DEL in China: the D antigen among serologic RhD-negative individuals

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Abstract

Background: Providing RhD-negative red cell transfusions is a challenge in East Asia, represented by China, Korea, and Japan, where the frequency of RhD-negative is the lowest in the world.

Findings: Among 56 ethnic groups in China, the RhD-negative frequency in Han, the prevalent ethnicity, is 0.5% or less, similar to most other ethnic groups. The Uyghur ethnic group has the highest reported RhD-negative frequency of up to 4.7%, as compared to 13.9% in the US. However, an estimated 7.15 million RhD-negative people live in China. The RhD-negative phenotype typically results from a loss of the entire RHD gene, causing the lack of the RhD protein and D antigen. The DEL phenotype carries a low amount of the D antigen and types as RhD-negative in routine serology. The DEL prevalence in RhD-negative individuals averages 23.3% in the Han, 17% in the Hui and 2.4% in the Uyghur ethnicities. The Asian type DEL, also known as RHD*DEL1 and RHD:c.1227G>A allele, is by far the most prevalent among the 13 DEL alleles observed in China.

Conclusion: The purpose of this review is to summarize the data on DEL and to provide a basis for practical strategy decisions in managing patients and donors with DEL alleles in East Asia using molecular assays.

Keywords: Rh blood group, RhD-negative, Ethnicity, Pregnancy, Transfusion, RhIG

Introduction

The transfusion of RhD-negative red cells, often in short supply, poses a particular challenge in East Asia [1, 2]. The lowest frequency of RhD-negative in the world is found in China, Korea and Japan [2, 3]. The first Rh typing for mainland China occurred in 1949 [4], Taiwan in 1956 [5], Macao in 1959 [6] and Hong Kong in 1961 [7], with reported RhD-negative frequencies of 1.92% or less. Even some of these low rates have overestimated the true frequency of RhD-negative, as the quality of the anti-D reagent may have varied. The RhD-negative frequencies are much greater in any of the other world populations, ranging from typically 10% in Africa, somewhat greater in North America, to up to 17% in Europe, where the greatest rate exceeding 20% occurs in the Basque population of Northern Spain.

The DEL phenotype is a D variant with a low number of D antigens per red cell [1, 8, 9]. Patients and donors with a DEL phenotype are routinely typed as RhD-negative in blood group serology, although they carry the D antigen. They should be considered RhD-positive for several clinically relevant applications [9–12]. DEL has been reviewed before [3, 13–15], albeit not with specific focus on East Asian populations.

RhD-negative phenotype

Ethnicities in China

China recognizes 56 ethnic groups [16]. Among them, the Han group accounts for 91.13% of the whole population (Table 1) [17–25]. The Han group itself has a certain genetic diversity and substructure [26, 27]. Molecular data for the RHD gene among RhD-negative Han individuals have been established in various localities, but not in...
all provinces of China. A few results are known for only 2 other ethnic populations in China (Fig. 1).

The reported frequency of an RhD-negative phenotype in Han ranges from 0.24% to 0.50% (Table 2) [17, 18, 28–34]. Among a total of 1,929,664 Han individuals tested in 8 studies (Table 2), only 5771 carried the RhD-negative phenotype, resembling an average of 0.30%, similar to that of many other ethnic groups in China (Table 1).

The Uyghur group has the greatest reported RhD-negative frequency (3.3–4.7%) [19, 21], followed by the Kazakhs group (2.9%) [20], as compared to 13.9% in the USA [3]. The Uyghur and Kazakhs groups reside in the northwestern parts of China and likely descent from Central Asian ancestry, with more recent common ancestors with Caucasian ethnicities. This may explain their greater rates of RhD-negative phenotypes compared to ethnic groups further east and south.

Up to an estimated 7.15 million individuals in China are RhD-negative (Table 1).

RhD-negative, DEL and the RHD gene

More than 70% of the RhD-negative phenotypes in the Han ethnicity are explained by a loss of the entire RHD gene [35–37]. These individuals lack the RhD protein and have no D antigen. The remainder of the serologic RhD-negative Han individuals actually carry the RHD gene [9, 17]. Most of them express a DEL phenotype [9, 17]. They type as RhD-negative in serologic routine testing and, using serology, can only be detected by labor-intensive adsorption and elution techniques [8, 9, 12] or a limited association with distinct serologic Rh types [38]. We summarize published data that detection by molecular diagnostic is straightforward.

DEL phenotype and alleles

Serologic DEL phenotype among Han

The DEL prevalence was first surveyed in 1993 in Hong Kong [34]. Since then, the DEL phenotype has been carefully documented in serologic RhD-negative Han using an adsorption-elution method [17, 18, 28–31, 39–47]. The reported frequency of the DEL phenotype in RhD-negative Han ranges from 16.3% to 32.6% (Table 3). Among a total of 6,470 RhD-negative individuals tested in 16 studies (Table 3), 1505 tested positive by adsorption-elution. Hence, an average 23.3% of RhD-negative Han individuals carry DEL phenotype. Up to an estimated 1,666,000 individuals are DEL positive in China. And 5.45 million lack the RhD protein, which puts them at risk of anti-D immunization, similar to RhD-negative Caucasians [48].

Table 1: Estimate of RhD-negative among the 56 ethnic populations in China

| Ethnicity | Population sizea | RhD-negative individuals in the ethnicity | References |
|-----------|------------------|------------------------------------------|------------|
|           | n                | %                                        | way of population | Reporte | Estimate (n) | |
| Han       | 1,220,844,520    | 91.13%                                   | 0.24–0.50%       | 2.93–6.10 million | [17, 18] |
| Zhuang    | 16,926,381       | 1.26%                                    | 0.49%            | 82,939 | [19] |
| Hui       | 10,586,087       | 0.79%                                    | 0.80–1%          | 84,689–105,860 | [19, 20] |
| Manchu    | 10,387,958       | 0.78%                                    | 0.40%            | 41,552 | [19] |
| Uyghur    | 10,069,346       | 0.75%                                    | 3.30–4.70%       | 332,288–473,259 | [19, 21] |
| Miao      | 9,426,007        | 0.70%                                    | 0.70%            | 65,982 | [19] |
| Yi        | 8,714,393        | 0.65%                                    | 1.30%            | 113,287 | [19] |
| Tibetan   | 6,282,187        | 0.47%                                    | 0.60%            | 37,693 | [22] |
| Mongolian | 5,981,840        | 0.45%                                    | 0.30–0.50%       | 17,946–29,909 | [19, 20] |
| Dong      | 2,879,974        | 0.21%                                    | 0.10%            | 2,880 | [23] |
| Buyi      | 2,870,034        | 0.21%                                    | 0.40%            | 11,480 | [23] |
| Kazakhs   | 1,462,588        | 0.11%                                    | 2.90%            | 42,415 | [20] |
| Shui      | 411,847          | 0.03%                                    | 0.10%            | 412 | [23] |
| Othersb   | 32,881,690       | 2.43%                                    | ~0.10%           | 32,882 | Estimate |
| Total     | 1,339,724,852    | 100%                                     | N/A              | 3.80–7.15 million | N/A |

N/A not applicable

a Calculation based on the 6th National Population Census of the People’s Republic of China of 2010 [24, 25]
b The other 43 ethnicities in order of population sizes (proportion) are the Tuji (0.63%), Yao (0.21%), Bai (0.15%), Korean (0.13%), Hani (0.12%), Li (0.11%), Dai (0.09%), She (0.05%), Lisu (0.05%), Dongxiang (0.05%), Gelao (0.04%), Lahu (0.04%), Wa (0.03%), Napo (0.02%), Qiang (0.02%), Tu (0.02%), Mulao (0.02%), Xibe (0.01%), Kyrgyz (0.01%), Jingpo (0.01%), Daur (0.01%), Salar (0.01%), Mongol (0.01%), Maonan (0.01%), Tajik (< 0.01%), Uighur (< 0.01%), Amdong (< 0.01%), Achang (< 0.01%), Nu (< 0.01%), Evenki (< 0.01%), Gini (< 0.01%), Jino (< 0.01%), De’ang (< 0.01%), Bonan (< 0.01%), Ross (< 0.01%), Tujia (< 0.01%), Uyghur (< 0.01%), Yidu (< 0.01%), Xibe (0.01%), Hezhen (< 0.01%), etc. (< 0.01%), and Tatars (< 0.01%)
Molecular basis of DEL phenotype among Han

Among 1,266 individuals with molecular data including PCR-SSP (polymerase chain reaction with sequence specific priming) or nucleotide sequencing or both for molecular signals of the *RHD* gene (Table 4), 96.7% were found to carry one distinct variant of the *RHD* gene, designated "Asian type" DEL [49]. The Asian type DEL is also known as *RHD*DEL1 and *RHD:c.1227G>A* allele [15]. It had originally been described as *RHD*(K409K) in 2001 [9].

The Asian type DEL is by far the most prevalent cause of the DEL phenotype in China. Among RhD-negative Han individuals (Table 4), 21.6% of these RhD-negative carried the (molecular) Asian type DEL, closely resembling the 23.3% reported to carry the (serologic) DEL phenotype (Table 3). A similar frequency of 22.0% Asian type DEL was observed among RhD-negative Han individuals who were tested by PCR-SSP only (Table 5).

The approach of molecularly testing all serologic RhD-negative individuals in 3 regions (Table 5) corroborated the results from the 13 studies that relied on testing adsorption-elution positive samples only (Table 4). These 13 studies could theoretically have missed some Asian type DEL. They did apparently not miss a clinically relevant number, if any, and the Asian
type DEL frequency has firmly been established for the Han population in China.

**Asian type DEL in other ethnic groups in China**

The knowledge of RHD alleles is quite limited in RhD-negative individuals of ethnic groups in China other than Han. The first systematic RHD allele screen among Chinese was conducted by Qing Wei, Tongji Medical College, Wuhan in 2005 [50]. Dr. Wei examined 50 randomly collected samples from Tibetans, described 4 novel RHD alleles and found 1 known variant RHD allele. Likely due to the small sample size, all individuals were RhD-positive and no Asian type DEL or other DEL allele was detected. Early systematic population screens by other Chinese researchers [51–53] were also productive in discovering novel RHD alleles.

Among the 55 other ethnic groups, RhD-negative individuals from only the Hui and Uyghur ethnicities were

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**Table 2** Prevalence of RhD-negative in Han

| Regions          | Province/city         | Subjects      | RhD-negative | Frequency | References |
|------------------|-----------------------|---------------|--------------|-----------|------------|
| Eastern China    | Shanghai              | 400,253       | Donor 1585   | 0.40%     | [28]       |
| Southeastern China | Guangdong             | 41,905        | Donor 102    | 0.24%     | [17]       |
|                  | Jiangsu, Guangdong, Guangxi | 42,306    | Patient 165  | 0.39%     | [29]       |
| Central China    | Anhui                 | 313,250       | Patient 808  | 0.26%     | [30]       |
|                  | Anhui                 | 30,799        | Donor 155    | 0.50%     | [18]       |
|                  | Henan                 | 38,526        | Donor 106    | 0.28%     | [31]       |
| Western China    | Shanxi                | 890,403       | Donor 2385   | 0.27%     | [32, 33] |
| Hong Kong        | N/A                   | 172,222       | Donor 465    | 0.27%     | [34]       |
| Total            | N/A                   | 1,929,664     | N/A 5771     | 0.30%     | N/A        |

N/A not applicable

* RhD-negative frequency among Han: 0.33% ± 0.09% (mean ± SD) for the 8 studies, 0.24–0.50% (range)

* Same dataset published in Chinese [32] and English [33]

**Table 3** DEL phenotype in serologic RhD-negative Han by adsorption-elution method

| Regions          | Province/city         | RhD-negative individuals (n) | Adsorption-elution (n) | DEL phenotype frequency | References |
|------------------|-----------------------|------------------------------|------------------------|-------------------------|------------|
| Eastern China    | Shanghai              | 1585                         | 1306                   | 17.6%                   | [28]       |
|                  | Shanghai              | 441                          | 369                    | 16.3%                   | [39]       |
|                  | Shandong              | 74                           | 52                     | 29.7%                   | [40]       |
| Northeastern China | Heilongjiang          | 374                          | 312                    | 16.6%                   | [41]       |
| Southeastern China | Zhejiang, Jiangsu    | 643                          | 488                    | 24.1%                   | [42]       |
|                  | Jiangsu, Guangdong, Guangxi | 165     | 124                    | 24.8%                   | [29]       |
|                  | Guangdong            | 102                          | 76                     | 25.5%                   | [17]       |
| Central China    | Anhui                 | 808                          | 630                    | 22.0%                   | [30]       |
|                  | Anhui                 | 515                          | 373                    | 27.6%                   | [43]       |
|                  | Anhui                 | 155                          | 124                    | 20.0%                   | [18]       |
|                  | Henan                 | 106                          | 78                     | 26.4%                   | [31]       |
| Hong Kong        | N/A                   | 465                          | 329                    | 29.2%                   | [34]       |
| Taiwan           | N/A                   | 395                          | 269                    | 31.9%                   | [44]       |
|                  | N/A                   | 294                          | 200                    | 32.0%                   | [45]       |
|                  | N/A                   | 230                          | 155                    | 32.6%                   | [46]       |
|                  | N/A                   | 118                          | 80                     | 32.2%                   | [47]       |
| Total            | N/A                   | 6470                         | 4965                   | 23.3%                   | N/A        |

N/A not applicable

* DEL phenotype frequency among RhD-negative Han individuals: 25.5% ± 5.5% (mean ± SD) for the 16 studies, 16.3–32.6% (range)
Table 4  Asian type DEL (RHD*DEL1) in serologic RhD-negative Han who tested positive in adsorption-elution method

| Regions            | Province/city               | RhD-negative individuals (n) | Allele identification (n) | RHD*DEL1 frequency among RhD-negativeb | Adsorption-elution positivea | Rh-negativeb |
|--------------------|-----------------------------|-----------------------------|---------------------------|----------------------------------------|------------------------------|--------------|
|                    |                             | Total                        | Neg | Pos | RHD*DEL1 | Others |                         |                          |               |
|                    |                             | Adsorption-elution positive  |    |    |          |        |                         |                          |               |
| Eastern China      | Shanghai                    | 1585                         | 11  | 268 | 268      | 11     | 96.1%                    | 16.9%                 | [28]           |
|                   | Shanghai                    | 441                          | 4   | 68  | 0        | 4      | 94.4%                    | 15.4%                 | [39]           |
|                   | Shandong                     | 74                           | 0   | 22  | ND       | ND     | 100%                     | 29.7%                 | [40]           |
| Northeastern China| Heilongjiang                 | 374                          | 1   | 61  | 0        | 1      | 98.4%                    | 16.3%                 | [41]           |
| Southeastern China| Zhejiang, Jiangsu           | 643                          | 0   | 155 | ND       | ND     | 100%                     | 24.1%                 | [42]           |
|                   | Jiangsu, Guangdong, Guangxi| 165                          | 4   | 37  | 0        | 4      | 90.2%                    | 22.4%                 | [29]           |
|                   | Guangdong                   | 102                          | 0   | 26  | 25       | 1      | 96.2%                    | 24.5%                 | [17]           |
| Central China      | Anhui                       | 808                          | 10  | 168 | 0        | 10     | 94.4%                    | 20.8%                 | [30]           |
|                   | Anhui                       | 515                          | 12  | 130 | 0        | 12     | 91.5%                    | 25.2%                 | [43]           |
|                   | Anhui                       | 155                          | ND  | ND  | 31       | 0      | 100%                     | 20.0%                 | [18]           |
| Taiwan             | N/A                         | 395                          | 0   | 126 | ND       | ND     | 100%                     | 31.9%                 | [44]           |
|                   | N/A                         | 294                          | 0   | 94  | 94       | 0      | 100%                     | 32.0%                 | [45]           |
|                   | N/A                         | 118                          | 0   | 38  | 38       | 0      | 100%                     | 32.2%                 | [47]           |
| Total              |                             | 5669                         | 42  | 1193| 456      | 43     | 96.7%c                   | 21.6%c                | N/A            |

ND not done, N/A not applicable

a RHD*DEL1 allele frequency among adsorption-elution positive Han individuals: 97.3% ± 3.4% (mean ± SD) for the 13 studies, 90.2–100% (range)
b RHD*DEL1 allele frequency among RhD-negative Han individuals: 24.0% ± 5.8% (mean ± SD) for the 13 studies, 15.4%—32.2% (range)
c Calculation: (1193 + 31) / 1266 × 100% = 96.7% and (1193 + 31)/5669 × 100% = 21.6%
examined so far (Table 6) in 2 studies [20, 39]. Asian type DEL was found in 17% of the RhD-negative individuals in Hui but only in 2.4% of that in Uyghur (Table 6); the others carrying an RHD deletion. The lower prevalence in the Uyghur group are interesting and pointed to the possibility that DEL frequency and DEL alleles may vary to some extent among the ethnic groups in China. It will be worthwhile to check a representative set of RhD-negative as well as RhD-positive individuals from each ethnic group to verify their RHD alleles. This approach ought to eventually confirm that clinical applications are safe for all ethnic groups in China.

**Molecular background of known DEL alleles in Han**

DEL alleles, other than the Asian type DEL, have been determined by nucleotide sequencing in 13 studies [17, 18, 28–30, 39–45, 47]. These studies identified to date a total of 12 additional DEL alleles in Han (Table 7) [54–60]. RHD*DEL2, also known as RHD:c.3G>A, represents the second most frequent DEL allele in Han, but at 1.11% it is much less frequent than the Asian type DEL. The remaining DEL alleles are even rarer. Of note, 3 of these rare alleles, RHD(28C>T), RHD-CE(4–7)-D and RHD-RHCE(10), were also observed outside of China (Table 7).

**Serology of DEL phenotype**

In 2005, Körmöczi and colleagues [61] suggested that DEL phenotype could be subdivided. They defined a partial DEL phenotype by drawing an analogy to the definition of partial D [62, 63]. In “complete” DEL, the majority of D epitopes were present; in “partial” DEL, the loss of some D epitope was documented [61]. Individuals with partial DEL phenotypes may produce anti-D [61]. According to published data, both the RHD*DEL1 [43] and the RHD*DEL2 alleles [30] lead to a complete DEL phenotype, while hybrid alleles, such as RHD-CE(4–9)-D [28, 30, 43], RHD-CE(4–7)-D [29, 43] and RHD-CE(2–5)-D [28–30, 43], are associated with a partial DEL phenotype [61].

This distinction between partial DEL and complete DEL has major clinical implications: Individuals who carry a partial DEL can develop anti-D when transfused with RhD-positive red cells; individuals who carry a complete DEL cannot develop anti-D.

### Table 5  Asian type DEL (RHD*DEL1) in serologic RhD-negative among Han by PCR-SSP only

| Regions in China | Province/city | RhD-negative individuals (n) | G1227A PCR-SSP (n) | RHD*DEL1 frequency | References |
|------------------|---------------|------------------------------|--------------------|-------------------|------------|
|                  |               |                              | Negative           | Positive          |            |
| southeastern     | Zhejiang      | 143                          | 102                | 41                | 28.7%      | [101]     |
| western          | Shanxi        | 2385                         | 1869               | 516               | 21.6%      | [32, 33]  |
|                  | Shanxi        | 30                           | 24                 | 6                 | 20.0%      | [102]     |
| total            | N/A           | 2558                         | 1995               | 563               | 22.0%      | N/A       |

* RHD*DEL1 allele frequency among RhD-negative Han individuals: 23.4% ± 3.8% (mean ± SD) for the 3 studies, 20.0%—28.7% (range)

N/A not applicable

### Table 6  DEL phenotype and RHD*DEL1 genotype in serologic RhD-negative individuals of ethnic groups in China

| Ethnicity | Total RhD-negative individuals (n) | Methods | G1227A PCR-SSP | RHD*DEL1 frequency among DEL phenotype frequency | References |
|-----------|-----------------------------------|---------|---------------|-----------------------------------------------|------------|
|           |                                   | Adsorption-elution |                | Sequencing | Others | Adsorption-elution positive | Rh-negative |            |
|           |                                   | Neg | Pos | Neg | Pos | RHD*DEL1 |                  |            |
| Han       | N/A                               | N/A | N/A | N/A | N/A | N/A | 96.7% | 21.7% | 23.3% | 21 studies |
| Hui       | 12                                | 10  | 2   | 0   | 2   | 2   | 100%  | 17%   | 17%   | 2 studies |
| Uyghur    | 127                               | 124 | 3   | 0   | 3   | 3   | 100%  | 2.4%  | 2.4%  | 2 studies |

N/A not applicable

* See Table 4

* Calculation: 

\[
\frac{(1193 + 31 + 563)/(5669 + 2558) \times 100\%}{21.7\%}, \text{ data from Tables 4 and 5}
\]

* See Table 3

* See Tables 3, 4 and 5. Data shown in this Table 6 for comparison
Clinical consequences

**DEL in Chinese transfusion recipients**

Mak and colleagues documented in 1993 [34] that anti-D rarely occurred in Hong Kong Chinese and speculated that this could be due to the presence of a very weak form of the D antigen [34]. In 2006, we suggested more specifically [11] that East Asians expressing a DEL phenotype and carrying the \( \text{RHD}^*\text{DEL1} \) allele might not form anti-D after exposure to RhD-positive blood. Wang and colleagues in 2014 [42] found in a retrospective study of 643 RhD-negative patients in China, that 72 pregnant women or transfusion recipients developed anti-D. None of them had a DEL phenotype associated with the \( \text{RHD}^*\text{DEL1} \) allele.

Our literature review documented that transfusion recipients with an Asian type DEL are not known to form anti-D when exposed to RhD-positive blood [42, 64]. Patients with a complete DEL phenotype, such as the \( \text{RHD}^*\text{DEL1} \) allele or Asian type DEL, may safely be managed as RhD-positive with regard to red cell transfusions [11]. Prospective observational studies, however, should monitor this practice [65].

Of course, our proposed strategy cannot be based on DEL phenotypes determined by serologic methods alone [65]. Patients with DEL alleles other than the \( \text{RHD}^*\text{DEL1} \) allele, causing the prevalent Asian type DEL, are known to be at risk and can develop allo-anti-D when stimulated by RhD-positive transfusion or pregnancy [61].

**DEL in Chinese pregnant women**

Following the suggestion in 2006 [11] that patients with the \( \text{RHD}^*\text{DEL1} \) allele would not develop anti-D, Shao and colleagues in 2010 [64] investigated 199 RhD-negative pregnant women, 38 of whom (19%) had anti-D. However, 44 other mothers (22%) had an \( \text{RHD}^*\text{DEL1} \) allele, but importantly, none of the pregnant women with the \( \text{RHD}^*\text{DEL1} \) allele had made an anti-D (p < 0.0001; \( 2 \times 2 \) contingency table, Fisher’s exact test, 2-tailed). Hence, all RhD-negative pregnant women who developed anti-D lacked the \( \text{RHD}^*\text{DEL1} \) allele. The conclusion of this study had previously been published as a letter [49]: pregnant...
women with Asian type DEL do not require RhIG (Rh immune globulin) prophylaxis. Similar results were later documented by Wang and colleagues in 2015 [43] who studied the outcome of 142 pregnant women with a DEL phenotype and RhD-positive newborns. Among them, 130 carried the Asian type DEL and did not develop anti-D. In the same report, anti-D was detected after delivery in 6 women with a DEL phenotype. All these 6 women had a partial DEL phenotype caused by \( RHD-CE-D \) hybrid alleles [43]. In the same year, Xu and colleagues [30] studied 178 pregnant women with a DEL phenotype and RhD-positive fetuses, who declared a history of gestations or birth. Among 176 pregnant women, 168 carried the \( RHD^*\text{DEL1} \) and 8 the \( RHD^*\text{DEL2} \) alleles, both being complete DEL, and had formed no anti-D. Anti-D alloimmunization was observed in the remaining 2 women with \( RHD-CE-D \) hybrid alleles, both being partial DEL. The authors concluded that only pregnant women with \( RHD-CE-D \) hybrid alleles, representing partial DEL phenotypes, are at risk of forming anti-D [30].

We concur that RhD-negative pregnant women with a complete DEL phenotype, notably the Asian type DEL, do not need RhIG prophylaxis, while pregnant women with a partial DEL phenotype should receive RhIG prophylaxis.

**DEL in Chinese blood donors**

Researchers recognized in 1993 the extremely weak expression of the D antigen by red cells with a DEL phenotype in China and considered it unlikely that transfusion of DEL red cells to RhD-negative individuals could elicit anti-D alloimmunization [34]. Since then, red cells from DEL positive donors have sporadically been reported in single cases from Austria [66], Japan [38, 67] and Korea [68] to induce anti-D. In Han, 1 case of primary and 2 cases of secondary anti-D immunizations in a total of 11 recipients have been reported, who had been transfused with red cells from donors with an Asian type DEL. In 2012, Chen and Liu [69] performed a retrospective study on 104 RhD-negative recipients who received DEL positive red cell transfusion. None of them had developed anti-D [69]. Blood centers have instituted screening blood donors for the \( RHD \) gene in Germany [12], Switzerland [70], Austria [71], USA [72] and Brazil [73]. None has published this approach to date in China.

Particularly for China, where RhD-negative red cell units are a scarce resource and the DEL phenotype is prevalent, we propose that red cell units from blood donors with any DEL allele may be labelled DEL positive, but not RhD-positive. The red cell genotyping for RhD-negative donors needs to be performed only once, for example at first-time donation. Such systematic red cell genotyping would capture the full set of DEL alleles.

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**Fig. 2** Prevalence of RhD-negative and DEL in clinical cohorts in China per year. The number of individuals with DEL who seek medical care every year, are estimated for the 3 clinical cohorts of transfused patients (upper row), pregnant women (middle row), and blood donors (lower row). Pie charts symbolize the total number (left column), serologic RhD-negative (second column), DEL phenotype (third column), and Asian type DEL (right column). Among the total number of individuals (orange, not to scale), the RhD-negative (blue) represent 0.30% (see Table 2). Approximately 23.3% (see Table 3) of the serologic RhD-negative carry a DEL phenotype (green). The vast majority (96.7%, see Table 4) of all DEL alleles (causing a DEL phenotype) is represented by the Asian type DEL (yellow). The small fraction of other DEL alleles (red) has a cumulative frequency of 3.3% (see Table 4).
in Han individuals and also in the other ethnicities in China, for whom few data are available at this time. The DEL red cell units should still be considered RhD-negative for transfusion purposes, unless the transfusion recipient has formed anti-D before or is currently pregnant. RhD-negative children and women of childbearing age can also be considered.

Benefits of red cell genotyping for DEL in China
An appreciation of the benefits depends on the number of patients, pregnant women and blood donors involved in China at the national level (Fig. 2). Approximately 19.5 million units of 200 ml red cells were transfused in China in 2013 [74]. Assuming an average of 3 units were transfused to each patient [75], we estimated a total of 6,500,000 recipients per year in China. There were approximately 13.3 million pregnancies in 2019 in China, based on the report of 14.6 million live births and adjusted for multiple birth per pregnancy [76]. The reported number of blood donors was 9.7 million in 2008 in China [77, 78] and we estimated an increase to greater 15 million blood donors per year in 2021 [79].

RhD-negative patients
We propose that red cell genotyping for DEL can be performed when a serologic RhD-negative patient is identified, who may require transfusion. Almost a quarter of all RhD-negative patients carry a complete DEL phenotype. The accumulated clinical data in China indicate that these patients could be managed as RhD-positive.

Among the 19,500,000 units red cell units transfused to 6,500,000 recipients [74], an estimated 58,500 units and 19,500 recipients (0.3%) were (serologic) RhD-negative. Also, approximately 13,600 units and 4500 recipients (23.3%) have a DEL phenotype, of which the vast majority carry the Asian type DEL, being a complete DEL phenotype (Fig. 2, upper row). The benefit of an approach to systematic red cell genotyping of all RhD-negative patients is twofold.

First, the transfusion recipients with Asian type DEL will enjoy a much larger supply of red cell units. Any possible delay causing the—unnecessary—procurement of RhD-negative red cells for these patients will be avoided, because an almost unlimited supply of RhD-positive red cells will promptly be available. If blood group antigens, other than the D antigen, need to be matched, because of allo-antibodies, the choice of red cell units for matching is much enlarged. It is known from Western blood supply that patient care is infringed if rare units are needed [80]. Avoiding issues with rare unit supply will hence improve patient care and eventually patient safety.

Second, our proposal will benefit the supply of RhD-negative red cell unit for patients with the definite need for such red cell units [81]. Patients with Asian type DEL will be transfused with RhD-positive red cells. However, red cell units from donors with the Asian type DEL or any other DEL alleles are still contributing to the pool of RhD-negative red cell units for transfusion. Hence, the supply of RhD-negative red cell units for truly RhD-negative recipients would be increased by 30% (calculation: 0.23/0.77 = 0.299).

RhD-negative pregnant women
Red cell genotyping for DEL can be performed once a pregnancy is recognized in a serologic RhD-negative woman. Pregnant women with a complete DEL phenotype, do not need RhIG prophylaxis. Pregnant women with a partial DEL phenotype should still receive RhIG prophylaxis.

Of the 13.3 million pregnancies per year [76], approximately 40,000 were expected to occur in (serologic) RhD-negative women. Approximately 9300 (23.3%) of them will have a DEL phenotype, of which 9000 (96.7%) carry a complete DEL phenotype, represented by the RHDL*DEL1 or RHDL*DEL2 alleles (Fig. 2, middle row).

At present, most pregnant women with RhD-negative in the USA will receive at least one antepartum injection of RhIG and a quarter of them may receive a second injection [81]. RhIG may be unavailable or not regularly obtained at public hospitals on mainland China, although it can occasionally be sourced through select international medical facilities at extra expense [82]. Some RhD-negative pregnant women may need to cover the costs without reimbursement from insurance [82]. RhIG from private hospitals in big cities like Shanghai can cost 700 US$ per injection or from pharmacies in Hong Kong 300 US$ per injection [83].

The positive cost–benefit of using red cell genotyping to guide RhIG prophylaxis among pregnant women with RhD-negative phenotype has been described for the USA [84]. The cost–benefit in China is much greater as 21.6% (Table 4) of the RhIG doses can be saved compared to less than 5% in the USA [81, 84–86]. For China, if red cell genotyping for DEL alleles were performed in RhD-negative women with a complete DEL phenotype, approximately 18,000 doses of RhIG annually can be avoided and hence up to 12,600,000 US$ can be saved annually.

RhD-negative blood donors
We propose to explore if red cell genotyping for DEL can routinely be performed in donors. Red cell units from blood donors with any DEL allele should be labelled DEL
positive. Such DEL red cell units can still be considered RhD-negative for most transfusion purposes.

Of the 12 million blood donors in China per year, approximately 36,000 are expected to be (serologic) RhD-negative. We estimated that approximately 8300 donors (23.3%) have a DEL phenotype, of which the vast majority carry the Asian type DEL (Fig. 2, lower row).

An estimated 1.7 million individuals in China carry the DEL phenotype. If these individuals could be explored as potential DEL donors to establish a separate DEL donor pool, this strategy would significantly lessen the demand for RhD-negative blood and would have a profound effect on the supply of red cell units for transfusion to RhD-negative recipients.

**Practical strategy for resolving RhD-negative individuals with DEL phenotype**

We propose that red cell genotyping for DEL can be performed whenever a (serologic) RhD-negative phenotype is detected in potential transfusion recipients, pregnant women and blood donors. Recipients and pregnant women with a complete DEL phenotype, notably the Asian type DEL, should be managed as RhD-positive with regard to transfusion or RhIG administration or both. Blood donors with any DEL phenotype can still be considered RhD-negative for most transfusion purposes. Systematic red cell genotyping has often been reviewed [87–96], beginning in 2001 for clinical applications [12] and red cell unit management [97, 98].

**Algorithm**

To facilitate implementation of our proposals, we developed an algorithm to resolve red cell genotyping results among serologic RhD-negative individuals (Fig. 3). PCR-SSP of RHD intron 4 and exon 7 had a greater positive predictive value for the presence of RHD gene [9, 88] than screening for other exons, such as RHD exon 10 [99, 100]. PCR-SSP of the RHD(1227G > A) single nucleotide variant (SNV) in exon 9 has successfully been applied to detect Asian type DEL in many studies [9, 17, 18, 28, 29, 32, 33, 39–42, 44, 45, 47, 88, 101–106].

Complete DEL alleles, other than the Asian type DEL, and all partial DEL alleles can be specifically detected by nucleotide sequencing [9], if desired. A need for nucleotide sequencing would occur in less than 4% of all RhD-negative individuals (Table 4). Nucleotide sequencing can, however, be particularly informative in ethnic groups, other than Han, where the DEL frequency and alleles may vary from the known situation in Han and remains currently largely unexplored.

**Cost-effectiveness of red cell genotyping**

Eventually, manufacturers of RHD genotyping assays and systems may offer cost-effective red cell genotyping tests designed to identify the presence of the RHD gene and the Asian type DEL. Our literature review documented that the accuracy to identify Asian type DEL in Han by PCR-SSP is at least comparable to adsorption-elution methods and similar to nucleotide sequencing.

Kacker and colleagues [84] evaluated the financial implication of RHD genotyping to guide RhIG
prophylaxis for pregnant females in the USA and found a saving for the medical care system at any cost of 256 US$ or below. The national insurance system in China reimburses 80–90% of the cost if the medical service is approved by the China Ministry of Health, while most genetic tests, including any red cell genotyping, are currently not approved and thus not paid by the insurance [107]. A cost-effectiveness analysis may be performed, as in other health care systems before [84], and be used to develop recommendations in a commissioned report by the government or in a ‘white paper’.

**Complexity of red cell genotyping for DEL**

Red cell genotyping in general and DEL screening in particular are not widely available and accessible in China. Currently, molecular DEL typing is mainly performed through the clinical laboratories affiliated with few top-ranked hospitals in major cities such as Shenzhen [17], Shanghai [28], Hefei [18], Nanjing [29], and similar institutions. Most local hospital-affiliated laboratories may not be outfitted for such test. Although DEL molecular typing could also be offered as part of a research program by universities and other institutions, no such diagnostic result reported by research laboratories is generally authorized for clinical care [107]. The increased market demands have stimulated development of private commercial laboratories, where advanced hardware and data management could be harnessed for DEL molecular testing. Medical genetic professionals should be trained in this precision medicine application and could be employed much wider than in a few public hospitals. Presently, samples for red cell genotyping, such as DEL molecular testing, can be sent to reference laboratories, where standardization of laboratory methods is promoted and the economy of large-scale testing can be achieved [81].

**Conclusion**

The DEL phenotype is a D variant with low amounts of D antigens on the red cell surface occurring among RhD-negative individuals, who are rare in East Asia. The prevalence of DEL and its molecular bases are well characterized for the Han population, whereas sufficient information for other ethnic groups in China is lacking. Red cell genotyping for DEL is predicted to lessen the demand for RhD-negative blood, save money for RhIG injection and increase the pool of red cell donors available for RhD-negative transfusion. The distinction of complete DEL and partial DEL is required and can—as a practical routine approach—only be achieved by molecular methods. We propose to explore a timely introduction and full integration of molecular typing for DEL into the clinical practice of China.

**Statement of Disclaimer**

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**Authors’ contributions**

QY collated the data and wrote drafts of the manuscript. WAF conceptualized the study, contributed to data collection, wrote parts of the manuscript, edited and finalized the manuscript. All authors read and approved the final manuscript.

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