na	na	235	12	5.1	25
Brazil	2012	PCR-SSP	intron 4, exon 10	239	22	9.2	18	11	61.1	26

Brazil	2012	PCR-SSP	intron 4, exon 7	2,450	101	4.1	na	na	na	27
Tunisia	2012	PCR-SSP	exon 10	448	11	2.5	45	10	22.2	28
Brazil	2012	real-time PCR	exons 7, 10	203	25	12.3	na	na	na	29
China	2012	PCR-SSP	5′-UTR, intron 4, exons 7, 10	155	35	22.6	na	na	na	30
Germany	2012	real-time PCR	exon 7	46,756	122	0.3	na	na	na	Northern region[31]
Poland	2013	real-time PCR	intron 4, exons 7, 10	31,200	63	0.2	na	na	na	32
Tunisia	2013	PCR-SSP	exons 3 to 7, 9	223	5	2.2	na	na	na	33
China	2013	PCR-SSP	exons 1, 4, 5, 6, 8, 9	2,493	808	32.4	1,093	723	66.1	34
Switzerland	2014	PCR-SSP	exons 3, 5, 10	25,370	120	0.5	na	na	na	35
Switzerland	2014	PCR-SSP	exons 3, 5, 10	26,243	65	0.2	na	na	na	36
Argentina	2014	PCR-SSP	intron 4 and 3′-UTR	1,314	27	2.1	114	19	16.7	37
Australia	2014	real-time PCR	exons 4, 5, 10	na	na	na	2,027	44	2.2	38
Tunisia	2014	PCR-SSP	exon 10	400	10	2.5	38	7	18.4	39
China	2014	PCR-SSP	exons 1 to 10	165	41	24.8	na	na	na	40
Brazil	2014	PCR-SSP	intron 4, exon 10	na	na	na	520	18	3.5	41
Tunisia	2014	PCR-SSP	exon 10	na	na	na	100	25	25.0	42
Netherlands	2016	MLPA	5′-UTR, exons 1 to 10	37,782	270	0.7	na	na	na	43
India	2018	QMPSF	exons 1 to 10	171	53	31	171	53	31	44
Brazil	2019	PCR-SSP	intron 4, exon 7	1,403	102	7.3	517	261	50.5	45
Iran	2019	real-time PCR	exons 5, 7, 10	200	2	1.0	16	2	12.5	46
United States	2019	ASOH	exons 1 to 10	1,163	48	4.1	81	5	6.2	BioArray *RHD* BeadChip[47]
Thailand	2019	QMPSF	exons 1 to 10	200	50	25	100	50	50	48
Iran	2021	real-time PCR	exons 5, 7, 10	200	2	1.0	na	na	na	49
Korea	2021	PCR-SSP	promoter, exons 4, 7, 10	na	na	na	177	109	61.6	50
China	2022	PCR-SSP	exon 10	104	26	25.0	na	na	na	51
India	2022	PCR-SSP	intron 4, exon 4, exon 7	200	5	2.5	190	4	2.1	52
Thailand	2022	QMPSF	exons 1 to 10	1270	307	24.2	598	305	51	53
Thailand	2022	PCR-SSP	exon 4	191	59	30.9	na	na	na	54
Congo	2024	PCR-SSP	exons 4, 5, 7, 10	59	53	89.8	na	na	na	55
BH[b]	2024	real-time PCR	exons 3, 5, 10	12,827	40	0.31	481	40	8.32	RBC-FluoGene[56]
Brazil	2024	PCR-SSP	intron 4, exon 7	648	17	2.6	na	na	na	57
Saudi Arabia	2024	PCR-SSP	exons 3, 4, 7	66	0	na	4	0	na	58

United States	2024	real-time PCR	intron 4, exons 5, 7	2,254	42	1.9	115	10	8.69	this study
Total				290,287	3,690	na	16,238	2,954	na	

a Multicenter study including Austria, Germany, Slovenia, Switzerland and Russia
b Bosnia and Herzegovina
na, not available or not applicable
PCR=polymerase chain reaction; PCR-SSP=PCR with sequence-specific primers; UTR=untranslated region; PCR-RFLP=PCR-restriction fragment length polymorphism; MLPA=multiplex ligation-dependent probe amplification; ASOH=allele specific oligonucleotide hybridization; QMPSF=quantitative multiplex polymerase chain reaction (PCR) of short fluorescent fragments

References

1. Okuda H, Kawano M, Iwamoto S, Tanaka M, Seno T, Okubo Y, et al. The *RHD* gene is highly detectable in RhD-negative Japanese donors. J Clin Invest 1997;100:373-9.
2. Fukumori Y, Hori Y, Ohnoki S, Nagao N, Shibata H, Okubo Y, et al. Further analysis of D_{el} (D-elute) using polymerase chain reaction (PCR) with *RHD* gene-specific primers. Transfus Med 1997;7:227-31.
3. Sun CF, Chou CS, Lai NC, Wang WT. *RHD* gene polymorphisms among RhD-negative Chinese in Taiwan. Vox Sang 1998;75:52-7.
4. Lan JC, Chen Q, Wu DL, Ding H, Pong DB, Zhao T. Genetic polymorphism of RhD-negative associated haplotypes in the Chinese. J Hum Genet 2000;45:224-7.
5. Wagner FF, Frohmajer A, Flegel WA. *RHD* positive haplotypes in D negative Europeans. BMC Genet 2001;2:10.
6. Rodrigues A, Rios M, Pellegrino J, Jr., Costa FF, Castilho L. Presence of the *RHD* pseudogene and the hybrid *RHD-CE-D*^{s} gene in Brazilians with the D-negative phenotype. Braz J Med Biol Res 2002;35:767-73.
7. Shao CP, Maas JH, Su YQ, Köhler M, Legler TJ. Molecular background of Rh D-positive, D-negative, D_{el} and weak D phenotypes in Chinese. Vox Sang 2002;83:156-61.
8. Xu Q, Zhang J, Wang Q, Zhang S, Si G. *RHD* gene polymorphism among RhD-negative Han Chinese. Chin Med J (Engl) 2003;116:1539-43.
9. Peng CT, Shih MC, Liu TC, Lin IL, Jaung SJ, Chang JG. Molecular basis for the RhD negative phenotype in Chinese. Int J Mol Med 2003;11:515-21.
10. Lee YL, Chiou HL, Hu SN, Wang L. Analysis of *RHD* genes in Taiwanese RhD-negative donors by the multiplex PCR method. J Clin Lab Anal 2003;17:80-4.
11. Chen JC, Lin TM, Chen YL, Wang YH, Jin YT, Yue CT. *RHD 1227A* is an important genetic marker for RhD_{el} individuals. Am J Clin Pathol 2004;122:193-8.
12. Gassner C, Doescher A, Drnovsek TD, Rozman P, Eicher NI, Legler TJ, et al. Presence of *RHD* in serologically D-, C/E+ individuals: a European multicenter study. Transfusion 2005;45:527-38.
13. Xu Q, Grootkerk-Tax MG, Maaskant-van Wijk PA, van der Schoot CE. Systemic analysis and zygosity determination of the *RHD* gene in a D-negative Chinese Han population reveals a novel D-negative *RHD* gene. Vox Sang 2005;88:35-40.
14. Kim JY, Kim SY, Kim CA, Yon GS, Park SS. Molecular characterization of D- Korean persons: development of a diagnostic strategy. Transfusion 2005;45:345-52.
15. Li Q, Ye LY, Guo ZH, Qian M, Zhu ZY. [Study on the molecular background of Del phenotype in Chinese population]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2006;23:486-91.
16. Luettringhaus TA, Cho D, Ryang DW, Flegel WA. An easy *RHD* genotyping strategy for D- East Asian persons applied to Korean blood donors. Transfusion 2006;46:2128-37.
17. Polin H, Danzer M, Hofer K, Gassner W, Gabriel C. Effective molecular *RHD* typing strategy for blood donations. Transfusion 2007;47:1350-5.
18. Flegel WA, von Zabern I, Wagner FF. Six years' experience performing *RHD* genotyping to confirm D- red blood cell units in Germany for preventing anti-D immunizations. Transfusion 2009;49:465-71.
19. Li Q, Hou L, Guo ZH, Ye LY, Yue DQ, Zhu ZY. Molecular basis of the *RHD* gene in blood donors with DEL phenotypes in Shanghai. Vox Sang 2009;97:139-46.
20. Polin H, Danzer M, Gaszner W, Broda D, St-Louis M, Pröll J, et al. Identification of *RHD* alleles with the potential of anti-D immunization among seemingly D- blood donors in Upper Austria. Transfusion 2009;49:676-81.
21. Touinssi M, Chapel-Fernandes S, Granier T, Bokilo A, Bailly P, Chiaroni J. Molecular analysis of inactive and active *RHD* alleles in native Congolese cohorts. Transfusion 2009;49:1353-60.

22. Christiansen M, Sørensen BS, Grunnet N. *RHD* positive among C/E+ and D- blood donors in Denmark. Transfusion 2010;50:1460-4.
23. St-Louis M, Perreault J, Lavoie J, Émond J, St-Laurent J, Long A, et al. [Genotyping of 21,000 blood donors in Quebec and *RHD* analysis]. Transfus Clin Biol 2010;17:242-8.
24. Krog GR, Clausen FB, Berkowicz A, Jørgensen L, Rieneck K, Nielsen LK, et al. Is current serologic RhD typing of blood donors sufficient for avoiding immunization of recipients? Transfusion 2011;51:2278-85.
25. Londero D, Fiorino M, Miotti V, de Angelis V. Molecular RH blood group typing of serologically D-/CE+ donors: the use of a polymerase chain reaction-sequence-specific primer test kit with pooled samples. Immunohematology 2011;27:25-8.
26. Cruz BR, Chiba AK, Moritz E, Bordin JO. *RHD* alleles in Brazilian blood donors with weak D or D-negative phenotypes. Transfus Med 2012;22:84-9.
27. Mota M, Dezan M, Valgueiro MC, Sakashita AM, Kutner JM, Castilho L. *RHD* allelic identification among D-Brazilian blood donors as a routine test using pools of DNA. J Clin Lab Anal 2012;26:104-8.
28. Moussa H, Tsochandaridis M, Chakroun T, Jridi S, Abdelneji B, Hmida S, et al. Molecular background of D-negative phenotype in the Tunisian population. Transfus Med 2012;22:192-8.
29. Szulman A, Nardozza LM, Barreto JA, Araujo Júnior E, Moron AF. Investigation of pseudogenes *RHDΨ* and *RHD-CE-D* hybrid gene in D-negative blood donors by the real time PCR method. Transfus Apher Sci 2012;47:289-93.
30. Chen Q, Li M, Li M, Lu XS, Lü R, Sun J, et al. Molecular basis of weak D and DEL in Han population in Anhui Province, China. Chin Med J (Engl) 2012;125:3251-5.
31. Wagner FF MI, Bittner R, Döscher A. *RHD* PCR of blood donors in Northern Germany: use of adsorption/elution to determine D antigen status (abstract 3C-S8–04). Vox Sang 2012;103:15.
32. Orzińska A, Guz K, Polin H, Pelc-Kłopotowska M, Bednarz J, Gieleżyńska A, et al. *RHD* variants in Polish blood donors routinely typed as D-. Transfusion 2013;53:2945-53.
33. Ouchari M, Jemni-Yaacoub S, Chakroun T, Abdelkefi S, Houissa B, Hmida S. *RHD* alleles in the Tunisian population. Asian J Transfus Sci 2013;7:119-24.
34. Ye SH, Wu DZ, Wang MN, Wu XY, Xu HG, Xu H, et al. A comprehensive investigation of *RHD* polymorphisms in the Chinese Han population in Xi'an. Blood Transfus 2014;12:396-404.
35. Crottet SL, Henny C, Meyer S, Still F, Stolz M, Gottschalk J, et al. Implementation of a mandatory donor RHD screening in Switzerland. Transfus Apher Sci 2014;50:169-74.
36. Gowland P, Gassner C, Hustinx H, Stolz M, Gottschalk J, Tissot JD, et al. Molecular *RHD* screening of RhD negative donors can replace standard serological testing for RhD negative donors. Transfus Apher Sci 2014;50:163-8.
37. Trucco Boggione C, Luján Brajovich ME, Tarragó M, Mattaloni SM, Biondi CS, Muñiz-Díaz E, et al. Molecular structures identified in serologically D- samples of an admixed population. Transfusion 2014;54:2456-62.
38. Scott SA, Nagl L, Tilley L, Liew YW, Condon J, Flower R, et al. The *RHD(1227G>A)* DEL-associated allele is the most prevalent *DEL* allele in Australian D- blood donors with C+ and/or E+ phenotypes. Transfusion 2014;54:2931-40.
39. Sassi A, Ouchari M, Houissa B, Romdhane H, Abdelkefi S, Chakroun T, et al. *RHD* genotyping and its implication in transfusion practice. Transfus Apher Sci 2014;51:59-63.
40. Gu J, Wang XD, Shao CP, Wang J, Sun AY, Huang LH, et al. Molecular basis of DEL phenotype in the Chinese population. BMC Med Genet 2014;15:54.
41. Costa S, Martin F, Chiba A, Langhi D, Jr., Chiattone C, Bordin J. *RHD* alleles and D antigen density among serologically D- C+ Brazilian blood donors. Transfus Med 2014;24:60-1.

42. Moussa H, Tsochandaridis M, Kacem N, Chakroun T, Abdelkefi S, Gabert J, et al. *RHD* positive among C/E+ and D-negative blood donors in Tunisia. Transfus Clin Biol 2014;21:320-3.
43. Stegmann TC, Veldhuisen B, Bijman R, Thurik FF, Bossers B, Cheroutre G, et al. Frequency and characterization of known and novel *RHD* variant alleles in 37,782 Dutch D-negative pregnant women. Br J Haematol 2016;173:469-79.
44. Kulkarni SS, Gogri H, Parchure D, Mishra G, Ghosh K, Rajadhyaksha S, et al. RHD-Positive Alleles among D- C/E+ Individuals from India. Transfus Med Hemother 2018;45:173-7.
45. de Paula Vendrame TA, Prisco Arnoni C, Guilhem Muniz J, de Medeiros Person R, Pereira Cortez AJ, Roche Moreira Latini F, et al. Characterization of *RHD* alleles present in serologically RHD-negative donors determined by a sensitive microplate technique. Vox Sang 2019;114:869-75.
46. Khosroshahi BN, Oodi A, Namjou S, Gholamali T, Amirizadeh N. *RHD* genotyping by molecular analysis of hybrid rhesus box in RhD-negative blood donors from Iran. Indian J Hematol Blood Transfus 2019;35:119-24.
47. Perez-Alvarez I, Hayes C, Hailemariam T, Shin E, Hutchinson T, Klapper E. *RHD* genotyping of serologic RhD-negative blood donors in a hospital-based blood donor center. Transfusion 2019;59:2422-8.
48. Thongbut J, Raud L, Férec C, Promwong C, Nuchnoi P, Fichou Y. Comprehensive Molecular Analysis of Serologically D-Negative and Weak/Partial D Phenotype in Thai Blood Donors. Transfus Med Hemother 2020;47:54-60.
49. Sadeghi-Bojd Y, Amirizadeh N, Oodi A. *RHD* genotyping of Rh-negative and weak D phenotype among blood donors in Southeast Iran. Int J Hematol Oncol Stem Cell Res 2021;15:213-20.
50. Kim T, Park Y, Shin L, Jung YS, Youn M, Kim Y. The experience of *RHD* genotyping in D-negative blood donors. Korean J Blood Transfus 2021;32:91-101.
51. Ren Q, Cao L, Xia Y, Xu H, Xie Y, Mi Z, et al. Genetic background of RhD negative blood donors. Zhongguo shuxue zazhi 2022;35:1014-6.
52. Khetan D, Shukla JS, Chaudhary RK. Molecular basis of RhD-negative phenotype in North Indian blood donor population. Indian J Med Res 2022;155:286-92.
53. Nuchnoi P, Thongbut J, Bénech C, Kupatawintu P, Chaiwanichsiri D, Férec C, et al. Serologically D-negative blood donors in Thailand: molecular variants and diagnostic strategy. Blood Transfus 2023;21:209-17.
54. Simtong P, Phothi R, Puapairoj C, Leelayuwat C, Romphruk AV. *RHD* 1227 A and hybrid Rhesus box analysis in Thai RhD+ and RhD- blood donors: Prevalence, *RHD* zygosity, and molecular screening. Transfus Apher Sci 2022;61:103496.
55. Fouti L, Mpelle L, Ondzia F, Senda L, Bikoue A, Boumba A, et al. Molecular characterization of *RHD* in Rh-negative blood donors in Congo Brazzaville. J. Biosci. Med. 2024;12:299-309.
56. Lilić M, Guzijan G, Jovanović Srzentić S. Rare occurrence of *RHD* null alleles with Del expression among serologically D-negative blood donors. Scripta Medica 2024;55:307-15.
57. Rodrigues ES, Santos FLS, de Paula Vendrame TA, Cuter TB, Romagnoli A, Mendes V, et al. Rh D-positive genotypes in Brazilian blood donors with D-negative phenotype. Ann Blood 2024;9:19-22.
58. Alalshaikh MA, Alsughayir AH, Alsaif AS, Ababtain SA, Aloyouni SY, Aldilaijan KE, et al. Molecular background of RhD-positive and RhD-negative phenotypes in a Saudi population. Saudi J Med Med Sci 2024;12:210-5.