18 Gerbich Blood Group System

18.1 Introduction

The Gerbich system consists of 12 antigens, seven of very high frequency and five of low frequency (Table 18.1). They are located on either or both of the red cell membrane sialoglycoproteins glycophorin C (GPC, CD236C) and glycophorin D (GPD, CD236D), or on closely related glycoproteins. GPD is a truncate version of GPC. GPC and GPD are produced by the same gene, *GYPC*, as a result of initiation of mRNA translation at two sites. *GYPC* consists of four exons.

There are three rare 'Ge-negative' phenotypes in which the red cells lack one or more of the high frequency antigens, Ge2, Ge3, and Ge4. Ge:–2,3,4 (Yus phenotype) and Ge:–2,–3,4 (Gerbich phenotype) result from deletions of *GYPC* exon 2 and exon 3, respectively. Ge:– 2,–3,–4 (Leach or Ge-null phenotype) usually results from a deletion of exons 3 and 4, although a single nucleotide deletion was involved in one case. Ls^a arises from a duplication or triplication of *GYPC* exon 3. The remaining four low frequency antigens and absence of the other four high frequency antigens result from point mutations in *GYPC*.

GYPC is located on chromosome 2q14-q21.

18.2 Glycophorin C (GPC) and glycophorin D (GPD), and *GYPC*, the gene that encodes them

18.2.1 Red cell membrane sialoglycoproteins

Glycophorin is a name given to several sialic acid-rich glycoproteins of the red cell membrane that are detected after SDS PAGE by periodic acid-Schiff (PAS) staining. The major sialoglycoproteins are glycophorin A (GPA) and glycophorin B (GPB), homologous structures carrying the antigens of the MNS system (Chapter 3). The minor sialoglycoproteins, glycophorin C (GPC) and glycophorin D (GPD), are another pair of homologous structures, which represent about 6% and 1% of PAS staining material, respectively [1]. GPA and GPB are not genetically related to GPC and GPD, which carry the Gerbich system antigens.

Several synonyms have been used for GPC (CD236C, β-sialoglycoprotein, component D, PAS–2′) and GPD (CD236D, γ-sialoglycoprotein, component E) (Table 3.3).

18.2.2 GPC and GPD

The apparent MWs of GPC and GPD on SDS PAGE are 40 kDa and 30 kDa, respectively (reviews in [2–4]). By the

Human Blood Groups, Third Edition. Geoff Daniels.

Table 18.1 Antigens of the Gerbich system.

Antigen			Molecular basis*		
No.	Name	Frequency	Nucleotides	Exon	Associated glycoprotein
GE2	Ge2	High	(Deletion exon 2)	2	GPD N-ter region
GE3	Ge3	High	(Deletion exon 3)	3	GPC 42–50, GPD 21–29
GE4	Ge4	High	(Deletion exon 3 & 4) or (131G>T, 134delC)	2 & 3 or 3	GPC N-ter region
GE5	Wb	Low	23A>G	1	GPC Asn8Ser
GE6	Ls[a]	Low	Duplicated or triplicated exon 3		GPC/GPD, junction of product of 2 exons 3
GE7	An[a]	Low	67G>T	2	GPD Ala2Ser
GE8	Dh[a]	Low	40C>T	1	GPC Leu14Phe
GE9	GEIS	Low	95C>A	2	GPC Thr32Asn, GPD Thr11Asn
GE10	GEPL	High	134C (T)	3	GPC Pro45 (Leu), GPD Pro24 (Leu)
GE11	GEAT	High	56A (T)	2	GPC Asp19 (Val)
GE12	GETI	High	80C (T)	2	GPC Thr27 (Ile), GPD Thr6 (Ile)
	GERW	High	173A (T)	3	GPC Asp58 (Val), GPD Asp37 (Val)

*Molecular basis of antigen-negative phenotype in parentheses.
Obsolete: GE1.

use of Fab fragments of monoclonal antibodies, the number of molecules per red cell has been estimated as 143 000 for GPC and 225 000 for GPC plus GPD [5].

The first 47 amino acid residues of GPC were determined by manual sequencing [6]. Colin *et al.* [7] used a mixture of 32 synthetic oligonucleotides, each of which represented the sequence of amino acid residues 19–23 of GPC, as radioactive hybridisation probes in order to isolate *GYPC* cDNA from a human reticulocyte cDNA library.

GPC has three domains: a glycosylated N-terminal extracellular domain (residues 1–57) containing one *N*-linked oligosaccharide at Asn8 and sites for 12 *O*-linked oligosaccharides; a hydrophobic membrane-spanning domain (58–81); and a C-terminal cytoplasmic domain (82–128) [7,8]. The cytoplasmic domain of GPC interacts with the red cell membrane skeleton (Section 18.7). An N-terminal signal peptide is often associated with nascent transmembrane glycoproteins, including GPA and GPB, and cleaved from the mature protein. No such signal peptide is encoded by *GYPC* [8].

For reasons discussed below (Section 18.2.3), GPD is a truncate version of GPC, lacking the N-terminal 21 amino acid residues of GPC and identical to residues 22–128 of GPC. This is consistent with the following details:

1 GPD has no *N*-glycosylation;
2 GPD lacks epitopes present on the N-terminal domain of GPC [9];
3 Ge3 antigen, which represents a region around amino acid residues 40–50 of GPC, is also present on GPD [10] (Section 18.3.2.2);
4 antigenic determinants detected by monoclonal and polyclonal antibodies produced in animals are present on the cytoplasmic domains of both GPC and GPD [11–13] (see Figure 18.2).

GPC is part of the putative 'junctional' membrane-protein complex [14] (see Sections 10.7 and 18.7, and Figure 10.2).

18.2.3 GPC and GPD are encoded by the same gene

Despite the high level of homology between GPC and GPD, no homologous gene could be detected in genomic DNA using *GYPC* cDNA as a probe. This led to proposals that a separate GPD gene does not exist and that GPC and GPD are both produced by *GYPC* [15,16]; the result of initiation of translation of *GYPC* mRNA at two different sites, to produce two polypeptides.

The process of protein synthesis, in which the nucleotide sequence of mRNA is translated into an amino acid sequence, commences at an AUG codon (a methionine

codon, ATG in DNA), although a 10-nucleotide consensus sequence including the AUG codon is also important for effective initiation of translation. Newly processed polypeptides have methionine at their N-terminus, although this is usually cleaved from the mature protein. The RNA sequence around the start codon for GPC (CCAGGA **AUG** U) does not conform closely to the consensus start sequence (CC(A/G)CC **AUG** G) found in eukaryote transcripts; the sequence around a downstream AUG triplet (CCG GGG **AUG** G), the codon for Met22 of GPC, is a closer fit to the consensus sequence [16]. A process referred to as 'leaky initiation of translation' occurs in which the initiation site at the codon for Met1 of GPC is sometimes missed during the scanning of *GYPC* mRNA. Scanning continues along the mRNA until the second initiation site at Met22 is reached, where translation begins to produce GPD, a shorter molecule comprising amino acid residues 22–128 of GPC (Figure 18.1). *GYPC* cDNA transfected into COS-7 cells produced GPC and GPD, cDNA with a deletion of ATG at position 1 produced only GPD, cDNA with an ATG to ACG mutation at position 22 produced only GPC, and cDNA with ATG to ACG mutations at positions 1 and 22 produced neither glycoprotein [17]. A mutation at nucleotide 4 (ATG **T** to ATG **G**), creating a more consensus motif around the first ATG codon, resulted in a doubling of expression of GPC compared with GPD [17].

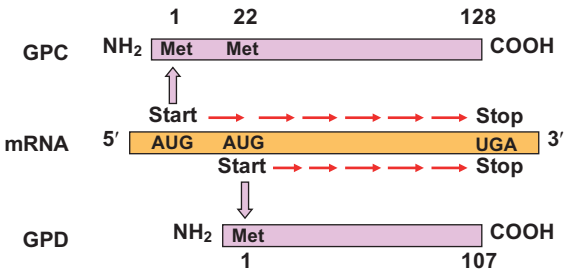

Figure 18.1 Production of GPC and GPD from a single gene by a process of leaky initiation of mRNA translation. When translation commences at the first AUG start codon a 128 amino acid GPC polypeptide is produced (above). If the first AUG codon is missed, then translation may commence at a second AUG site and a shorter 107-residue GPD polypeptide is produced (below).

18.2.4 Organization of *GYPC*

The 13.5 kb *GYPC* gene is organised into four exons (Table 18.2) [10,18]. Exons 1 to 3 encode the extracellular domain of GPC, exon 4 the membrane-spanning and cytoplasmic domains. Exons 2 and 3 show a high level of homology, probably arising from exon duplication, although exon 3 contains an insert of 27 nucleotides not present in exon 2, encoding amino acid residues 42–50 of GPC. The intronic flanking regions of exons 2 and 3 demonstrate an even higher level of homology than do the coding regions.

The upstream promoter region of *GYPC* contains the ubiquitous *cis*-acting elements CACC, TATA, and Sp1, plus three erythroid-specific GATA-1 binding sites and a binding site for an erythroid/megakaryocyte-specific factor, NF-E6 [19,20].

GYPC was localised to 2q14-q21 by *in situ* hybridisation [21].

18.3 The high frequency antigens Ge2, Ge3, and Ge4, and the Gerbich-negative phenotypes

18.3.1 Serological history

Gerbich began as a simple, inherited blood group antigen of very high frequency when, in 1960, Rosenfield *et al.*

Table 18.2 Organisation of *GYPC*.

	Amino acid residues		
Exon	GPC	GPD	Characteristics
1	1–16		N-terminus and part of extracellular domain of GPC; *N*-glycan; Ge4.
2	17–35	1–14	GPC Met22 (translation initiation site for GPD); part of extracellular domain of GPC and GPD including N-terminus of GPD; Ge2 on GPD.
3	36–63	15–42	Part of extracellular domain of GPC and GPD; Ge3 on GPC and GPD; trypsin cleavage site on GPC and GPD.
4	64–128	43–107	Membrane-spanning and cytoplasmic domains of GPC and GPD.

[22] described antibodies of apparently identical specificity in three women (including Mrs Gerbich). The simplicity of the Gerbich system was short-lived: in 1961 Cleghorn [23,24] found that the red cells of Mrs Yus., a Turkish Cypriot woman, failed to react with two of the original anti-Gerbich sera, but did react with the other, the antibody of Mrs Gerbich herself. The serum of Mrs Yus. contained an antibody that reacted with all cells tested apart from her own and those of the original three Gerbich-negatives. Adsorption of Mrs Gerbich's serum with Mrs Yus.'s cells removed all antibody. Although the Yus type is not strictly Gerbich-negative, as the cells react with the antibody of Mrs Gerbich, it is generally considered Gerbich-negative because the cells fail to react with the majority of antibodies made by Gerbich-negative people [25]. Both of these Gerbich-negative phenotypes are exceedingly rare in most populations.

Further complexities of the Gerbich system arose from studies of the Melanesians of Papua New Guinea. Not only was Gerbich found to be polymorphic among some populations of the north-east coast of New Guinea [26,27], but another Gerbich-related antibody was found in a Ge+ Melanesian [27]. This antibody failed to react with Gerbich-negative cells of Gerbich and Yus types, but also did not react with red cells of up to 15% of Ge+ Melanesians. Booth and McLoughlin [27] proposed a numerical notation for Gerbich phenotypes, shown in modified form in Table 18.3. Red cells of the Melanesian phenotype (Ge:–1,2,3) and the two examples of anti-Ge1 [27,28] have not been widely used and are not available for further investigation. Consequently, Ge1 has been declared obsolete and the Melanesian phenotype is omitted from Table 18.3.

Some monoclonal antibodies were shown to be related to the Gerbich system because they agglutinated all red cells except those of the Ge:–2,–3 (Gerbich) and Ge:–2,3 (Yus) phenotypes, although they reacted with these cells by an antiglobulin test [29,30]. Anstee *et al.* [30,31] found that red cells of two unrelated Ge:–2,–3 women failed to react with the Gerbich-related monoclonal antibodies by any technique. This new Gerbich-negative phenotype was called the Leach phenotype. An alloantibody in the serum of a Leach phenotype patient [32] behaved in a very similar manner to the monoclonal antibodies and became anti-Ge4. Leach phenotype red cells are Ge:–2,–3,–4, the Gerbich-null phenotype; all other red cells have Ge4 (Table 18.3).

Table 18.3 Gerbich-negative phenotypes.

Ge:–2,3,4	Yus phenotype
Ge:–2,–3,4	Gerbich phenotype
Ge:–2,–3,–4	Leach phenotype

18.3.2 High frequency antigens Ge2, Ge3, and Ge4

18.3.2.1 Ge2

Anti-Ge2 is the antibody characteristic of the Ge:–2,3,4 phenotype, but is also the most frequently encountered antibody in the Ge:–2,–3,4 and Ge:–2,–3,–4 phenotypes. Of 17 antibodies from Ge:–2,–3 people, only four were anti-Ge3, the other 13 were anti-Ge2 [25]; of the six Leach phenotype individuals with antibody, four had anti-Ge2, one anti-Ge3, and one anti-Ge4 [30,32–35].

Immunoblotting with numerous examples of alloanti-Ge2 demonstrated that the Ge2 antigen is located on GPD, but not on GPC [12,36]. It is on the N-terminal tryptic peptide of GPD (residues 1–27) [36]. Treatment of intact red cells with trypsin or papain destroys Ge2; chymotrypsin and pronase do not. About 50% of anti-Ge2 show a reduction in strength of reaction with sialidase-treated cells [25].

GPD is a shorter version of GPC and does not have any amino acid sequence that is not present in GPC. Ge2 is usually at the N-terminus of GPD. Anti-Ge2 might recognise an amino acid sequence only when it is in the conformation of the N-terminus of GPD and not when it is an internal sequence within GPC. Alternatively, the Ge2 determinant could involve the free amino group of GPD and the adjacent amino acid sequence. Some anti-Ge2 do not react with red cells after acetylation of membrane protein with acetic anhydride, suggesting that a free amino group is involved in the epitope detected by those antibodies [37]. Anti-Ge2 probably represents a heterogeneous collection of antibodies that react with epitopes at the N-terminal region of GPD.

18.3.2.2 Ge3

Anti-Ge3 has been found in immunized Ge:–2,–3,4 and Ge:–2,–3,–4 individuals, but is far less common than anti-Ge2 [25,33]. Like Ge2, Ge3 is destroyed by trypsin and not by chymotrypsin or pronase. Unlike Ge2, Ge3 is resistant to treatment of intact red cells with papain [25]. Consequently, papain-treated cells can be used for distinguishing anti-Ge2 and -Ge3 in the absence of the very rare Ge:–2,3,4 cells (although one anti-Ge3 in a Ge:–2,–3,4 patient detected a papain-sensitive Ge3 antigen [38]).

Table 18.4 Frequency studies performed by testing red cells of random individuals with Gerbich antibodies.

Antibody	Population tested	No. tested	No. negative	References
Anti-Ge2	English, Danes, New Zealanders, Californians	28 331	0	[22,23,42,43]
Anti-Ge3	New Yorkers*	11 000	0	[22]
Anti-Ge3	French	5912	1	[43,44]
	Total	45 943	1	
Anti-Ge2	PNG† Sepik Region	748	182	[27]
Anti-Ge2	PNG† Morobe Region	1014	517	[27]
Anti-Ge2	PNG† Highlands	1348	1	[27]
Anti-Ge2‡	PNG† Port Moresby donors	116	13	[45]
Anti-GPC‡	Ethiopean Jews	980	1	[46]
Anti-Ge2	Japanese	22 000	0	[47]

*Including at least 1500 black people and 100 Asiatics, mostly Chinese.
†Melanesians of Papua New Guinea.
‡Monoclonal antibody.

Immunoblotting with human alloantibodies and rodent monoclonal antibodies showed that Ge3 is present on both GPC and GPD [5,12,36,39,40]. Alloanti-Ge3 eluted from either GPC or GPD on an immunoblot detected both GPC and GPD on a separate immunoblot, confirming that anti-Ge3 is a single antibody to an antigen common to both proteins [12].

Ge3 is encoded by exon 3 of *GYPC*. Ge3 is missing in those rare phenotypes resulting from deletion of exon 3, but not from those resulting from a deletion of exon 2 (Section 18.3.3). Exon 2 and exon 3 are very similar apart from a 27 nucleotide insert in exon 3, representing amino acids 42–50 of GPC (21–29 of GPD) (Section 18.2.4), so Ge3 must be in this region, as confirmed by haemagglutination inhibition with fragments of GPC [36].

18.3.2.3 Ge4

The only example of alloanti-Ge4 was identified in the serum of a woman with the Ge:–2,–3,–4, Leach phenotype [32]. Anti-Ge4 was also found in the serum of a patient with a transient GPC deficiency [41]. Numerous monoclonal antibodies to GPC have been produced that behave serologically as anti-Ge4 (Section 18.5.3).

Ge4 is usually situated near the N-terminus of GPC and, therefore, is not on GPD [9,30,31,41]. Detailed analyses of monoclonal antibodies specific for GPC have shown that some require the amino group of Met1 of GPC for binding, whereas others detect other epitopes within the first 21 amino acids of GPC, but not involving Met1 [9,40]. Most required normal *O*-glycosylation of the GPC for effective binding. Ge4 is destroyed by trypsin and papain treatment of the red cells.

18.3.3 Gerbich-negative phenotypes

Outside Papua New Guinea (PNG), Gerbich-negative phenotypes are very rare. Screening of over 44 000 blood samples from white populations with anti-Ge2 or -Ge3 produced only one Gerbich-negative (Table 18.4). Molecular genotyping revealed frequencies for *GYPC* with a deletion of exon 3 (*GYPC.Ge*) of 0.46 and 0.18 in the Wosera and Liksul areas of PNG, respectively, with 22% and 3% homozygous for *GYPC.Ge*, predicting the Ge:–2,–3,4 phenotype [48].

18.3.3.1 Ge:–2,3,4: the Yus phenotype

Ge:–2,3 has been found in people of European origin, Middle Eastern Jews and Arabs, and in people of African origin, including Ethiopian Jews (Table 18.4); it has not been found in PNG.

Ge:–2,3,4 red cells contain no GPC or GPD, but they do have a GPC-like structure (GPC.Yus), represented on SDS PAGE by a broad, diffusely staining band of apparent MW 32.5–36.5 kDa, situated between the positions for GPC and GPD [12,30,49]. This structure carries Ge4 and Ge3 [12,30] (Figure 18.2). GPC, GPD, and GPC.Yus are

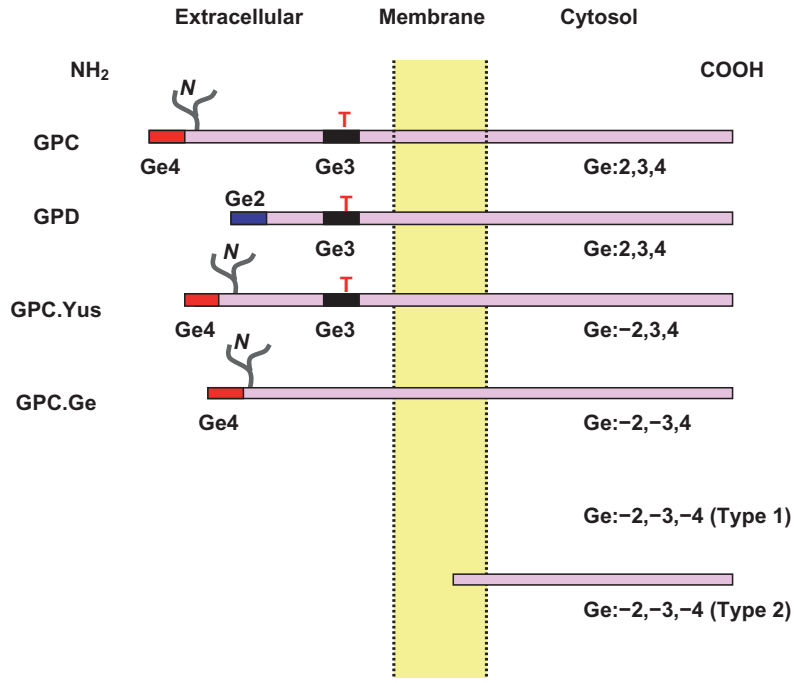

Figure 18.2 Diagram representing GPC, GPD, and related structures characteristic of Gerbich-negative phenotypes. Ge:2,3,4 cells have both GPC and GPD; Ge:–2,3,4 and Ge:–2,–3,4 cells have GPC.Yus and GPC.Ge, respectively, but no GPC or GPD. In most Ge:–2,–3,–4 (Leach phenotype) cells, no GPC, GPD, or related molecule is present (Type 1), but in one example a GPC/D C-terminal fragment was detected (Type 2). T, trypsin cleavage site at Arg48.

present in red cell membranes from individuals heterozygous for *GYPC* and *GYPC.Yus* [50].

Ge:–2,3,4 individuals are homozygous for a *GYPC* gene (*GYPC.Yus*, *GYPCΔex2*, or *GE*01.–02*) in which the second exon is deleted [10,51,52] (Figure 18.3). The protein product is a GPC-like molecule (GPC.Yus) lacking amino acid residues 17–35, with no loss of Ge4 or Ge3 (Figure 18.2). The second translation initiation site (Met22) is lost, so no GPD is formed and, therefore, no Ge2 is expressed.

Ge:–2,3,4 may also result from heterozygosity for *GYPC.Yus* and *GYPC.Ge* [55,56]. Five of 10 Ge:–2,3 propositi were found to have both GPC.Yus and GPC.Ge [55]. Heterozygosity for *GYPC* with 80C >T (*GE*01.–12*) and for *GYPC* with g >a in the 3′ nucleotide of intron 1 (IVS2−1g >a) was found in a brother and sister with the GETI– phenotype (Section 18.4.6.3) [57]. IVS2−1g>a would cause splicing out of exon 2, encoding GPC.Yus.

18.3.3.2 Ge:–2,–3,4: the Gerbich phenotype

Ge:–2,3,4 is the typical Gerbich-negative phenotype, the probable phenotype of Mrs Gerbich, although her cells were never tested with anti-Ge4. Ge:–2,–3 is polymorphic in certain regions of PNG [27,45,48] (Table 18.4). Ge:–2,–3 is rare in all other populations tested, but has been found among people of European and African origin, Iraqi Jews, Native Americans, Japanese, and Polynesians.

Like the Ge:–2,3,4 phenotype, Ge:–2,–3,4 cells have no GPC or GPD, but have a diffusely staining abnormal GPC-like structure (GPC.Ge) of mobility between that of GPC and GPD [12,30,49]. The apparent MW of GPC.Ge is 30.5–34.5 kDa, slightly less than that of GPC.Yus. GPC.Ge carries Ge4, but no Ge3 [12,30] (Figure 18.2). GPC.Ge is trypsin-resistant, unlike GPC, GPD, and GPC.Yus [30]. The composition of the *N*-glycan of GPC.Ge differs from that of GPC, with a higher mannose content [58].

Ge:–2,–3,4 results from a *GYPC* gene (*GYPC.Ge*, *GYPCΔex3*, or *GE*01.–03*) with a deletion of exon 3 [10,18,51,56,59] (Figure 18.3), encoding a GPC-like structure (GPC.Ge) lacking amino acid residues 17–35 of GPC. GPC.Ge is slightly smaller than GPC.Yus because exon 3 is larger than exon 2 owing to a 27 nucleotide insert. Loss of exon 3 also explains absence of Ge3 and of

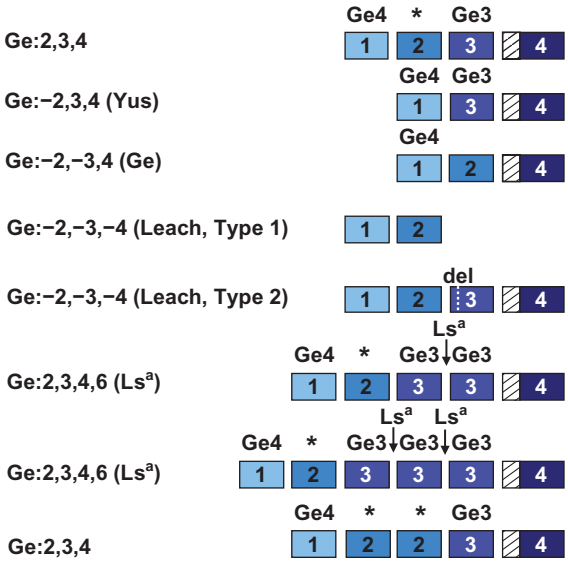

Figure 18.3 The genomic organisation of *GYPC* and variants of *GYPC* responsible for rare Gerbich phenotypes. The boxes represent exons and the shaded area in exon 4 represents the region encoding the membrane-spanning domain. *Second translation start codon (Met22). *GYPC* with duplication of exon 2 does not result in any qualitative change to the Gerbich phenotype [53,54]; triplication and quadruplication of exon 2 is not shown.

the trypsin cleavage site at Arg48 (Figure 18.2). It does not, however, explain the absence of GPD and, therefore, of Ge2. Colin *et al.* [18] suggested that a GPD molecule lacking most of its extracellular domain might not be transported to the membrane or might be unstable and rapidly degraded. GPC.Ge and GPC.Yus carry *N*-glycans with variable numbers of repeating lactosamine units, explaining their diffuse appearance on electrophoresis gels [12]. They may also have altered *O*-glycosylation [60].

It is probable that *GYPC* genes with deletions of exon 2 or exon 3 arose from intragenic unequal crossing-over, an event that would have simultaneously produced genes with duplications of exons 2 or exon 3 [10,18] (see Figure 18.4). This topic is returned to in the section on Ls^a (Section 18.4.2).

18.3.3.3 Ge:–2,–3,–4: the Leach phenotype

The Leach phenotype is the null phenotype of the Gerbich system. Ge:–2,–3,–4 red cells do not react with any Gerbich antibodies or related monoclonal antibodies. Six propositi with the Ge:–2,–3,–4 phenotype have been reported, all white English or North Americans [30, 32–35]. Ge:–2,–3,–4 red cells are totally devoid of GPC and GPD [10,12,30,33,34] (Figure 18.2).

Ge:–2,–3,–4 has at least two genetic backgrounds. Five unrelated Ge:–2,–3,–4 individuals were homozygous for a deletion of exons 3 and 4 of *GYPC* (*GE*01N.01*) [10,52,61,62] (Figure 18.3). Surprisingly, mRNA derived from this deleted gene was detected in reticulocytes from Ge:–2,–3,–4 individuals, despite the gene lacking the normal polyadenylation signal [62]. If any protein were produced by this gene, it would lack the membrane-spanning and cytoplasmic domains and could not be inserted into the membrane. One other Ge:–2,–3,–4 individual [32] was homozygous for *GYPC* with a single nucleotide deletion within codon 45 in exon 3 (134delC), resulting in a frameshift and premature generation of a stop codon after codon 55 (*GE*01N.02*) [61] (Figure 18.3). Most of exon 3 and all of exon 4 would not be translated and so viable GPC could not be produced. A 12 kDa component was detected, which bound a monoclonal antibody to an epitope on the cytoplasmic domains of GPC and GPD and appeared to represent the C-terminal domain of GPC/D [63] (Figure 18.2). This led to speculation that translation is reinitiated at an alternative start sequence overlapping the premature stop codon.

GPC and GPD are associated with the membrane skeleton, acting as a link between the membrane and the skeletal proteins (Section 18.7). One characteristic of the Ge:–2,–3,–4 phenotype is elliptocytosis [12,30,31,33,34]. Between 20 and 61% of the red cells of five Ge:–2,–3,–4 phenotype individuals were classed as elliptocytes [33]. Two Ge:–2,–3,–4 brothers had a longstanding history of mild anaemia [35]. Ge:–2,–3,–4 phenotype membranes have reduced mechanical stability [64,65] and may be released into the circulation as normal discocytes, but become distorted when exposed to shear stress and elongation [66]. Elliptocytes were present in a patient with a temporary reduction in red cell membrane GPC content and normal GPD content, but were not present when her GPC levels returned to normal [41]. Ge:–2,–3,4 and Ge:–2,3,4 cells show no sign of elliptocytosis [30] and have normal membrane stability [65], despite GPC and GPD deficiency, presumably because of the presence of the GPC-like molecules, GPC.Ge and GPC.Yus.

18.3.4 Associations with other blood group systems

Several reports link the Ge:–2,–3 phenotype with a depression of Kell-system antigens, although the effect is variable [25,30,32,33,44,47]. The phenomenon may

Table 18.5 Frequencies of low frequency Gerbich antigens.

Antigen	Population	No. tested	No. positive	Antigen frequency	References
Wb GE5	White Australians	3550	2	0.0006	[70]
	English	15 815	3	0.0002	[43]
	Welsh	10 117	8	0.0008	[71]
Ls^a GE6	Finnish	1113	18	0.0162	[43,72]
	Norwegians	7151	8	0.0011	[73]
	African Americans	110	1	0.0091	[43]
	Black West Indians	878	9	0.0103	[43]
	West Africans	81	2	0.0204	[43]
	Japanese	200 000	8	<0.0001	[74]
	English	5887	0		[43]
An^a GE7	Finnish	10 000	6	0.0006	[75]
	Swedish	3266	2	0.0006	[75]
Dh^a GE8	Danish	2493	0		[76]
GEIS GE9	Japanese	32 852	3	0.0001	[77]

affect all high frequency Kell antigens and K, if present [44]. Alternatively, K11 antigen may be the only antigen to show weakness [25]. Nine of 11 Ge:–2,–3 samples showed different degrees of weakening of Kell system antigens, whereas none of six Ge:–2,3 samples showed any such Kell depression [25]. At least four Ge:–2,–3, –4 propositi have depression of Kell system antigens [30,32,33]. One murine monoclonal antibody (MIMA-9) that did not react with K_o or Kp(a+b–) red cells, reacted only weakly with K+k–, McLeod phenotype, and Ge:–2, –3 cells [67].

Two Gerbich-related antibodies have suggested some association between Gerbich and Rh [68,69]. In addition to being non-reactive with most Ge-negative red cells, they also failed to react with Rh_{null}, D––, and cells with some other variant Rh phenotypes, and one of them also failed to react with D–, k–, and Kp(b–) cells. GPC, the Kell glycoprotein, and the Rh proteins may belong to the same 'junctional' protein complex in the membrane [14]. A possible association between Gerbich and the Vel antigen is described in Section 30.2.2, so Vel may also be part of that complex.

18.4 Other Gerbich antigens

18.4.1 Wb (GE5)

The original anti-Wb (Webb) was found in ABO grouping serum [70]; no further example was found in 2000 Australian sera, but three examples were detected in 7544 British sera [43,71] (Table 18.5). Wb is sensitive to trypsin and sialidase treatment, but resistant to chymotrypsin treatment [64,78,79].

Wb results from Asn8Ser in GPC (*GE*01.05*) [51,80] (Table 18.1). Asn8 in GPC is *N*-glycosylated and loss of this *N*-glycosylation results in an abnormal GPC (GPC.Wb), with an apparent MW 3 kDa lower than that of GPC [78,79]. Treatment of Wb– red cells with endoglycosidase F, which cleaves *N*-linked oligosaccharide chains, results in a reduction in the apparent MW of GPC to that of GPC.Wb; treatment of Wb+ cells with endoglycosidase F has no effect on GPC.Wb. Whether Ser8 of GPC.Wb is *O*-glycosylated has not been determined.

18.4.2 Ls^a (GE6)

Ls^a (Lewis II) [43] is the same as Rl^a [73,81] and is polymorphic in Finns and people of African origin, but rare in other populations (Table 18.5). The original anti-Ls^a was found in an anti-B reagent; other examples were found in sera containing multiple antibodies to low frequency antigens [73] and during pre-transfusion cross-matching [82]. Anti-Ls^a was associated with HDFN necessitating exchange transfusion, but not proven to be the cause [83]. One anti-Ls^a was found by screening over 4000 normal sera from Europeans [43,72] and 19 examples were found in 44 000 Japanese donor sera [74]. Ls^a is trypsin-sensitive, but resistant to ficin and sialidase [84].

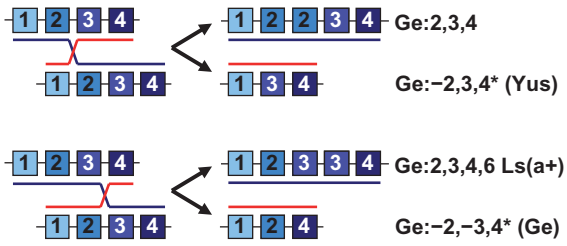

Figure 18.4 Diagram showing two intragenic unequal crossing-over events resulting in four abnormal *GYPC* genes, two with exon deletions and two with exon duplications. A duplication of exon 3 is associated with Ls^a antigen. *The Gerbich-negative phenotypes only occur when there is homozygosity for the aberrant gene (or heterozygosity for two aberrant genes).

Ls(a+) red cells have, in addition to normal GPC and GPD, abnormal GPC and GPD molecules, each with an apparent MW 5.5 kDa greater than its normal counterpart [84]. Both abnormal structures immunostain very strongly with anti-Ge3. GPC.Ls^a also binds monoclonal anti-Ge4 and GPD.Ls^a binds anti-Ge2. *GYPC* from Ls(a+) individuals has a duplication of exon 3 (*GE*01.06.01*) (Figure 18.3) [10,85]. The presence of an extra exon 3 explains why GPC.Ls^a and GPD.Ls^a are of increased size and have strongly expressed Ge3 antigens.

Intragenic crossing-over has been suggested as a mechanism to explain the origin of the *GYPC* exon 2 and exon 3 deletions responsible for the Ge:–2,3,4 and Ge:–2,–3,4 phenotypes, respectively [10,18]. Each unequal crossing-over event would also generate *GYPC* with a duplication of either exon 2 or exon 3 (Figure 18.4). The junction of two contiguous exons 3 would produce a unique amino acid sequence, probably representing the Ls^a antigen. A synthetic peptide (TPTIMDIVVIA-EPDPG) representing the last 11 amino acids encoded by the 3′ end of exon 3 followed by the first five amino acids encoded by the 5′ end of exon 3 inhibited anti-Ls^a [86].

A monoclonal antibody (CBC-96) to an epitope on GPC agglutinated Ls(a+) cells at a significantly higher dilution than that required to agglutinate cells of common Gerbich phenotype, presumably because the epitope is more exposed on the extended molecule [54]. Uchikawa and his colleagues used an appropriate dilution of this antibody to screen red cells of 200 000 Japanese donors. Of the 60 samples strongly agglutinated, eight were Ls(a+) [74]. One of these eight samples had enhanced Ls^a expression and had GPC and GPD molecules with an apparent MW 6 kDa greater than GPC.Ls^a and GPD.Ls^a, resulting from triplication of *GYPC* exon 3 (*GE*01.06.02*) [54] (Figure 18.3). Forty samples (0.02%) had *GYPC* with a duplication of exon 2, the putative result of the unequal crossing-over event responsible for the exon 2 deletion of GPC.Yus (Figure 18.4), and six (0.003%) had a quadruplication of exon 2 [53,54]. As predicted [10], no low frequency antigen associated with GPC and GPD molecules containing multiple products of exon 2 of *GYPC* was found, as this does not create a unique amino acid sequence.

18.4.3 An^a (GE7)

An^a (Ahonen) was found in about 0.06% of Finns and Swedes (Table 18.5) [75]. Anti-An^a has been found in about 1 in 1000 normal sera and does not appear to require red cell stimulation [75]. An^a is destroyed by trypsin, papain, and sialidase [37].

An^a is located on GPD, but not on GPC. It results from an alanine to serine substitution at position 23 of GPC and at position 2 of GPD (*GE*01.07*) [37]. This raises the question of why An^a is only expressed on GPD when the amino acid substitution occurs in both GPC and GPD. An^a does not appear to involve the free amino group at the N-terminus of GPD as acetylation of red cell surface proteins does not affect An^a expression [37]. Consequently, Daniels *et al.* [37] suggested that anti-An^a recognises a conformational difference in the N-terminal region of GPD resulting from the amino acid substitution, which is not apparent when the same substitution occurs within the GPC chain. GPD.An^a in human embryonic kidney cells expressed both An^a and Ge2, as determined by two alloanti-An^a and two alloanti-Ge2 [87].

18.4.4 Dh^a (GE8)

The first anti-Dh^a (Duch) was an IgM 'naturally occurring' antibody found when the serum reacted with the red cells of a Danish blood donor during pre-transfusion compatibility testing [76] (Table 18.5). Identification of anti-Dh^a in sera of two blood donors led to the discovery of an English family with eight Dh(a+) members in three generations [88]. Treatment of intact red cells with trypsin, papain, ficin, pronase, or sialidase destroys Dh^a; chymotrypsin does not [76,88].

Dh^a is located on GPC, but not on GPD, and results from Leu14Phe in GPC (*GE*01.08*) [89,90]. Dh^a is not affected by endoglycosidase F treatment of the cells and so is not dependent on the presence of the *N*-glycan at Asn8 [89]. Dh^a on red cells is sialidase-sensitive, suggesting the necessity for normal *O*-glycosylation of GPC, but anti-Dh^a was specifically inhibited by a synthetic peptide

representing amino acid residues 8–21 of GPC.Dh[a] (NSTAWPFSLEPNPG) [86].

18.4.5 GEIS (GE9)

A very rare antigen initially named Is results from Thr32Asn on GPC and Thr11Asn on GPD (*GE*01.09*) [77] (Table 18.5). GEIS was affected by papain, trypsin, or sialidase treatment of red cells, but not by chymotrypsin. Three examples of anti-GEIS were found in 5447 Japanese.

18.4.6 Four Gerbich antigens of high frequency associated with *GYPC* missense mutations

Antibodies resembling anti-Ge2 or -Ge3 have led to the identification of four antigens of high frequency, absence of which result from missense mutations in *GYPC* [57,91] (Table 18.1).

18.4.6.1 GEPL (GE10)

GEPL– phenotype was associated with a Ge:2,3,4 phenotype with aberrant expression of Ge3 and homozygosity for 134C>T encoding Pro45Leu in GPC and Pro24Leu in GPD (*GE*01.–10*). Immunoblotting revealed apparently normal GPC and GPD, plus additional bands indicating abnormal GPC and GPD molecules 2 kDa lower than GPC and GPD. Anti-GEPL resembled anti-Ge3 [57].

18.4.6.2 GEAT (GE11)

GEAT– phenotype was associated with a Ge:2,3,4 phenotype, with the red cells giving weak reactions with some Gerbich antisera, and with homozygosity for 56A>T encoding Asp19Val in GPC (*GE*01.–11*). Immunoblotting revealed apparently normal GPC and GPD. Anti-GEAT resembled anti-Ge3, but gave variable or weak reactions with Ge:–2,3,4 red cells [57].

18.4.6.3 GETI (GE12)

GETI– phenotype was associated with a Ge:–2,3,4 phenotype, apart from the cells reacting with one autoanti-Ge2, and with homozygosity for 80C>T encoding Thr27Ile in GPC and Thr6Ile in GPD (*GE*01.–12*). Immunoblotting revealed apparently normal GPC and GPD. The proximity of the Thr6Ile substitution in GPD is probably responsible for the loss of Ge2. Anti-GETI resembled anti-Ge2. In addition, a brother and sister with the GETI– phenotype were heterozygous for *GYPC* with the 80C>T mutation and for an intron 1 splice site mutation (IVS2–1g>a), which would be expected to cause splicing out of exon 2, consistent with Ge:–2,3,4 (Section 18.3.3.1) [57].

18.4.6.4 GERW

GERW– phenotype was associated with a Ge:2,3,4 phenotype, except the cells did not react with one alloanti-Ge2, one autoanti-Ge3, and one autoanti-Ge4. Immunoblotting revealed GPC, GPD, and GPC.Ge. The GERW– propositus was heterozygous for (1) *GYPC* encoding Asp58Val in GPC and Asp37Val in GPD, in the region of the insertion of these molecules into the membrane and (2) *GYPC.Ge* (*GYPC* with a deletion of exon 3). The antibody of the propositus did not react with her own cells or with those of four of her eight siblings. It immunostained GPC on blots of normal red cell membranes and appears to react with GPC with Asp58, but not with Val58. It is unclear why this antibody did not react with GPD or with GPC.Yus, both of which have aspartic acid at the position equivalent to amino acid 58 of GPC [91].

18.5 Gerbich antibodies

18.5.1 Alloantibodies

Gerbich antibodies are usually stimulated by pregnancy or transfusion, but some are apparently 'naturally occurring' [27,42,43,47,56]. Eighty-nine (13%) of 664 sera from Gerbich-negative Melanesians had Gerbich antibodies and the frequency of anti-Gerbich was about the same in men as in women [27]. Some Gerbich antibodies may be IgM, but the majority are IgG, mostly IgG1 [92]. The clinical significance of Gerbich antibodies is reviewed in [93].

Gerbich antibodies have not caused a serious HTR and least incompatible red cells can usually be selected for transfusion. A Ge:–2,–3 patient with anti-Ge3 had clinical and laboratory signs of a mild, acute HTR following transfusion of two units of Ge+ red cells [93]. The patient had been transfused uneventfully with Ge+ red cells several years earlier, when the result of a monocyte monolayer assay increased from 2.2 to 79.5% reactivity, predicting the potential to cause an HTR.

Several examples of Gerbich antibodies have caused mild neonatal jaundice, treated by phototherapy (see [94]). Anti-Ge3 has been responsible for late-onset severe anaemia in three babies, two of whom were from the same mother [94,95]. The anaemia was not apparent and did not become severe until 2–4 weeks after delivery. Therefore, pregnancies involving Gerbich antibodies

should be monitored for several weeks. The characteristics of HDFN caused by anti-Ge3 resemble those resulting from Kell antibodies, with only small elevations in reticulocyte and bilirubin levels and unresponsiveness to erythropoietin (EPO), suggesting suppression of erythropoiesis [94,96] (see Section 7.3.5.2). Like the Kell glycoprotein, glycophorin C appears on erythroid progenitors at an early stage of erythropoiesis [97,98], so fetal and neonatal erythroid cells may be phagocytosed by monocytes before they produce haemoglobin. Studies on K562 cells, however, suggest that binding of antibody to GPC causes cytoskeletal rearrangement through protein 4.1R and interference with the EPO signalling cascade, resulting in unresponsiveness to EPO [99]. In addition, ligation of GPC on red cells with murine monoclonal anti-GPC led to exposure of phosphatidylserine (PS) on their surface, a characteristic associated with eryptosis (red cell death) [100].

18.5.2 Autoantibodies

There are four reports of Gerbich autoantibodies causing severe AIHA: three resembled anti-Ge2 (one was IgA, another IgM) [101–103], the other resembled anti-Ge3 [104]. Gerbich-positive red cells of a patient with anti-Ge2 in his serum gave a negative DAT, but anti-Ge2 could be eluted [105]. Immunoblotting with this antibody and with another Ge2-like autoantibody showed that they differed from most alloanti-Ge2 because they bound GPC, but not GPD [105,106]. Both patients had reduced expression of target antigen on their cells. Autoanti-Ge3 behaved like alloanti-Ge3 on immunoblots by binding GPC and GPD [106].

An antibody resembling anti-Ge4 in a Ge:2,3,4 patient with aplastic anaemia and with elliptocytes in her peripheral blood did not cause autoimmune haemolysis [41]. The antibody bound GPC, GPC.Ge, and GPC.Yus, but not GPD. The patient's red cells had a substantially reduced content of GPC, but normal GPD. Two years later, antibody was no longer present in the patient's serum, the GPC content of her red cell membranes had returned to normal, no elliptocytes were present, and the antibody from an earlier sample reacted with her red cells.

18.5.3 Monoclonal antibodies

Many monoclonal antibodies associated with Gerbich define epitopes close to the N-terminus of GPC and behave serologically as anti-Ge4 [5,9,29,30,31,40,53,97, 107–110]. Production of recombinant GPC and its variants in heterologous expression systems has been valuable for analysing epitopes [87,111]. The epitopes of most include Met1 of GPC, but some involve amino acids 16–23 [53,109,110]. One monoclonal anti-Ge4 also reacted with MNS-variant Dantu+ red cells (Section 3.14.1). Several rodent monoclonal anti-Ge3 have been produced [5,39,40]. Rodent monoclonal antibodies behaving serologically as anti-Ge2 bound GPC [60,109] or GPC and GPD [109,112]. An IgM monoclonal anti-Ge2 defined an epitope involving amino acids 15–22 (SLEPDPGM) on GPC, yet did not react with GPC.Ge, possibly because of altered *O*-glycosylation [60]. This antibody cross-reacted with an epitope (amino acids 22–27, EDPDIP) on the cytoplasmic N-terminal domain of band 3. A human monoclonal anti-Ls^a has been produced [110].

18.6 Development and distribution of Gerbich antigens

Ge2 and Ge3 are well developed at birth [22]. Gerbich antigen (type unspecified) was detected in 19 fetuses aged 17.5–28 weeks [43]. During erythropoiesis, GPC and GPD are present on erythroid cells at an early stage of differentiation, though glycosylation may not be complete [97,113]. GPC, detected by a monoclonal antibody (BRIC4) to a glycosylation-dependent epitope, was strongly expressed on 84% of $CD34^+$ cells derived from cord blood [98].

GPC and GPD are not erythroid-specific, though the level of expression and the degree of glycosylation of the proteins may differ in erythroid and non-erythroid cells. Initiation of transcription of *GYPC* probably occurs at different sites in erythroid and non-erythroid cells [19]. GPC has been detected on T-lymphocytes and weakly on B-lymphocytes and platelets; GPC was not detected on granulocytes or platelets [19,97], although GPC was expressed during both erythroid and neutrophil differentiation of $CD34^+$ haemopoietic progenitors [113]. *GYPC* mRNA was detected in human erythroblasts, erythroleukaemic cell lines, and fetal liver, but not in adult liver [7], and in human kidney [8]. Immunostaining with a monoclonal antibody to the cytoplasmic C-terminal domain of GPC and GPD, selected to avoid any effect of differential glycosylation, was apparent on fetal liver (mostly on cells of erythroid lineage), sinusoids of adult liver, kidney glomeruli, and neural cells in the brain [13].

18.7 Functional aspects: association of GPC and GPD with the membrane skeleton

The shape, flexibility, and deformability of red cells is maintained by a submembranous matrix containing the proteins α- and β-spectrin, actin, protein 4.1R, adducin, dematin, tropomyosin, and tropomodulin [114,115]. Transmembrane protein band 3 (anion exchanger) is linked to the cytoskeleton through ankyrin and protein 4.2, representing the major site of attachment of the skeletal network to the membrane (Section 10.7, Figure 10.2). GPC and GPD serve a similar function. Protein 4.1R links GPC and GPD to the spectrin/actin junction, with the phosphoprotein membrane-palmitoylated protein 1 (MMP1, p55) functioning to stabilise the interaction. The Arg-His-Lys tripeptide at positions 86–88 of GPC bind to a sequence within the 30 kDa FERM domain of 4.1R; the tripeptide Tyr-Phe-Ile at the C-terminus of GPC binds to the PDZ domain of MMP1; and the D5 domain of MMP1 binds to 4.1R [116–124]. Protein 4.1R also binds calmodulin and increased levels of Ca^{++} decreases affinity of 4.1R interactions with MMP1 and GPC [124]. Phosphorylation of 4.1R results in dissociation of GPC from the membrane skeleton [125]. Protein 4.1R, therefore, plays an important role in regulating the GPC–4.1R–MMP1 complex. Palmitoylation of MMP1 is essential for membrane organisation and rare individuals who lack the active transferase responsible for MMP1 palmitoylation have an unusual form of haemolytic anaemia [126].

There are about 200 000 molecules of 4.1R per red cell [9], about the same as the total number of GPC and GPD molecules [5]. Patients with hereditary elliptocytosis caused by 4.1R deficiency have a 70–90% reduction of GPC and GPD, are MMP1 deficient, and have complete elliptocytosis [118,127–129]. GPC- and GPD-deficient Ge:–2,–3,–4 red cells have about 25% reduction in 4.1R and about 98% reduction in MMP1 [118]. Red cells of 4.1R knockout ($4.1R^{-/-}$) mice are deficient in GPC and MMP1 [130], and have reduced levels of Rh, Duffy, and Xk proteins as well as possible conformational changes in band 3 and Kell, suggesting the presence of a membrane protein complex linked to the spectrin-actin junction through GPC and 4.1R [14] (Figure 10.2). In $4.1R^{-/-}$ mice GPC sorts to the erythroblast nuclear membrane, rather than the surface membrane, and is lost from reticulocytes during enucleation [131].

The functions of the extracellular domains of GPC and GPD are unknown. Like GPA and GPB, an important function of these heavily sialylated structures could be to contribute to the glycocalyx (see Section 3.23).

18.8 Malaria

The level of invasion of Ge:–2,–3,–4 red cells by the malaria parasite *Plasmodium falciparum* was only 57% of that of Gerbich-positive cells [132]. *P. falciparum* expresses four genes encoding proteins of the Duffy binding-like (DBL) family. These proteins recognise different red cell ligands as a result of mutations in the receptor region of two of the four genes [133]. One of the variants, BAEBL (VSTK) (also known as EBA-140 and PfEBP-2), binds GPC on red cells and could be inhibited from binding to red cells by soluble GPC, but does not bind GPC.Ge, which lacks the product of *GYPC* exon 3 [58,133–136] (although failure to bind Ge:–2,–3 red cells has been disputed [135]). Binding of BAEBL (VSTK) to GPC is dependent on the presence of the single *N*-glycan on GPC, which differs in composition from that on GPC. Ge; neither *N*-deglycosylated GPC nor isolated *N*-glycans inhibited binding of BAEBL (VSTK) to red cells [58]. A selection advantage through the reduced ability of some strains of *P. falciparum* to invade Ge:–2,–3 red cells, could explain the high frequency of this phenotype in parts of PNG, where malaria is endemic, although no evidence for reduced infection of Ge:–2,–3 individuals (as determined by genotyping) was found in a population study [137].

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