

亚洲型DEL形成的分子机制及临床应用

广州血液中心 付涌水



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Guangzhou Blood Center



TRANSFUSION MEDICINE

Patients with Asian-type DEL can safely be transfused
with RhD-positive blood

Blood. 2023 Apr 27;141(17):2141-2150.



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Comment on *Ji et al*, page 2141

Safe transfusion in Asian-type DEL

John R. Hess | University of Washington

In this issue of *Blood*, **Ji and colleagues** assemble evidence to justify transfusion of RhD⁺ red cells to individuals with RhD⁻ cells in conventional typing but have the common Asian-type DEL mutant.¹ Their evidence comes from many sources including direct clinical challenge, epidemiologic studies, and genomic and transcriptomic analysis and has potential implications for millions of people.



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A

Conventional hemagglutination testing

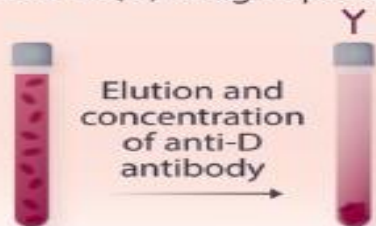
With addition of appropriate antibody RBC agglutinate, otherwise they stay in suspension



B

D eluate cells hemagglutination testing

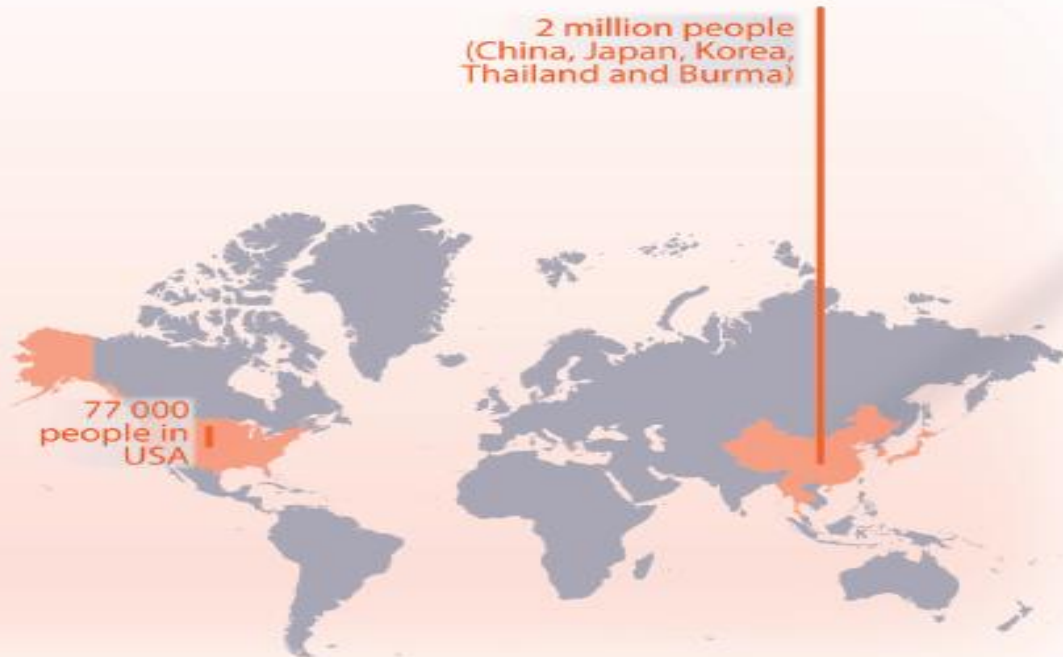
DEL or D eluate cells do not agglutinate with reagent anti-Rh(D) antibody, but antibody eluted from them and concentrated with will agglutinate Rh(D) antigen positive cells



C

Asian-type DEL in the world

Common Asian-type DEL produces many RhD protein isoforms including a small fraction of the full normal protein which seems to protect these individuals from making alloanti-D.





AABB @AABB · 22h



Patients with Asian-type DEL can safely be transfused with RhD-positive blood, according to findings published last week in @BloodJournal. bit.ly/3NApkvZ

[#Transfusion](#) [#BloodTransfusion](#)
[#Alloimmunization](#)



Patients with Asian-type DEL can safely be transfused with...
ashpublications.org



4



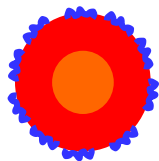
10



613



Background



RhD 阳性



DEL



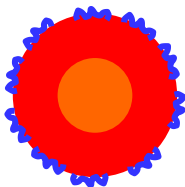
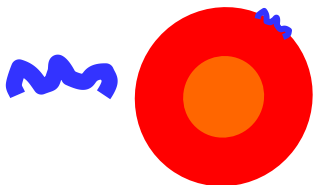
- ✓ 传统方法中，仅能通过**吸收放散实验**能够鉴定出来
- ✓ 但由于吸收放散方法未常规开展，故DEL血型个体在临床长期被作为**RhD阴性**个体对待



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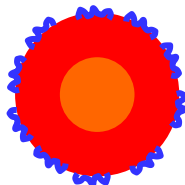
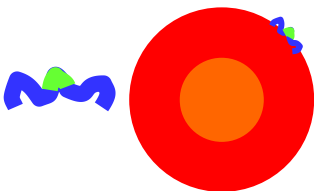
Patient

D+ Donor



No anti-D formation

➤ Complete DEL



Anti-D formation

➤ Partial DEL



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ISBT总结的已报道的DEL血型等位基因

Del defined as weak D expression primarily detectable only by adsorption and elution

Del	RHD*01EL.01 RHD*DEL1	c.1227G>A	9	p.Lys409Lys	PMID: 11495631	JQ424879	rs549616139	Splice site affected
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.....

Del	RHD*01EL.44 RHD*DEL44	RHD-CE(4-9)-D	4-9	Hybrid allele	PMID: 19490579			
Del	RHD*01EL.45 RHD*DEL45	c.721A>C	5	p.Thr241Pro			rs1553143921	
Del	RHD*01EL.46 RHD*DEL46	c.884T>C	6	p.Met295Thr			rs1553144518	
DEL Partial D	RHD*01EL.47 RHD*DEL47	c.510_511insG	4	p.His171Alafs*28	PMID: 28714065	KR611039		also known as c.510dupG
DEL Partial D	RHD*01EL.48 RHD*DEL48 RHD*Ex9del RHD*DKG	Exon 9 deletion	9	p.Gly385Valfs*80	PMID: 29214630	KX793704		
Del	RHD*01EL.49 RHD*DEL49	c.1016G>T	7	p.Gly339Val				
Del	RHD*01EL.50 RHD*DEL50	c.1151C>G	8	p.Thr384Arg				



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亚洲型DEL血型 (Asian-type DEL)

Transfusion of RhD-Positive Blood in “Asia Type”
DEL Recipients

Shao CP. N Engl J Med. 2010;362(5):472-3

Asian-type DEL的分布

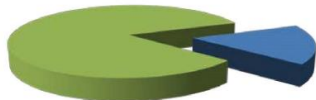
■ 占RhD初筛阴性人群的25%

Countries



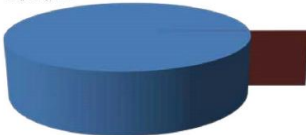
Whole population

513,400,000



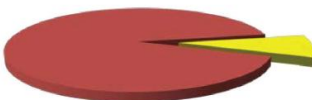
Rh negative population

87,291,000



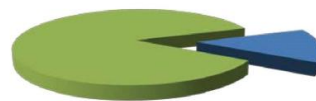
DEL population

139,000

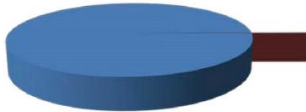


1万

327,100,000



45,362,000

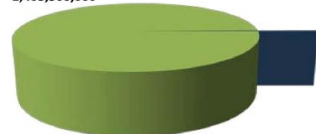


118,000



9万

1,403,500,000



5,557,000



978,000



约100万

约25万

日本、韩国、泰国

Asian-type DEL是Complete DEL ?

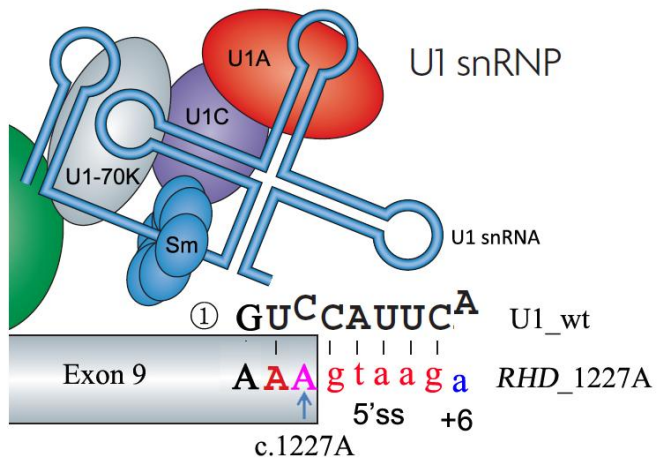
Günther F. Körmöcz, et al. Transfusion, 2005

Anti-D MoAb	D epitope*	RHD(K409K) (n = 3)
LHM70/45	1.2	+
P3×249	2.1	+
P3×290	3.1	+
ESD1	4.1	+
P3×35	5.4	+
P3×241	5.4	+
BRAD-3	6.2	+
HM16	6.4	+
P3×61	6.4	+
HM10	6.6	+
BRAD-5	6.8	+
P3×212-11F1	8.2	+
P3×212-23B10	9.1	+
Birma D6	9.1	+

Gu J, et al. Blood Transfus 2014

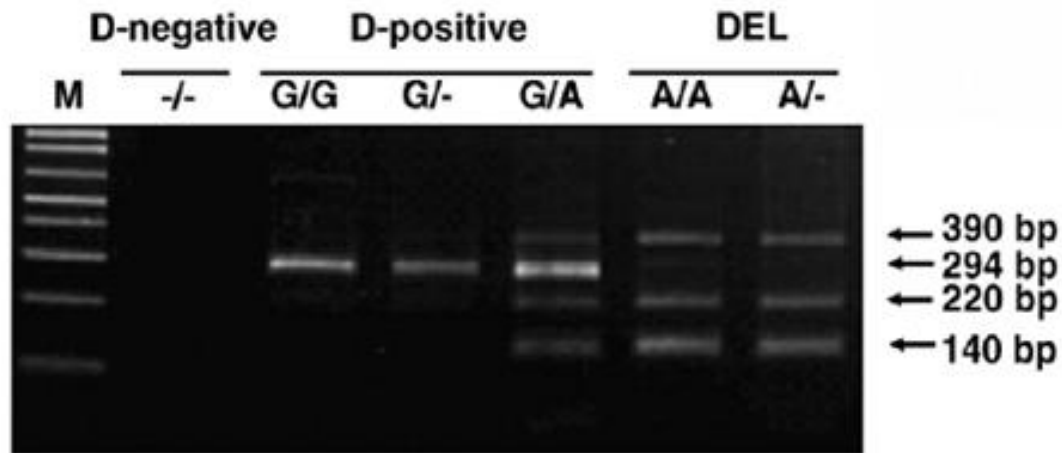
D epitope		Monoclonal anti-D	
1-9	1-16	IgG	IgM
1	1.2	LHM169/81	
1	1.2	LHM70/45	LHM174/102
2	2.1	P3×249	
3	3.1	P3×290	LHM76/55
4	4.1	ESD1	
5	5.4	P3×35	
5	5.4	P3×241	
6	6.3	LHM169/80	
6	6.4		P3×61
6	6.4	HM16	
6	6.6		HM10
8	8.1	LHM76/58	LHM59/19
8	8.2		P3×21211F1
9	9.1	LHM77/64	P3×21223B10

Not produce alloanti-D?

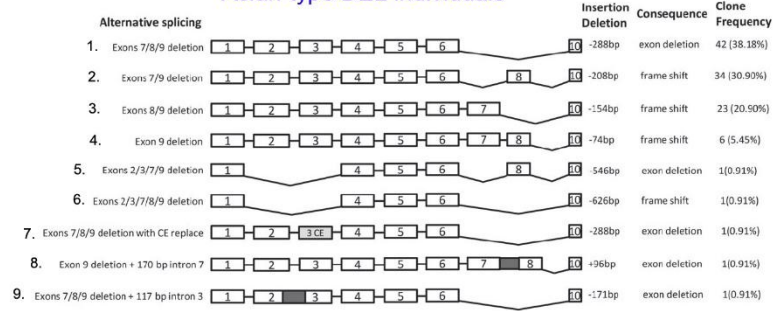


1227A位于外显子9的5'ss剪接位点，
导致RHD基因mRNA异常剪切

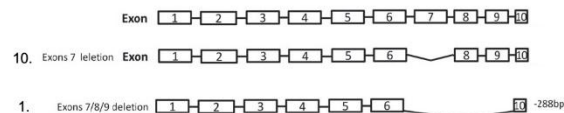
RHD Exon 7-10



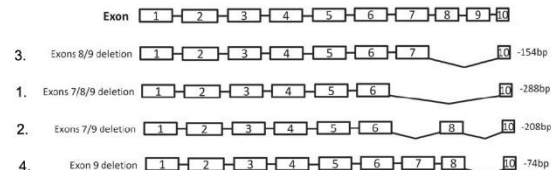
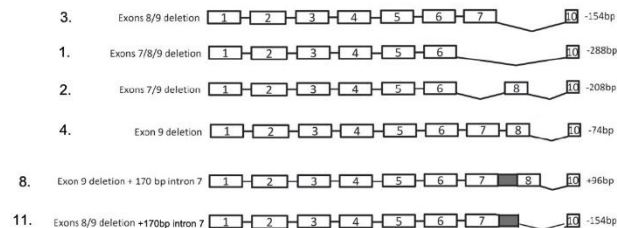
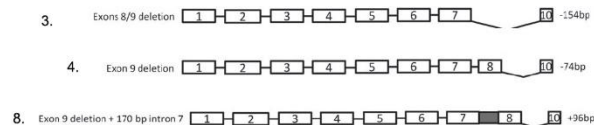
Asian-type DEL individuals



D+ individuals



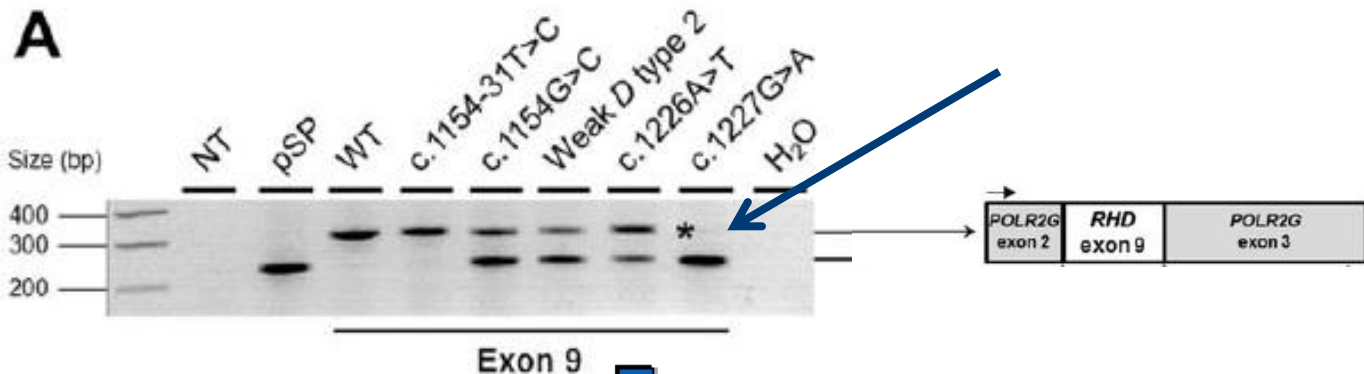
某种缺乏外显子9的低拷贝 *RHD* 基因转录本
表达了表位完整的RhD抗原？



Extensive functional analyses of *RHD* splice site variants: insights into the potential role of splicing in the physiology of Rh

Transfusion, 2015

♦ Minigene splicing assay in vitro



1227A突变载体体外可以表达外显子9，但量非常少

Asian-type DEL个体可能表达包含外显子9的少量全长转录本，表达表位完整的RhD抗原？

临床证据表明Asian-type DEL血型 孕妇不发生抗-D同种免疫

◆ Asian-type DEL血型孕妇共计500例左右，无1例产生抗-D

- Shao CP. *N Engl J Med* 2010 (n = 44)
- Wang QP, et al. *Blood Transfus* 2014 (n = 86)
- Xu W, et al. *Transfus Med Hemother* 2015 (n = 168)
- Wang M, et al. *Transfus Med* 2015 (n = 130)
- 吴筱莹,等.《中国现代医学杂志》2017 (n=41)
- 徐华, 等.《中国输血杂志》2010 (n=20)

◆ 真阴性孕妇产生抗-D的频率在 18.1-40% 之间

- Wang QP, et al. *Blood Transfus* 2014 (61/330, 18.5%)
- Shao CP. *N Engl J Med* 2010 (38/155, 24.5%)
- 吴筱莹,等.《中国现代医学杂志》2017 (28/155, 18.1%)
- 徐华, 等.《中国输血杂志》2010 (40/100, 40%)

缺乏Asian-type DEL血型患者输注D+红细胞的安全性
的系统研究，即大样本的直接证据

Transfusion of RhD-Positive Blood in “Asia Type” DEL Recipients

Shao CP. N Engl J Med. 2010;362(5):472-3

Planned Transfusion of D-Positive Blood Components in an Asia Type DEL Patient: Proposed Modification of the Korean National Guidelines for Blood Transfusion

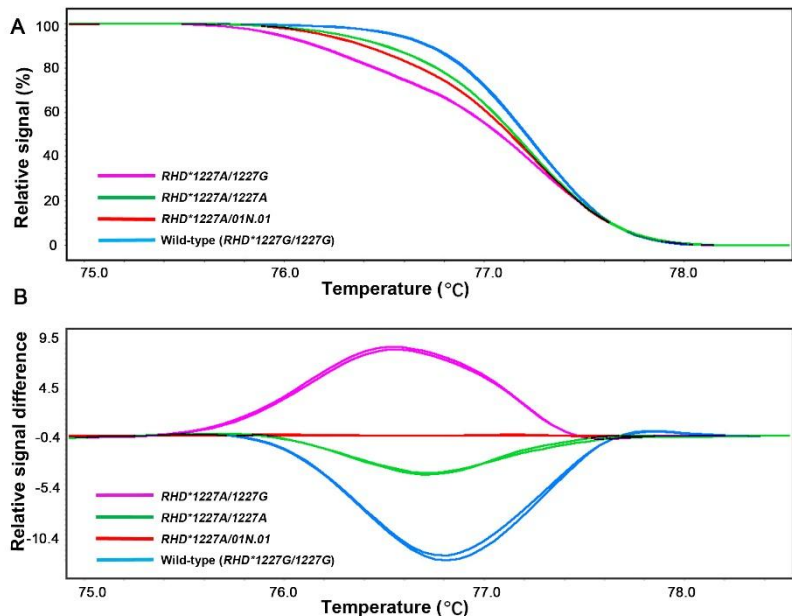
Soojin Choi, M.D.^{1*}, Sejong Chun, M.D.^{2*}, Ji Young Seo, M.T.¹, Ji-Hyuk Yang, M.D.³, and Duck Cho , M.D.¹

Ann Lab Med 2019;39:102-104

Methods and Results

Asian-type DEL常规鉴定方法的建立

基因分型方法



吸收放散方法

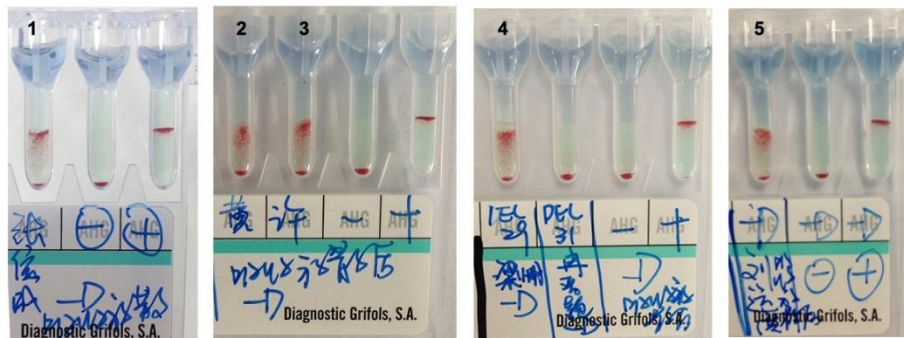


Table S1. Results of *RHD* genotyping for 1036 Chinese pregnant women and 541 patients with DEL phenotype

Individuals with DEL phenotype	Number of samples	Frequency	<i>RHD</i> _Allele 1	<i>RHD</i> _Allele 2
Pregnant women (n=1036)	863	83.1%	<i>RHD</i> *1227A	-
	102	9.8%	<i>RHD</i> *1227A	<i>RHD</i> *1227A
	53	5.1%	<i>RHD</i> *1227A	<i>RHD</i> *D- <i>CE</i> (2-9)-D
	6	0.6%	<i>RHD</i> *1227A	<i>RHD</i> *711delC
	2	0.2%	<i>RHD</i> *1227A	<i>RHD</i> *D- <i>CE</i> (2-7)-D
	1	0.1%	<i>RHD</i> *1227A	<i>RHD</i> *D- <i>CE</i> (2-10)
	4	0.4%	<i>RHD</i> *1227A	<i>RHD</i> * <i>CE</i> (1-9)-D
	1	0.1%	<i>RHD</i> *1227A	<i>RHD</i> *680_684delTGCTG *
	1	0.1%	<i>RHD</i> *744T	-
	1	0.1%	<i>RHD</i> *1222C	-
	1	0.1%	<i>RHD</i> *761T *	-
	1	0.1%	<i>RHD</i> *1227delG *	-
Patients (n=541)	464	85.8%	<i>RHD</i> *1227A	-
	50	9.2%	<i>RHD</i> *1227A	<i>RHD</i> *1227A
	12	2.2%	<i>RHD</i> *1227A	<i>RHD</i> *D- <i>CE</i> (2-9)-D
	3	0.6%	<i>RHD</i> *1227A	<i>RHD</i> *711delC
	3	0.6%	<i>RHD</i> *1227A	<i>RHD</i> *D- <i>CE</i> (2-10)
	1	0.2%	<i>RHD</i> *1227A	<i>RHD</i> * <i>CE</i> (1-9)-D
	1	0.2%	<i>RHD</i> *1227A	<i>RHD</i> *D- <i>CE</i> (2-7)-D
	1	0.2%	<i>RHD</i> *1227A	<i>RHD</i> *D- <i>CE</i> (4-9)-D
	1	0.2%	<i>RHD</i> *1227A	<i>RHD</i> *D- <i>CE</i> (4-7)-D
	1	0.2%	<i>RHD</i> *1227A	<i>RHD</i> *421delG *
	1	0.2%	<i>RHD</i> *1227A	<i>RHD</i> *IVS5+2G *
	1	0.2%	<i>RHD</i> *1227A	<i>RHD</i> *1155C *
	1	0.2%	<i>RHD</i> *D- <i>CE</i> (4-7)-D	-
	1	0.2%	<i>RHD</i> *222A *	-

* Novel *RHD* alleles identified.

For the total of 1571 "Asia type" DEL pregnant women and patients identified, RhCE typing data showed that they were all positive for RhC antigen (Ccee, n = 1210; CCee, n = 329; CcEe, n = 25; CCEe, n = 7).

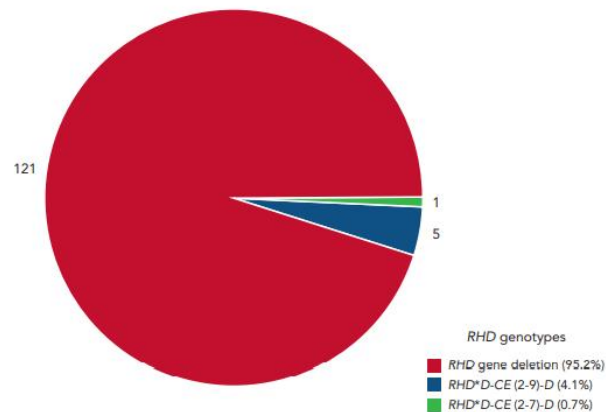
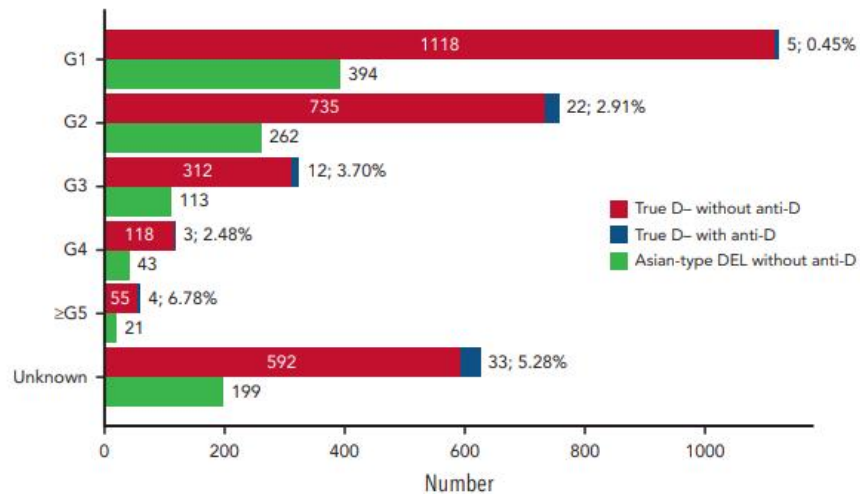
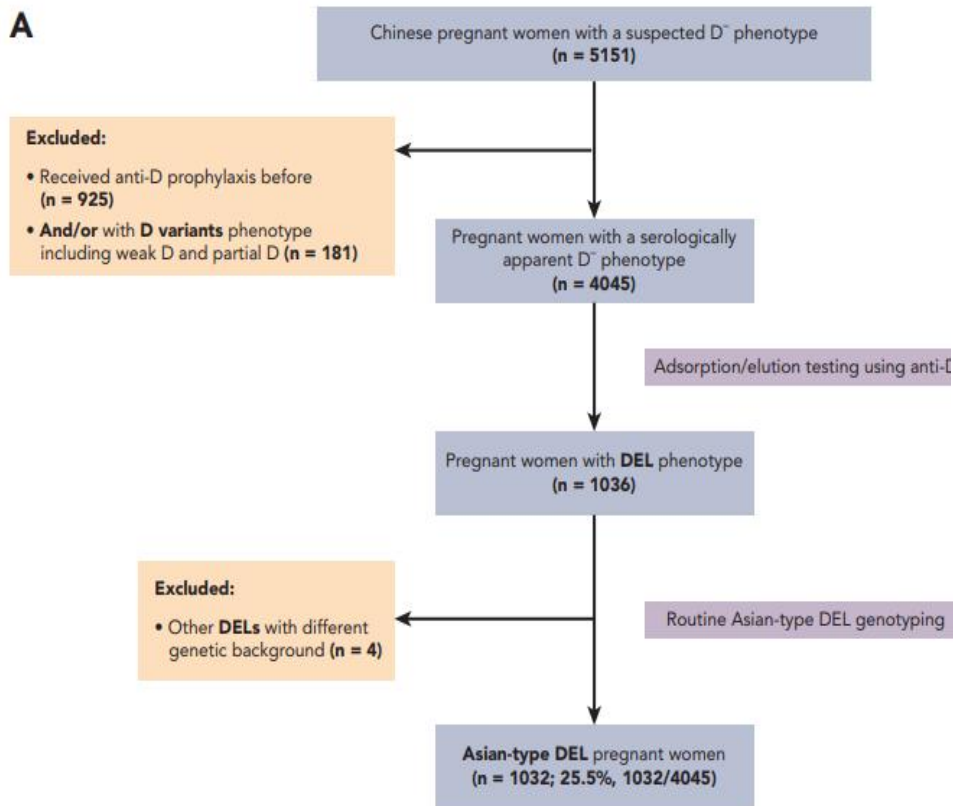
Methods and Results

1. 大样本证实Asian-type DEL孕妇不会发生抗-D同种免疫

- 收集RhD真阴性及Asian-type DEL血型孕妇，统计其孕次及抗-D发生率
- 收集已经产生抗-D的孕妇，鉴定是否有Asian-type DEL的分布



A





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2. Clinical trial Methods and Results

NIH U.S. National Library of Medicine

ClinicalTrials.gov

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ID: DEL

RhD+ Blood Transfusion to "Asia Type" DEL Recipients

Record Summary

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Record Status

In Progress → Entry Completed → Approved → **Released** → PRS Review → Public

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Record Owner: Jiyanti

Last Update: 01/12/2022 22:10 by Jiyanti

Initial Release: 10/26/2018

Initial Results Release: 01/12/2022

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PRS Review: Pending [Review History](#)

Public Site: Last Public Release: 10/09/2019 [View on ClinicalTrials.gov](#)

FDAAA: Non-ACT (No FDA-regulated drug/device)

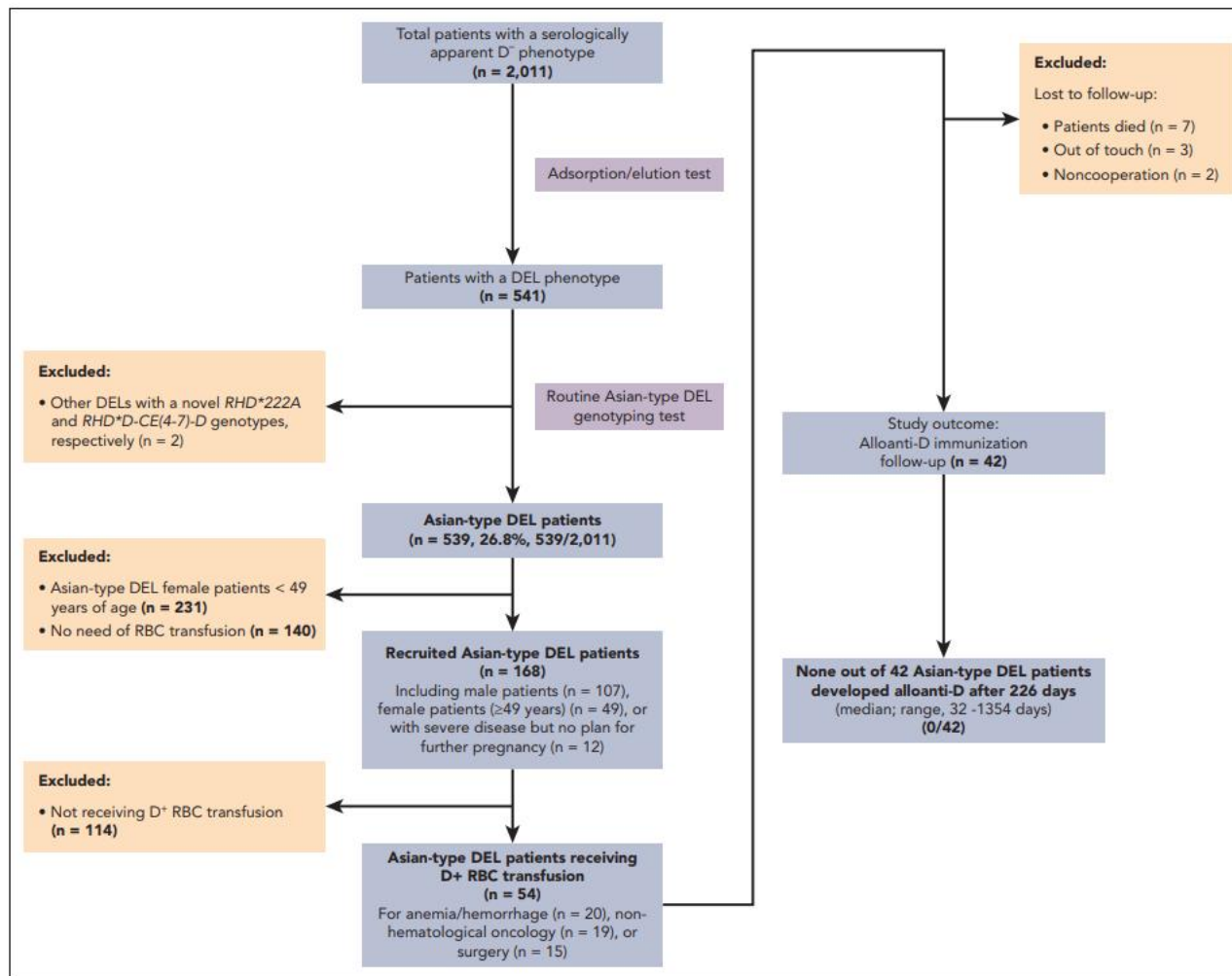


Table 1. Demographics of 42 Asian-type DEL recipients with D⁺ RBC transfusion and their follow-up examinations

Characteristic	Data
Number of male, female	30, 12
Male recipient age (y)	
Mean ± SD	51.5 ± 24.9
Median	58.5
Range	0.3-86
Female recipient age (y)	
Mean ± SD	52.1 ± 10.8
Median	51
Range	30-75
Number of units of D ⁺ RBCs transfused (U)	
Mean ± SD	6.1 ± 5.4
Median	4
Range	0.5-28
Transfusion episodes of D ⁺ RBCs (times)	
Mean ± SD	2.6 ± 2.9
Median	1
Range	1-17
Number of units of D ⁺ RBCs per episode (U)	
Mean ± SD	2.7 ± 1.4
Median	2
Range	0.5-8

Length of serologic follow-up (d)	
Mean ± SD	306.5 ± 264.9
Median	226
Range	32-1354
Number of antibody screening test after D ⁺ RBC transfusion	
Mean ± SD	2.2 ± 1.5
Median	1
Range	1-7
Transfusion treatment of duration (d)	
Mean ± SD	82.6 ± 238.0
Median	1
Range	1-1355
Case of recipients re-exposed to D ⁺ RBCs	19

SD, standard deviation.

Table S3. Information of 54 Asian-type DEL recipients who received D+ RBC transfusion

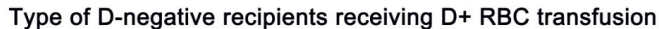
Recipients	Age (years)	Gender	Pregnancy history	Diagnosis	Blood group typing			Transfused D+ RBCs units †	Alloantibody test after D+ RBCs transfusion		Effectiveness of transfusion (Hb changes)	
					DEL Serological A/E testing	RhCE phenotype	RHD genotypes		Observation time (days) ‡	Results		
1	DEL-18	76	M	-	Abdominal surgery	2+	<u>Ccee</u>	RHD*1227A	4U; 2U (2 days)	28; 344	<u>Neg</u>	4U: 141g/L→76g/L (Intraoperative blood loss 2000ml); 2U (2 days): 76g/L→87g/L
2	DEL-76	72	M	-	Coronary heart disease	2+	<u>CCee</u>	RHD*1227A/1227A	2U	6; 192	<u>Neg</u>	124g/L→128g/L (Intraoperative blood loss <u>unknown</u>) 106g/L→103g/L
3	DEL-168	64	F	G4P4	Ovarian cancer	2+	<u>Ccee</u>	RHD*1227A	2U	167	<u>Neg</u>	(Intraoperative blood loss 500ml)
4	DEL-177	30	F	G5P5	Hepatic metastasis of colonic carcinoma	2+	<u>Ccee</u>	RHD*1227A	4U	171	<u>Neg</u>	62g/L→78g/L
5	DEL-211	63	M	-	Liver cancer	3+	<u>Ccee</u>	RHD*1227A	4U; 2U (5 days)	230	<u>Neg</u>	4U: 75g/L→81g/L; 2U (5 days): 69g/L→78g/L
6	DEL-230	52	F	G2P2	Uremia	+	<u>Ccee</u>	RHD*1227A	1U	206; 224; 487	<u>Neg</u>	55g/L→60g/L
7	DEL-231	66	M	-	Lung cancer	+	<u>Ccee</u>	RHD*1227A	2U	222	<u>Neg</u>	57g/L→83g/L



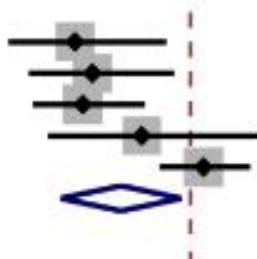
Incidence of anti-D alloimmunization in D-negative individuals receiving D-positive red blood cell transfusion: A systematic review and meta-analysis

Affiliations + expand

PMID: 35014050 DOI: [10.1111/vox.13232](https://doi.org/10.1111/vox.13232)



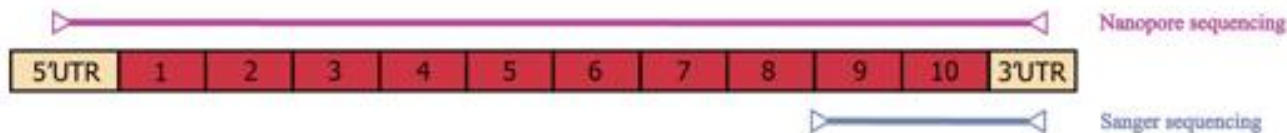
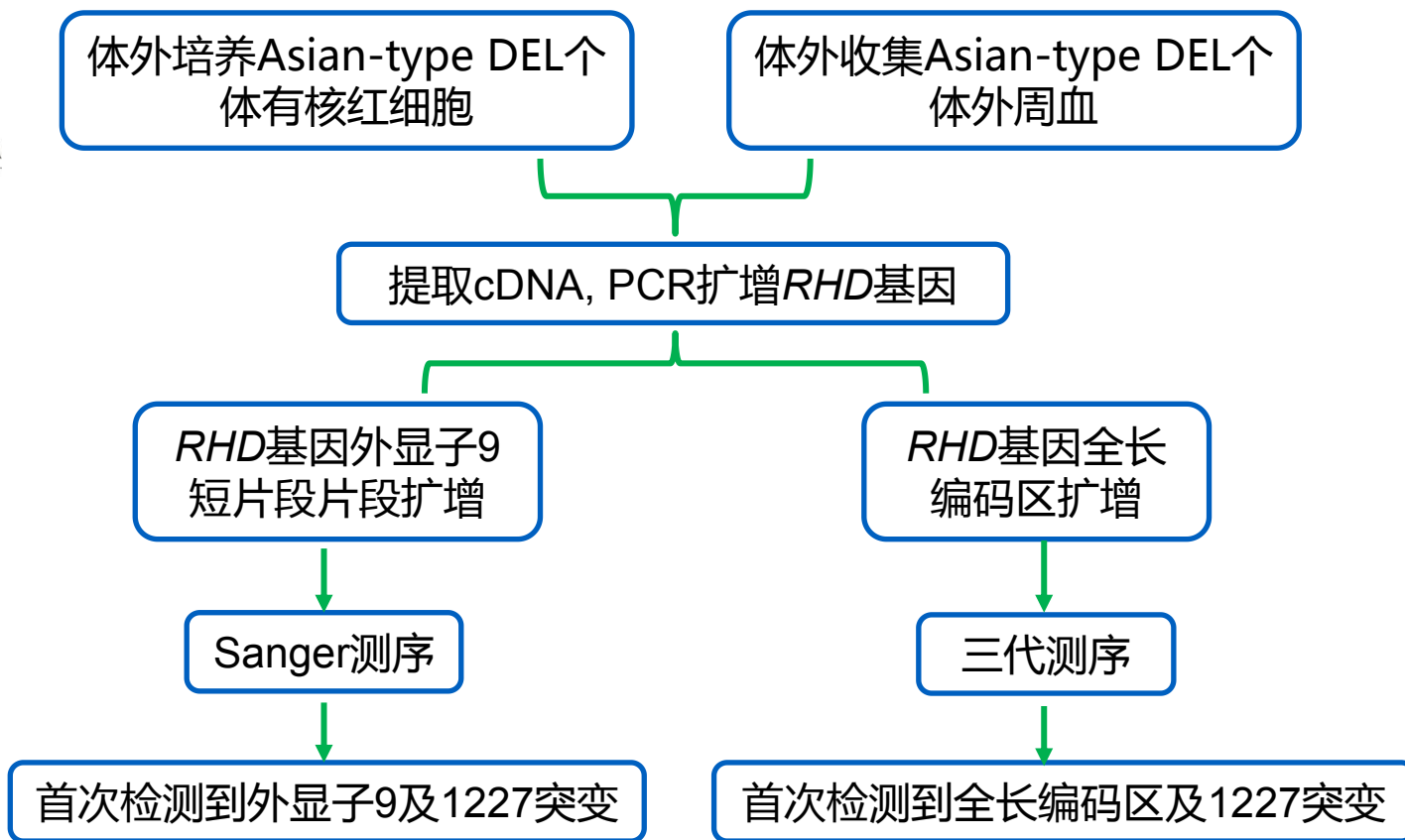
Mixed patients			
Frohn et al, 2003	16	78	0.21 (0.13-0.31)
Yazer et al, 2007	22	98	0.22 (0.15-0.32)
Gonzalez-Porras et al, 2008	34	159	0.21 (0.16-0.28)
Brien et al, 2014	14	50	0.28 (0.17-0.42)
Yazer et al, 2021	117	335	0.35 (0.30-0.40)
Total	203	720	0.26 (0.19-0.32)
Heterogeneity: $I^2=74.06\%$, $P=0.00$			



Methods and Results

3. 机理研究

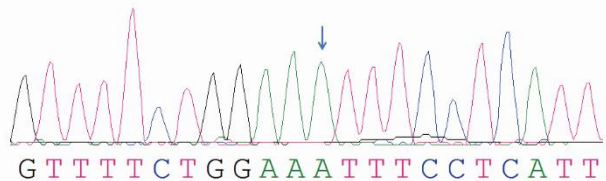
- 体内研究：某种缺乏外显子9的低拷贝RHD基因转录本表达了表位完整的RhD抗原？
- 体外研究推测：
Asian-type DEL个体可能表达包含外显子9的少量全长转录本
表达表位完整的RhD抗原



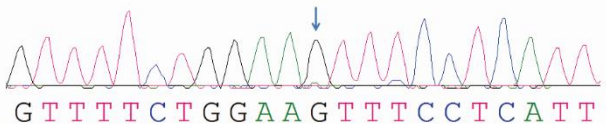


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Sanger测序结果

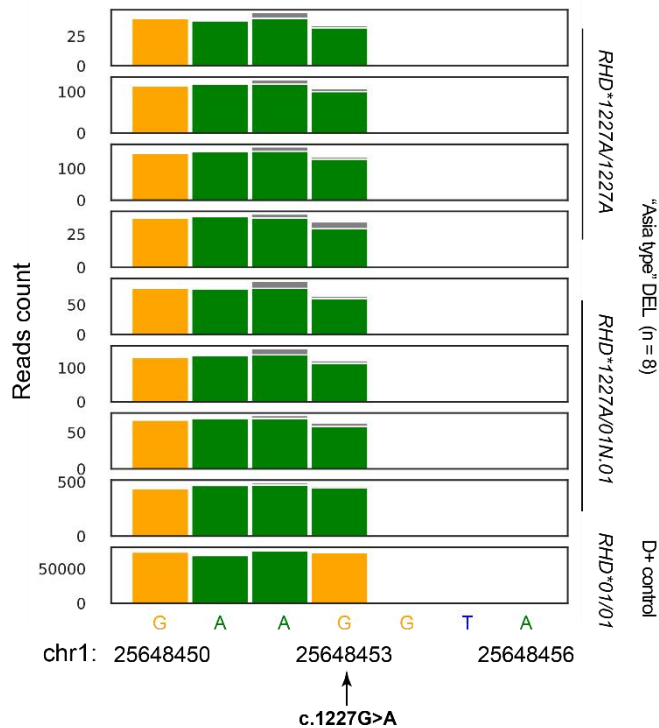


“Asia type” DEL, *RHD*_exon9, c.1227G>A

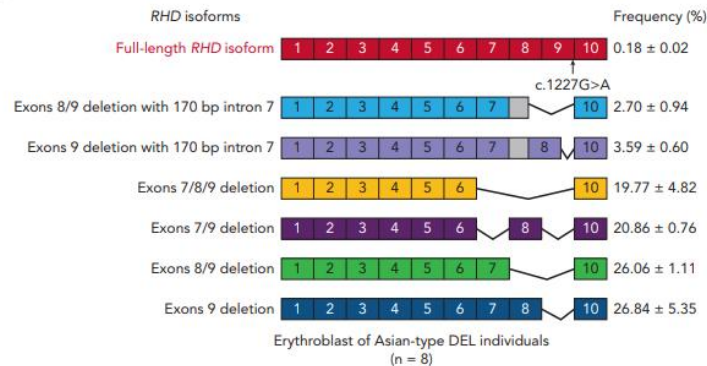


D+ wild-type control, *RHD*_exon 9, c.1227G

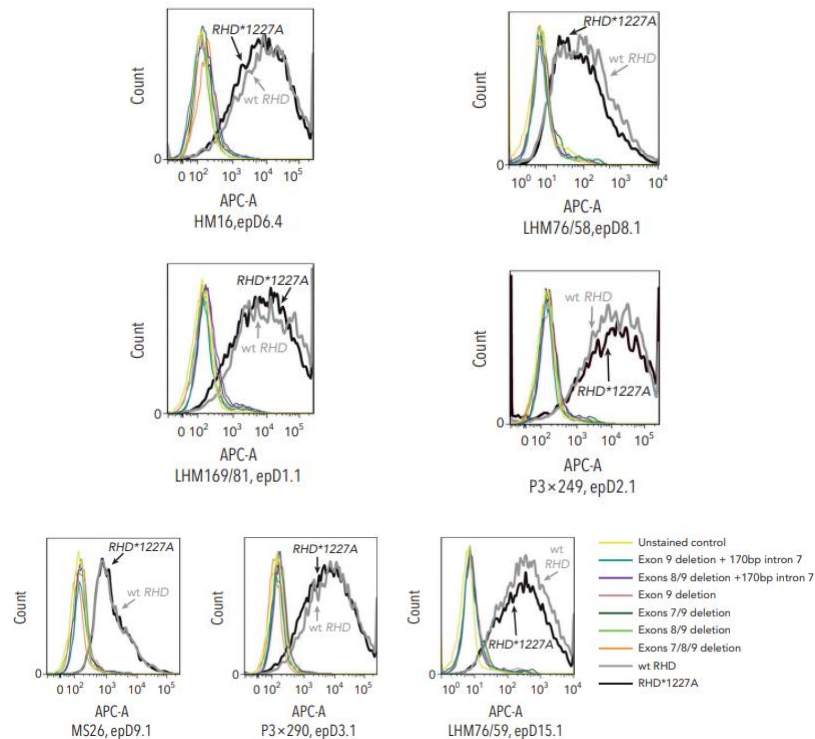
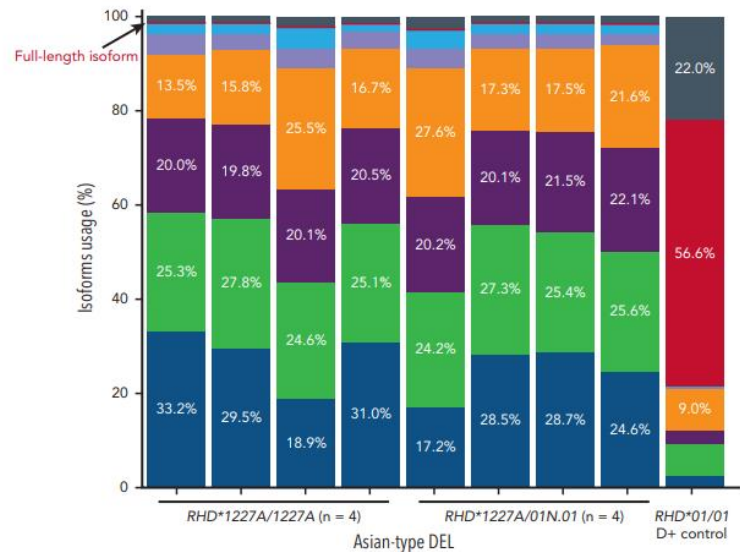
三代测序1227位点分析结果



B



C



Conclusions

This study provides clear evidence:

- 🔴 To establish new guideline for D+ RBC transfusion to Asian-type DEL patients
- 🔴 To discontinue the usage of rhesus immunoglobulin prophylaxis in Asian-type DEL pregnant women



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■ 谢谢！