# Occurrence of Rare Deletional Yus and Gerbich Alleles in Syria and Neighbouring Countries

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## Abstract

***Background:*** Gerbich-negative phenotypes of the Gerbich Blood Group System (ISBT 020) are very rare (with the exception of Papua New Guinea). The Gerbich-negative phenotypes Yus and Gerbich are negative for the antigens Ge2, and Ge2 and Ge3, respectively. In antigen-negative individuals, anti-Ge2 and anti-Ge3 antibodies can be naturally occurring, or are triggered during pregnancies and after transfusions. Previous studies suggested an elevated frequency of Gerbich-negative phenotypes for the Middle East. In the summer of 2015, a large-scale migration of people from the Middle East to Europe occurred raising the issue of question how to guarantee blood supply for patients and manage antenatal care for pregnant women from these countries. ***Materials and Methods:*** To investigate the frequency of rare Gerbich-negative phenotypes, 1,665 immigrants to Germany originating from the Middle East were genetically tested for the presence of rare Yus, i.e., *GE*01.-02*, and Gerbich, i.e., *GE*01-03*, alleles and compared to results obtained from 507 Germans. ***Results:*** Seven Yus *GE*01.-02.01* and one Gerbich *GE*01.-03.02* alleles were exclusively observed among people from the Middle East, with five of them clustering among 797 Syrians. No such alleles were observed in Germans. A cumulative Yus- and *GE*01.-03*-type allele frequency of 0.00314 and resultant overall Gerbich-negative phenotype frequency of one among 101,633 Syrians were calculated. ***Conclusion:*** This manuscript describes for the first time an exclusively genetic screening for carriers of Gerbich-negative alleles. In conclusion, the Gerbich blood group system should be considered as one causative agent of unusual antibodies to red cell antigens, in routine patients and pregnant women, especially when originating from the Middle East.

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## Introduction

Variable distribution of blood group antigens between one ethnic group and another is well documented. Such ethnically specific distribution of antigens can be observed for almost every blood group system.

For instance, within the blood group system ABO (ISBT 001), the ratio of blood group A to B individuals in the three major ethnic groups of Caucasians, Black Africans, and Asians is 43% (blood group A)–9% (blood group B), 27–20%, and 28–25%, respectively [1]. Still, additional major variation for ABO is documented within these main ethnic groups [2]. For MNS (ISBT 002), absence of the high-incidence antigen U (MNS5) and presence of the low-incidence antigen Mur (MNS10) is highly indicative for individuals of Black African and East Asian ancestry, respectively [3–5]. In general, frequency of the "low-incidence" GP.Mur glycophorin can be high-

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er than 80% in the Amis, the largest ethnic group among Taiwan aborigines [6]. Within the P1PK blood group system (ISBT 003) more recently, the G-allele of SNV rs5751348 has been described to cause P1 positivity versus negativity [7, 8]. Using genetic polymorphism and frequency data, homo- and heterozygotes expressing P1 may therefore be predicted from genetic data at frequencies of 0.50, 0.30, and 0.25 for Europeans, Africans, and Asians, respectively [9].

The exemplified population-dependent variation of an antigen prevalence within a blood group system can have importance in clinical transfusion medicine. For this reason, supplying one ethnic group with blood from another ethnic group can cause fundamental problems, e.g., statistically elevating the risk of alloimmunization. Additionally, certain patients may only be transfused with blood from donors of the same ethnicity. This may be exemplified for individuals with negativity for U (MNS5), or Js$^b$ (KEL7), both occasionally found among Black Africans, and individuals negative for Di$^b$ (DI2) sometimes encountered among ethnic groups of Mongoloid heritage [10]. Historical and current migration, especially on a large scale, and blood donation behaviour reasoned by sociocultural differences can therefore raise specific challenges for modern transfusion medicine. Consequently, such migration situations need to be identified in time and addressed accordingly.

In the summer of 2015, a large-scale migration movement took place from the Middle East towards Europe [11]. In Germany, 2015 marked the highest immigration and the highest net migration since 1992. German immigration increased by 46% in comparison to the previous year, and the absolute migration gain, subtracting onward travel to other EU countries, resulted in 1.14 million persons. Also in 2015, Syrians were by far the largest group of immigrants to Germany with 326,900 individuals. The same applies to the strong influx of immigrants from Afghanistan, Iraq, and Pakistan. The generally increased migration to Central Europe has put a focus on the question how to guarantee blood supply for patients from these countries [12]. In terms of blood transfusions, thalassaemia, which requires regular blood supply, plays a particularly important role, due to its higher frequency in patients from Asia in comparison to Central Europeans [13]. In an earlier study, we therefore determined common blood group allele types and their frequencies in individuals from Arabian countries and Iran by molecular typing and compared them to a German rare donor panel [12].

Here, we report on an overlapping collective of individuals mainly immigrating from the Middle East to Germany and on a molecular approach for frequency estimation of two specific Gerbich phenotypes, the Yus (Ge:−2,3,4) and Gerbich type (GE:−2,−3,4) of the Gerbich blood group system. Data from Middle Easterners were compared to a German control group. The designation of a specific phenotype within a blood group system and the naming of the blood group system itself with an identical word, namely, "Gerbich," is terminologically unfortunate and confusing. Therefore, although the term "Yus" will continue to be used in this manuscript, the term "Gerbich" will be omitted and GE:−2,−3,4 will be used to designate the Gerbich phenotype. Gerbich-negative phenotypes such as the Yus- and GE:−2,−3,4-type homo- and compound heterozygotes are very rare and were only observed in one individual among over 44,000 samples from white populations [4]. Otherwise, the GE:−2,−3,4 phenotype is remarkably common only in Melanesians, especially Papua New Guinea, suggesting that it protects against malaria [4, 14]. However, in a previous study, a vast majority of blood samples collected to investigate the exact molecular background for Yus and GE:−2,−3,4 phenotypes were from self-identified Caucasian individuals of Middle Eastern origin and from Eritrea, Northern Africa (Maghreb area), and the Balkan region of South-East Europe, suggesting a potentially higher frequency of these phenotypes in these geographic areas [15].

Of the 13 antigens of the Gerbich Blood Group System (ISBT 020) described in total so far, the Yus, GE:−2,−3,4, and Leach phenotypes type negative for the antigens Ge2, Ge2 and Ge3, and Ge2, Ge3, and Ge4, respectively [16]. The Yus phenotype is encoded by homo- or compound heterozygosity of four different Yus alleles, each characterized by deletions of exon 2 and adjacent introns [15, 17]. Heritability of the GE:−2,−3,4 phenotype is homo- or compound heterozygosity but composed of three different *GE*01.-03* alleles, with deletions of exon 3 and adjacent introns of *GYPC* [15, 17]. Molecular characterization of Leach-negative haplotypes either showed deletions of *GYPC* exons 3 and 4 or a single nucleotide frameshift deletion in exon 3 [16, 18].

Anti-Gerbich antibodies mostly do not cause serious haemolytic transfusion reactions and antigen-positive, serologically incompatible red cell units can usually be used for transfusion [4]. However, haemolytic disease of the fetus and newborn has been reported, especially in the presence of anti-Ge3 [4, 19]. In their association with a late-onset anaemia in neonates, anti-Ge3 antibodies are of particular clinical significance. Consequently, pregnancies and neonates involving such antibodies should be monitored for several weeks [4, 19, 20].

This study had two main objectives. Firstly, the exact description of the molecular background of Yus and GE:−2,−3,4 phenotypes has simplified their molecular detection and reliable heterozygous typing only recently [14, 15]. Since more numerous, typing for heterozygotes allows for higher accuracy in allele frequency estimates than serological screening for homozygous (and com-

**Table 1.** Primers used for PCR amplifications and sequencing (*primers according to [15])

| GYPC genetic region, type | Primer name | Primer sequence 5′→3′ | Primer 3′ at nt of NG_007479.1 | Amplicon length (wildtype/deletion) |
|---|---|---|---|---|
| Diagnostic amplicon, wildtype versus Yus (GYPC exon 2, NG_007479.1: 39,148–39,204) | GYPC_E2_Amp_F | AGCAGCACCTGGCATTTAGT | 37,042 | 5,827 bp/2,220 bp |
| | GYPC_E3+153R* | ATGTCCATTATGGTGGGGGTG | 42,806 | |
| Diagnostic amplicon, wildtype versus Ge (GYPC exon 3, NG_007479.1: 42,757–42,840) | GYPC-i2-3511F* | CCTCACCATAATGGAAACTGCCG | 39,246 | 4,963 bp/1,378 bp |
| | GYPC-i3-675R* | CATACCCCAGGACTGAACCTTGT | 44,164 | |
| Amplicon for sequencing, wildtype versus Yus (GYPC exon 2, NG_007479.1: 39,148–39,204) | GE1-e2-29F* | ACCACAATGCATACTACCAC | 39,196 | 5,130 bp/1,523 bp |
| | GE25-i3-503R* | TGCCTTCCTAAAATGATTCTCC | 44,285 | |
| Positive amplification control on PTX1 (CRP) | CRP-1 | CCAGCCTCTCTCATGCTTTTGGCCAGACAG | na | 448 bp |
| | CRP-2 | GGGTCGAGGACAGTTCCGTGTAGAAGTGGA | na | |
| Sequencing primer | | | | |
| Sequencing wildtype | GYPC_Yus02_Seq | AGGCTGCACTTGTTCACTGT | 37,379 | na |
| Sequencing Yus | GYPC_Yus01_SeqF | GGTGCACCACACTGTCTCTG | 39,026 | na |
| Sequencing wildtype | GYPC-i2-1015_F* | TCCTGAGCAGCCTTACAC | 40,235 | na |
| Sequencing Ge | GYPC_Ge02_SeqF | GCATTCTAAGAAAAGACAGA | 40,116 | na |

pound heterozygous) Yus and GE:–2,–3,4 phenotypes alone. Using this benefit of genetic Yus and GE:–2,–3,4 determination in comparison to serotyping, frequency of these alleles should be compared between a representative, mainly Syrian-influenced immigrant group with German Central Europeans. Secondly, observed allele frequencies do have a direct influence on the expected probability of antibody formation against the Blood Group System Gerbich antigens Ge2 and Ge3 in specific populations. The aim of this study was therefore also to indirectly predict whether anti-Ge2 and anti-Ge3 antibodies are to be expected more frequently in people from the Middle East, e.g., immigrants to Europe, than in Central Europeans.

## Materials and Methods

### DNA Isolation

DNA was isolated from EDTA anticoagulated blood by automated magnetic bead technology either by the EZ1 DNA Blood Kit (Qiagen, Hilden, Germany) or by DNA Blood Kit special (MSM1; Chemagen, Perkkin Elmer, Baesweiler, Germany) according to the manufacturers' instructions. Typical ranges of DNA concentration were 20–45 ng/μL.

### PCR and Sanger Sequencing

Identification of specific deletions within the GYPC gene was performed by two long-range PCR reactions covering either exon 2 (Yus phenotype) or exon 3 (GE:–2,–3,4 phenotype) according to published primers [15] with slight modifications (Table 1). PCR was performed in a final reaction volume of 15 μL containing 40–90-ng DNA, GoTaq Long PCR Master Mix (Promega, Walldorf, Germany) in a 33% concentration of the manufacturer's instruction, a total of 1.5-mM MgCl$_2$, 0.66-μM allele-specific sense and anti-sense primer each, and 0.07-μM CRP1 and CRP2 internal control primer (all primers provided by TibMolbiol, Berlin, Germany). The cycle protocol consisted of an initial denaturation at 95°C for 3 min, followed by 10 cycles at 93°C for 20 s and 71.2°C (exon 2 deletion), or 69°C (exon 3 deletion), respectively, for 5 min followed by 20 cycles at 93°C for 20 s and 65°C for 5 min, and a final extension at 72°C for 10 min (Life Touch thermal cycler; Bioer Technology, Hangzhou, China). In case of exon 2 or 3 deletions, the respectively reduced amplicon length was detectable in 1% agarose gels in 1 X TBE buffer at 140 V in 40 min running time.

The deletion breakpoints within the amplicons were detected by Sanger cycle sequencing. Amplicons of reduced length corresponding to an exon 2 or exon 3 deletion were extracted from the agarose gel using the Monarch Gel extraction kit (New England Biolabs, Frankfurt, Germany). Extracted amplicons were purified and amplified in 20-μL reactions with 4 μL of Big Dye Terminator v3.1 chemistry (Applied Biosystems/Thermo Fisher, Fisher Scientific GmbH, Schwerte, Germany) and 1 μL of 0.25 μM sequencing primers and were subjected to electrophoretic separation in an ABI Prism 310 Genetic Analyzer [21]. To identify the positions of the two repeat regions [15], Basic Local Alignment Search Tool (BLAST) algorithms from the National Center for Biotechnology Information (NCBI) were used. Primers for PCR and Sanger sequencing are given in Table 1.

### Designation of the Geographical Term "Middle East"

The Middle East is a geopolitical term [22] that refers to a region spanning the vast majority of Western Asia and all of Egypt (mostly in North Africa). It includes the political nations of the Arabian Peninsula (Bahrain, Oman, Qatar, Saudi Arabia, United Arab Emirates, and Yemen), Akrotiri and Dhekelia (Cyprus), Cyprus, Egypt, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Palestine, Syria, and Turkey [23]. Due to the geographic proximity and the common borders with Turkey and Iran, Azerbaijan was also included in the geographical area of the "Middle East" in this study. For geographical reasons as well, individuals from Egypt were excluded from the "Middle East" in this study and listed under "North Africans" together with people from Algeria, Eritrea, Libya, Morocco, and Tunesia.

**Table 2.** Study samples and results of Yus (*GE\*01.-02*) and Gerbich (*GE\*01.-03*) allele detection grouped in four geographical study regions Middle East, Central Asia, Northern Africa, and Germany

| Geographical region | Sum | Male | Female | Yus, *GE\*01.-02* | Ge, *GE\*01.-03* |
|---|---|---|---|---|---|
| Middle East | | | | | |
|    Syria | 797 | 741 | 56 | 4 (male) | 1 (male) |
|    Iran | 147 | 124 | 23 | 0 | 0 |
|    Turkey-1 | 124 | 94 | 30 | 1 (male) | 0 |
|    Turkey-2 | 471 | na | na | 0 | 0 |
|    Iraq | 49 | 41 | 8 | 0 | 0 |
|    Lebanon | 48 | 41 | 7 | 1 (male) | 0 |
|    Arabian Peninsula: Oman (1), Saudi Arabia (4), United Arab Emirates (1), Yemen (2) | 8 | 5 | 3 | 0 | 0 |
|    Jordan | 7 | 6 | 1 | 0 | 0 |
|    Israel | 6 | 4 | 2 | 0 | 0 |
|    Palestine | 5 | 5 | 0 | 0 | 0 |
|    Azerbaijan | 3 | 1 | 2 | 1 (female) | 0 |
|    Subtotal Middle East | 1,665 | 1,062 | 132 | 7 | 1 |
| Central Asia | | | | | |
|    Pakistan | 27 | 23 | 4 | 0 | 0 |
|    Afghanistan | 24 | 18 | 6 | 0 | 0 |
|    Kazakhstan | 3 | 2 | 1 | 0 | 0 |
|    Tajikistan | 2 | 0 | 2 | 0 | 0 |
|    Kyrgyzstan | 1 | 1 | 0 | 0 | 0 |
|    Subtotal Central Asia | 57 | 44 | 13 | 0 | 0 |
| Northern Africa | | | | | |
|    Morocco | 19 | 11 | 8 | 0 | 0 |
|    Egypt | 9 | 7 | 2 | 0 | 0 |
|    Tunesia | 7 | 4 | 3 | 0 | 0 |
|    Algeria | 5 | 5 | 0 | 0 | 0 |
|    Eritrea | 3 | 3 | 0 | 0 | 0 |
|    Lybia | 1 | 1 | 0 | 0 | 0 |
|    Subtotal Northern Africa | 44 | 31 | 13 | 0 | 0 |
| Germany (total) | 507 | na | na | 0 | 0 |
| Total | 2,273 | na | na | 7 | 1 |

*Sample Origin*

Samples from immigrants included in this study were collected in between June 2016 and April 2018 [12]. Participants self-identified their nationality. 265 Syrian volunteers were recruited and 8 mL of EDTA anticoagulated blood were collected for the study at social venues for refugees in Rhineland-Palatinate from June 2016 until October 2017. Among 139 of these 265 refugees with available self-declared places of residence, their proportion from different regions of Syria seemed relatively evenly distributed and in line with local settlement density. For example, in our study and per 1,000 inhabitants 3.9 were from Damascus (southwest), 2.5 from Aleppo (northwest), 9.8 from Al Hasakah (northeast), and 9.2 from Idlib (northwest). All other immigrant donors presented from July 2017 to April 2018 at regular blood donation activities of the German Red Cross Blood Service West in North Rhine-Westphalia, Rhineland-Palatinate, and Saarland. There was no preselection concerning ABO blood groups and Rhesus factors. All subjects gave their informed consent. Studies on blood group antigen frequency estimates in individuals immigrating to Germany were approved by the Ethics Committee of the Medical Chamber of Rhineland-Palatinate in Mainz, Germany (approval number 837.020.16). An additional 471 individuals included in this study were regular blood donors of Turkish origin residing in Germany and identified by their Turkish family names. The German control group consisted of 507 regular German blood donors. In total,

2,273 individuals from the Middle East ($n$ = 1,665), Central Asia ($n$ = 57), Northern Africa ($n$ = 44), and Germany ($n$ = 507) were included in the study (Table 2).

*Gerbich Yus- and GE:–2,–3,4-allele frequency estimates and additional statistics*

The population frequency of Gerbich Yus and GE:–2,–3,4 phenotype causative alleles was calculated from direct counts among individuals of investigated nationalities. Confidence intervals were calculated according to the Poisson distribution [24]. Donors were not screened for kinship. Fisher's Exact test was used for the comparison of categorical variables between groups. Statistical analyses were performed with the software package SPSS 26.0 for Windows (SPSS, Chicago, IL, USA). Statistical significance was defined as $p$ value <0.05.

## Results

Two diagnostic PCRs per sample were used for the discrimination of wildtype and deletional *GYPC* alleles, characterized by deletions of exon 2 and adjacent introns (deletion of 3.607 bp) in Yus and exon 3 and adjacent in-
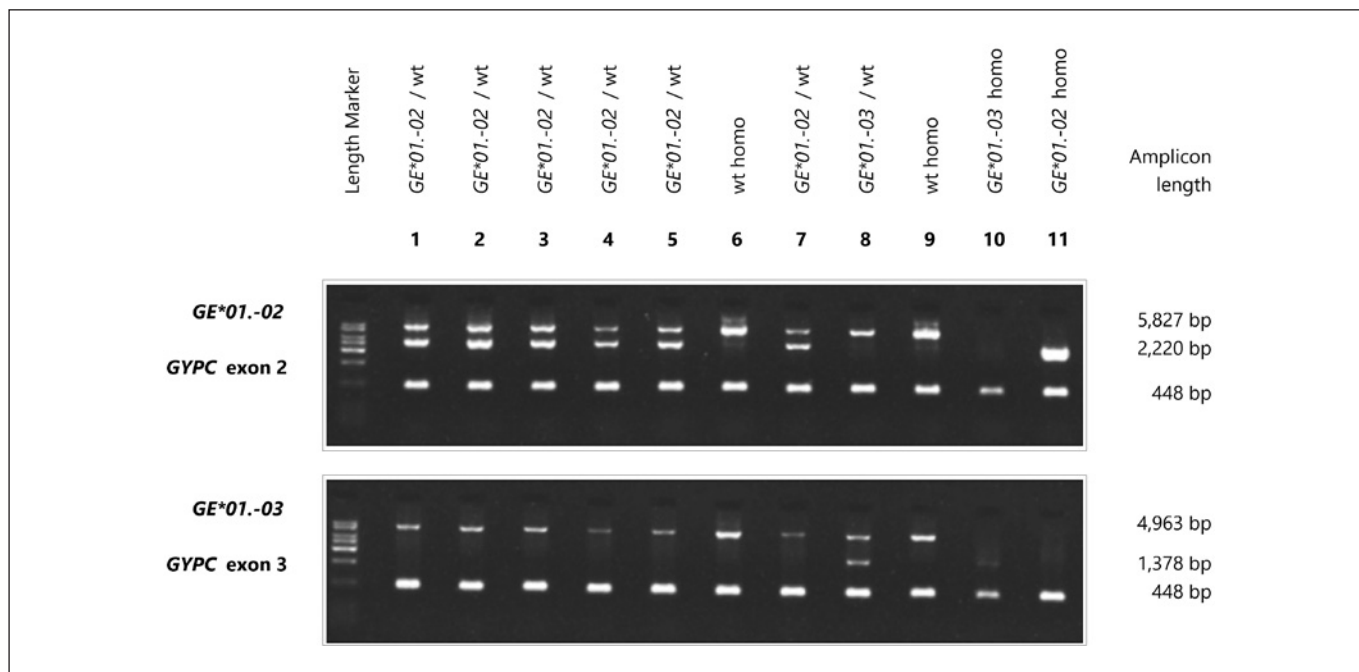
**Fig. 1.** Diagnostic PCR and agarose gel electrophoresis of exemplary study and control samples. Currently known Yus alleles, i.e., *GE*01.-02*, are identified in the upper panel by a length-shift of the diagnostic wildtype (wt, *GE*01*) amplicon from 5,827 bp to 2,220 bp in the presence of a Yus-specific 3,607-bp deletion, including *GYPC* exon 2. The length-shift of all currently known *GE*01.-03* alleles is 3,585 bp, includes *GYPC* exon 3, and reduces the diagnostic 4,963-bp wt amplicon to the deleted 1,378-bp *GE*01.-03* amplicon shown in the lower panel. Lanes 1–5 and 7 represent heterozygous *GE*01.-02*/GE*01* (Yus/wildtype), lanes 6 and 9 homozygous *GE*01/GE*01* (wt/wt), and lane 8 a heterozygous *GE*01.-03/GE*01* (Gerbich/wt) genotype samples of individuals identified during this study. Lane 10 represents a homozygous *GE*01.-03.02* control sample with suboptimal amplification in the upper panel due to depletion of DNA sample material. Lane 11 is a homozygous *GE*01.-02.01* control sample. Marker lane, both panels. Smallest visible band 500 bp, 1,000 bp, (most intense) 1,500 bp, (double band) 2,000 bp and 2,500 bp, (double band) 4,000 bp and 6,000 bp.

trons (deletion of 3,585 bp) in *GE*01.-03* alleles encoding the GE:–2,–3,4, e.g., "Gerbich" phenotype, respectively. Homozygous wildtype genotypes only delivered amplicons with regular lengths of 5,827 bp and 4,963 bp for the two diagnostic reactions. Presence of the various Yus alleles however was indicated by a reduction in length by 3,607 bp of the 5,827-bp diagnostic amplicon to 2,220 bp and a length-shift of the 4,963-bp diagnostic amplicon by 3,585 bp to 1,378 bp in the presence of any *GE*01.-03* allele. Both reactions were validated using known homozygous Yus, e.g., *GE*01.-02.01* and *GE*01.-03.02*, genotype samples. Positive amplification of every reaction was controlled by a 448-bp positive amplification product of *PTX1* (*CRP*).

Diagnosis of all 2,273 samples investigated in this study delivered seven individuals of apparent *GE*01* I *GE*01.-02* (Yus) and one individual of apparent *GE*01* I *GE*01.-03* heterozygous genotypes. Figure 1 shows photo-documentation of an agarose gel electrophoresis of all study samples with Yus and *GE*01.-03* allele detection, lacking one *GE*01* I *GE*01.-02* (Yus) individual due to sample depletion.

Of all heterozygous samples, both amplicons, e.g., of regular and reduced length, corresponding to *GYPC* exon 2 or exon 3 deletional alleles, were extracted from an agarose gel and sequenced for their wildtype and breakpoint region to identify their exact allelic types. Basic Local Alignment Search Tool (BLAST) algorithms from the National Center for Biotechnology Information (NCBI) and additional alignments confirmed full identity of all seven Yus and the single *GE*01.-03* alleles with previously reported *GE*01.-02.01* (Yus) and *GE*01.-03.02* alleles, respectively [15].

Individual samples were grouped into one of four geographical regions according to their self-declared or officially confirmed nationality. In total, 2,273 DNA samples of individuals from the Middle East (n = 1,665), Central Asia (n = 57), Northern Africa (n = 44), and Germany (n = 507) were included in this study (Fig. 2; Table 2). All deletional Yus and GE:-2,-3,4 encoding-alleles were exclusively found among individuals of Middle Eastern origin with a majority of them identified among individuals of Syrian nationality. The seven heterozygous carriers of *GE*01* I *GE*01.-02.01* (Yus) genotype were from Syria
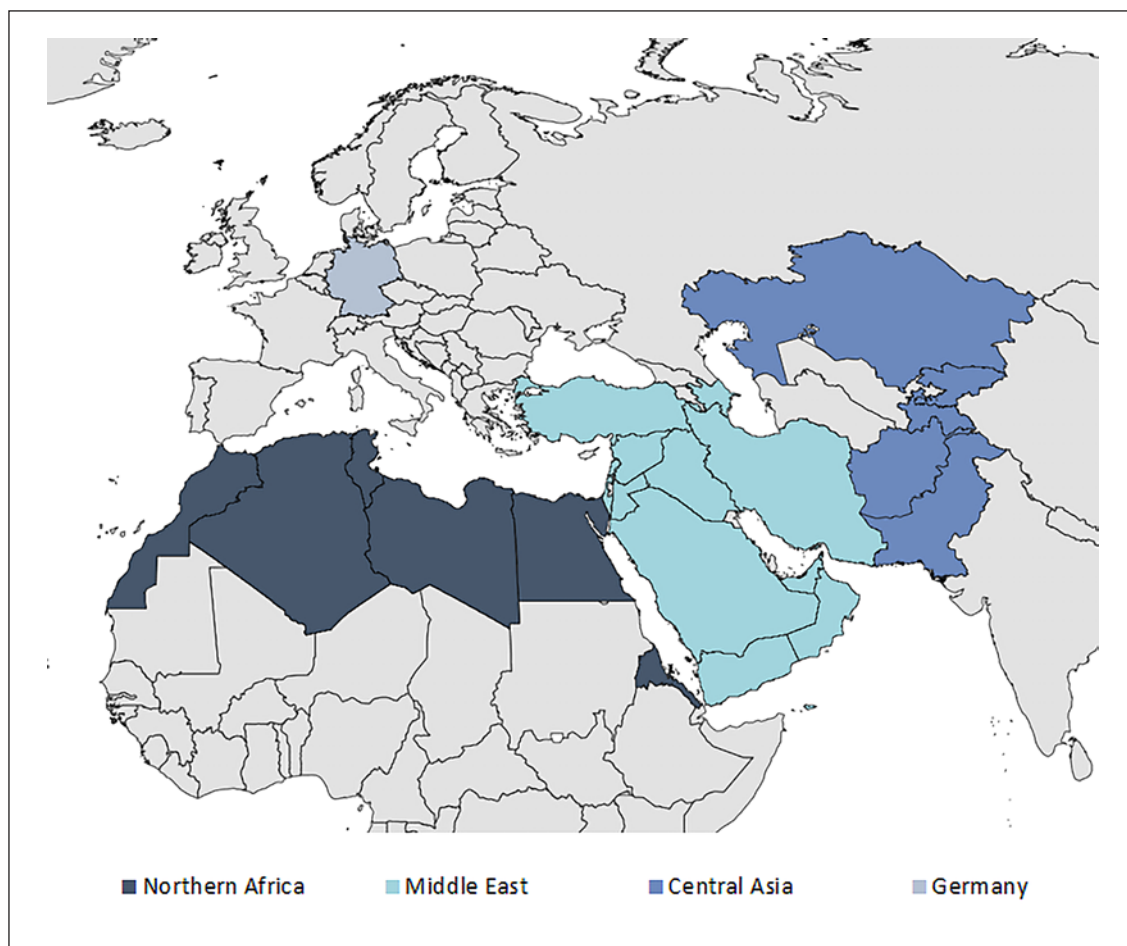
Gassner/Scherer/Zanolin-Purin/
Scharberg/Flesch

**Fig. 2.** Geographical map of the four geographical study regions: Middle East, Central Asia, Northern Africa, and Germany.

($n$ = 4), Turkey ($n$ = 1), Lebanon ($n$ = 1), and Azerbaijan ($n$ = 1) and were 7 males and 1 female, respectively. The single heterozygous carrier of *GE*01* I *GE*01.-03.02* genotype was male and originated from Syria ($n$ = 1) (Table 2).

Cumulative population frequency of four Yus and one *GE*01.-03* alleles among 797 individuals from Syria was 0.00314 with a lower and upper 95% confidence interval of 0.00124 and 0.00701, respectively. Using this cumulative allele frequency of 0.00314, the frequency of homozygotes and compound heterozygotes, i.e., phenotypically Gerbich-negatives of either GE:-2,3,4 or GE:-2,-3,4 phenotype, is calculated to one Gerbich-negative individual among 101,633 Syrians, or 0.986 per 100,000 Syrians. For all 1,665 individuals originating from the Middle East, respective numbers were 0.00240, 0.00099, and 0.00448. Among all 507 people from Germany, 44 from Northern Africa, and 57 from Central Asia, no deletional *GYPC* alleles were observed (Table 3). Cumulative allele frequency comparison of Syria with Germany was not statistically significant ($p$ = 0.085).

## Discussion

The designation of a specific phenotype within a blood group system and the naming of the blood group system itself with an identical word, namely, "Gerbich", is terminologically unfortunate and confusing. Therefore, the term "Gerbich" when addressing the respective phenotype was omitted throughout this manuscript and GE:–2,–3,4 used instead. The Gerbich-negative phenotype Yus (Ge:-2,3,4) has been found in Europeans, in the Middle East, and in people of African origin. Although the Gerbich-negative phenotype GE:–2,–3,4 has been detected in about one of two Melanesians in the Morobe region of Papua New Guinea, it has only been observed with exceeding rarity in Europeans and Africans and sporadically in people from Iraq, Native Americans, Japanese, and Polynesians. However, outside Papua New Guinea, Gerbich-negative phenotypes are very rare. This is also evidenced by the observation that only one individual of an unspecified Gerbich-negative phenotype has been observed among more than 44,000 samples from white populations [4].

**Table 3.** Yus (*GE*01.-02*)- and Gerbich (*GE*01.-03*)-allele frequency estimates for Syria and four geographical study regions: Middle East, Central Asia, Northern Africa, and Germany

| Geographical region | Individuals | Alleles | Yus, *GE*01.-02*, n | Ge, *GE*01.-03*, n | Sum, *GE*01.-02*, *GE*01.-03*, n | Allele FRQ | Allele FRQ (95% CI lower) | Allele FRQ (95% CI upper) |
|---|---|---|---|---|---|---|---|---|
| Syria | 797 | 1,594 | 4 | 1 | 5 | 0.00314 | 0.00124 | 0.00701 |
| Middle East | 1,665 | 3,330 | 7 | 1 | 8 | 0.00240 | 0.00099 | 0.00448 |
| Central Asia | 57 | 114 | 0 | 0 | 0 | 0.0 | 0.0 | 0.02882 |
| Northern Africa | 44 | 88 | 0 | 0 | 0 | 0.0 | 0.0 | 0.03733 |
| Germany | 507 | 1,014 | 0 | 0 | 0 | 0.0 | 0.0 | 0.00324 |

A more recent analysis of a collection of 29 rare samples according to their origin then led to the speculation that Gerbich-negative phenotypes may occur more frequently in people with ancestry from the Balkans, the Middle East, and Northern Africa [15]. This assumption was supported by an anti-Ge2 carrier case report from the United Arab Emirates [25]. Returning to one of the aims of this study, it can now be said that although antibodies against Gerbich-negative phenotypes are to be expected more frequently in Syria than in Germany, these are still likely to be very rare events considering a cumulative phenotype frequency from Yus homozygotes, or Yus/*GE*01.-03* compound heterozygotes of just under 1 (0.986) per 100,000 inhabitants. The study presented here was also intended as a response to the recent immigration movement from the Middle East to Europe, exacerbating the associated need to secure matchable blood supplies [11]. We therefore decided to investigate the frequency of *GYPC* alleles leading to Gerbich-negative phenotypes in a collective of immigrants to Europe and to compare the results with a German control group. For this purpose, we used a molecular method that has only recently allowed the determination of heterozygous carriers of such Gerbich-negative alleles and their unambiguous identification [15]. By determining the frequency of heterozygous carriers of Gerbich-negative alleles, the frequency of Gerbich-negative phenotypes can be calculated much more precisely than by serological determination alone.

Seven Yus alleles *GE*01.-02.01* and one "phenotype Gerbich" allele *GE*01.-03.02* were observed in total and exclusively among 1,665 people from the Middle East. Neither among 507 Germans nor – admittedly few – 57 Central Asians and 44 North Africans was a single Gerbich-negative allele identified. Within the people from the Middle East, there was an accumulation of Gerbich-negative alleles among immigrants from Syria. For 797 Syrians, a cumulative allele frequency of 0.00314 for Gerbich-negative alleles can therefore be given as the sum of four observed *GE*01.-02.01* (Yus) and one *GE*01.-03.02* with individual frequencies of 0.00251 and 0.00063.

The cumulative phenotype frequency calculated from our data, i.e., phenotypically Gerbich-negatives of either GE:-2,3,4 or GE:-2,-3,4 phenotype, is one Gerbich-negative individual among 101,633 Syrians. This frequency is even lower, instead of higher as expected, than indicated above for samples from white populations with one among more than 44,000 [4]. One effect that would correct this number upwards would be the presence of fully unexpressed *GYPC* null alleles; e.g., *GYPC* null alleles inactivated in their expression by missense, nonsense, splice-site, frame-shift mutations or large deletions. Such alleles were not specifically searched for in this study. In a previous study, the presence of the currently known *GYPC* null alleles, also referred to as Leach alleles *GE*01N.01* and *GE*01N.02* by the ISBT, were excluded from the samples. But the search for Leach alleles that might have a different molecular background than *GE*01N.01* and *GE*01N.02* was also omitted at that time [15]. In fact, a re-analysis of the genotypes of 28 samples with Yus or Gerbich phenotypes of that study revealed an implausible Hardy-Weinberg distribution (data not shown). In contrast, when the existence of a hypothetical Leach allele with a deletion of at least *GYPC* exon 2 and 3 plus an inactivating mutation was assumed, the serologically proven numbers of phenotypes remained unchanged, but the modelled number of genotypes improved towards the expected Hardy-Weinberg distribution. As in the previous study, the lack of identification of such a hypothetical Leach allele or a group of such Leach alleles and their detection is a limitation of this study [15].

In summary, the method described here is capable of recognizing all subtypes of the currently known four Yus and three GE:-2,-3,4 alleles. In combination with sequencing, the diagnostic amplicons are also suitable for detecting new alleles as long as their deletional mutations are within the (comprehensive) limits set by the primers used. However, unknown deletional *GYPC* alleles, including Leach alleles, may have remained undetected in the study, which would have led to an underestimation of the true frequency of the GE-negative phenotype in Syrians. Nevertheless, for the first time this manuscript describes an

exclusively genetic screening for carriers of alleles *GE\*01.-02* and *GE\*01.-03* and all their currently known subtypes, expressing the Gerbich-negative phenotypes Yus and GE:-2,-3,4. This way, this study provides the most robust frequency estimate for GE negativity in the Syrian population using our current knowledge of the Gerbich blood group system. Technically and as an alternative to our approach, modern gene-dose determination methods such as next generation sequencing may also have the potential to comparable results, e.g., an accurate detection of respective heterozygotes with one parental haplotype showing large deletions of *GYPC* [26]. The expected clustering of Yus (*GE\*01.-02*) and *GE\*01.-03* alleles among Middle Easterners could not be confirmed as statistically significant. However, in this study, the identified Gerbich-negative alleles *GE\*01.-02.01* and *GE\*01.-03.02* only occurred among people from the Middle East and accumulated among people from Syria. In conclusion, the fact remains that Gerbich-negative phenotypes are extremely rare, even in Syria and the wider Middle East.

## Acknowledgment

## Statement of Ethics

Studies on blood group antigen frequency estimates in individuals immigrating to Germany were approved by the Ethics Committee of the Medical Chamber of Rhineland-Palatinate in Mainz, Germany (837.020.16).

## Conflict of Interest Statement

C.G. acts as a consultant to inno-train Diagnostik GmbH, Kronberg i.T., Germany. Procedures for the molecular detection of GYPB deletions for S-s-U-phenotype diagnostics have been granted as a European patent (No. 3545102). Similar content patent US application is pending. All other authors declare no conflicts of interest.

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## Author Contributions

B.F., E.A.S., and C.G. designed and supervised the study; B.F. and E.A.S. contributed sample material; V.S. planned and performed experiments; B.F., V.S., D.P., and C.G. analysed data and discussed the results; D.P. and C.G. wrote the manuscript; and all authors edited the manuscript. The manuscript contains data of the master thesis of V.S.

## Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

## References

1 Reid ME, Lomas-Francis C, Olsson ML. The blood group antigen factsbook. 3rd ed. Oxford: Academic; 2012.

2 Chester MA, Olsson ML. The ABO blood group gene: a locus of considerable genetic diversity. Transfus Med Rev. 2001;15(3):177–200.

3 Wiener AS, Unger LJ, Gordon EB. Fatal hemolytic transfusion reaction caused by sensitization to a new blood factor U: report of a case. J Am Med Assoc. 1953;153(16):1444–6.

4 Daniels G. Human blood groups. 3rd ed. New York (NY): John Wiley & Sons; 2013.

5 Lin X, Rubio G, Patel J, Banerjee S, Frame T, Billups N, et al. Hybrid glycophorin and red blood cell antigen genotyping in Asian American type O blood donors with Mia phenotype. Transfusion. 2019;59(12):3767–75.

6 Broadberry RE, Lin M. The distribution of the MiIII (Gp.Mur) phenotype among the population of Taiwan. Transfus Med. 1996;6(2):145–8.

7 Westman JS, Stenfelt L, Vidovic K, Möller M, Hellberg Å, Kjellström S, et al. Allele-selective RUNX1 binding regulates P1 blood group status by transcriptional control of A4GALT. Blood. 2018;131(14):1611–6.

8 Yeh CC, Chang CJ, Twu YC, Hung ST, Tsai YJ, Liao JC, et al. The differential expression of the blood group P1 -A4GALT and P2 -A4GALT alleles is stimulated by the transcription factor early growth response 1. Transfusion. 2018;58(4):1054–64.

9 National Center for Biotechnology Information. rs-database [cited 2021 Oct 1]. Available from: URL: https://www.ncbi.nlm.nih.gov/snp/?term=rs5751348.

10 Issitt PD, Anstee DJ. Applied blood group serology, 1. reprint. 4th ed. Durham (NC): Montgomery Scientific Publ; 1998.

11 Bundesamt für Migration und Flüchtlinge. Migrationsbericht des Bundesamtes für Migration und Flüchtlinge im Auftrag der Bundesregierung: Migrationsbericht 2015; 2016 Dec 14 [cited 2021 Oct 1]. Available from.https: //www.bamf.de/SharedDocs/Anlagen/DE/Forschung/Migrationsberichte/migrationsbericht-2015.html?nn=403964.

12 Flesch BK, Scherer V, Just B, Opitz A, Ochmann O, Janson A, et al. Molecular blood group screening in donors from Arabian countries and Iran using high-throughput MALDI-TOF mass spectrometry and PCR-SSP. Transfus Med Hemother. 2020;47(5):396–408.

13 Kattamis A, Forni GL, Aydinok Y, Viprakasit V. Changing patterns in the epidemiology of β-thalassemia. Eur J Haematol. 2020;105(6):692–703.

14 Scott B, Easteal S. A single-step assay for the Gerbich-negative allele of glycophorin C. Blood Cells Mol Dis. 2008;41(1):1–4.

15 Gourri E, Denomme GA, Merki Y, Scharberg EA, Vrignaud C, Frey BM, et al. Genetic background of the rare Yus and Gerbich blood group phenotypes: homologous regions of the GYPC gene contribute to deletion alleles. Br J Haematol. 2017;177(4):630–40.

16 Red Cell Immunogenetics and Blood Group Terminology. Names for GE (ISBT 020) blood group alleles. 2020 Mar 1 [cited 2021 Oct 1]. Available from: https://www.isbtweb.org/fileadmin/user_upload/files-2015/red%20cells/blood%20group%20allele%20terminology/allele%20tables/_ISBT_020__GE_blood_group_alleles_v4.0_01-MAR-2020.pdf.

17 High S, Tanner MJ, Macdonald EB, Anstee DJ. Rearrangements of the red-cell membrane glycophorin C (sialoglycoprotein beta) gene. A further study of alterations in the glycophorin C gene. Biochem J. 1989;262(1):47–54.

18 Telen MJ, van Kim C, Chung A, Cartron JP, Colin Y. Molecular basis for elliptocytosis associated with glycophorin C and D deficiency in the Leach phenotype. Blood. 1991;78(6):1603–6.

19 Levitt RN, Gourri E, Gassner C, Banez-Sese G, Salam A, Denomme GA, et al. Molecular characterization and multidisciplinary management of Gerbich hemolytic disease of the newborn. Pediatr Blood Cancer. 2018;65(6):e27014.

20 Arndt PA, Garratty G, Daniels G, Green CA, Wilkes AM, Hunt P, et al. Late onset neonatal anaemia due to maternal anti-Ge: possible association with destruction of eythroid progenitors. Transfus Med. 2005;15(2):125–32.

21 Flesch BK, Reil A, Bux J. Genetic variation of the HNA-3a encoding gene. Transfusion. 2011;51(11):2391–7.

22 Beaumont P, Blake GH, Wagstaff JM. The Middle East: a geographical study. London: Fulton; 1988. Vol. 2.

23 Wikipedia. Middle East; 2021 [cited 2021 Oct 1]. Available from: https://en.wikipedia.org/wiki/Middle_East.

24 Sachs L. Angewandte Statistik. 7th ed. Berlin, Heidelberg: Springer Berlin Heidelberg; 1992.

25 Choi SJ, Lee E, Kim S, Lyu CJ, Kim HO. Identification of Anti-Gerbich antibody in an Emirati marrow hematopoietic progenitor cell donor with Fy(a-b-) phenotype. Yonsei Med J. 2018;59(10):1253–6.

26 Jakobsen MA, Dellgren C, Sheppard C, Yazer M, Sprogøe U. The use of next-generation sequencing for the determination of rare blood group genotypes. Transfus Med. 2019;29(3):162–8.