# Strains Responsible for Invasive Meningococcal Disease in Patients With Terminal Complement Pathway Deficiencies.

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**MAJOR ARTICLE**

# *Strains responsible for invasive meningococcal disease in patients with terminal complement pathway deficiencies*

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**Brief summary**

Invasive meningococcal strains isolated from patients with complement terminal pathway deficiencies (TPD) present similar characteristics to those isolated from the nasopharynx of asymptomatic carriers. This finding has implications in the management of patients with TPD.

## ABSTRACT

**Background.** Patients with terminal complement pathway deficiencies (TPD) are susceptible to recurrent invasive meningococcal disease (IMD). *Neisseria meningitidis* (Nm) strains infecting these patients are poorly documented in the literature.

**Methods.** We identified patients with TPD and available Nm strains isolated during IMD. We investigated the genetic basis of the different TPD and the characteristics of the Nm strains.

**Results.** We included n=56 patients with C5 (n=8), C6 (n=20), C7 (n=18), C8 (n=9) or C9 (n=1) deficiency. Genetic study was performed in 47 patients and 30 pathogenic variants were identified in the genes coding for C5 (n=4), C6 (n=5), C7 (n=12), C8 (n=7) and C9 (n=2). We characterized 61 Nm strains responsible for IMD in the 56 patients with TPD. The most frequent strains belonged to group Y (n=27; 44%), B (n=18; 30%) and W (n=8; 13%). Hyperinvasive clonal complexes (cc) (cc11, cc32, cc41/44 or cc269) were responsible for 21% of IMD cases. The cc23 predominates and represented 26% of all invasive isolates. Eleven out the 15 cc identified fit to 12 different cc belonging to carriage strains.

**Conclusions.** Unusual meningococcal strains with low level of virulence similar to carriage strains are most frequently responsible for IMD in patients with TPD.



**Key words**

*Neisseria meningitidis*; primary immunodeficiency; complement; terminal complement pathway; membrane attack complex

## INTRODUCTION

*Neisseria meningitidis* (Nm) is a leading cause of bacterial meningitis and septic shock. The portal of entry of this Gram-negative pathogen is the human nasopharynx, which is frequently (10-25%) colonized by low virulence strains called carriage strains [1, 2]. Multilocus sequence typing (MLST), a method analyzing the sequence of 7 representative housekeeping genes, has identified closely related genotypes groups (called clonal complexes) corresponding to hyperinvasive lineages and responsible for most cases of invasive meningococcal disease (IMD) worldwide [2]. In the general population, carriage isolates are highly genetically diverse and hyperinvasive lineages are less frequently found among carriage isolates compared to disease isolates [3]. The polysaccharide capsule is a key virulent factor, which allows Nm to resist complement-mediated lysis [4].

An effective complement system is a powerful effector arm of the innate immune defense against this invading pathogen [5]. Despite the co-optation of complement regulatory proteins from its human host to promote complement evasion [4, 6], the interaction of the complement with Nm results in C3 opsonization on the bacterial surface and formation of the membrane attack complex (MAC), two important steps for efficient bacterial killing. The MAC is a structure typically formed on the bacterial surface as a result of the activation of the host's alternative, classical or lectin pathway. The MAC disrupts the cell membrane of bacteria and forms transmembrane channels, leading to bacterial lysis. MAC assembly requires the sequential and irreversible association of complement proteins C5b, C6, C7, C8 (composed of C8β and C8αγ) and C9 [7].

The complement system is determinant in the immunity to Nm [8, 9]. Inborn errors in components of the alternative pathway such as properdin or factor D and of the terminal pathway (C5, C6, C7, C8 and C9) underlie susceptibility to meningococcal disease. Autosomal recessive terminal complement pathway deficiencies (TPD) are primary immune deficiencies [10]. The risk of IMD is 1 000 to 10 000–fold increased in patients with TPD compared to the general population [9]. This increased risk has also been described in patients with C9 deficiency [11], for which the prevalence is about 1/1000 in the Japanese population [12, 13]. To date, TPD has been reported in more than 350 individuals but most frequently without genetic study. TPD patients are more prone to be infected with Nm strains belonging to minor or uncommon groups in the general population, such as groups W, Y or non-groupable strains [9, 14]. However, the detailed characteristics of Nm strains infecting these patients have been poorly described [15].

Eculizumab, a monoclonal antibody that induces functional C5 deficiency, is approved for the treatment of patients with paroxysmal nocturnal hemoglobinuria and atypical hemolytic and uremic syndrome [16]. Therefore besides patients with hereditary TPD, an increasing number of patients are now experiencing secondary C5 deficiency due to C5–targeted therapy and are at risk for invasive Nm infections. Adverse events have already been reported, including in patients who have been vaccinated as recommended prior to therapy [17-21].

The characterization of invasive Nm isolates is an essential step to optimize prophylactic strategy in patients who lack the capacity to form the MAC. We here report 61 Nm isolates responsible for IMD in a cohort of 56 patients with hereditary TPD and we compare these data to previously reported genotypes from both carriage and invasive strains isolated from the general population.

## METHODS

### Patients' recruitment

160 patients with TPD were diagnosed between 1980 and 2015 by the Complement laboratory of the Broussais/European Georges-Pompidou Hospital from Paris. Among these patients, we identified those in whom Nm isolates or available samples collected during IMD were stored in the biobank of the French National Reference Center for Meningococci (NRCM) (see **Supplementary Figure 1**). This retrospective study was conducted with patients coming from 45 different French departments of pediatrics, internal medicine, intensive care and infectious diseases. History of Nm episodes from TPD patients was identified using both records of the Complement laboratory and the NRCM. Both documented IMD and undocumented but clinically suspected IMD (i.e. meningitis, sepsis, feverish *purpura*) were included in the description of patients' history.

### Complement exploration

EDTA plasma samples were stored at -70°C. TPD screening was made by determination of CH50 activity, which explores the functionality of the classical and the terminal complement pathways. Levels of terminal complement component (C5, C6, C7, C8 and C9) were measured by ELISA. The CH50 and ELISA assays were performed on plasma-EDTA as previously described, and their results were expressed as the percentage of the mean result obtained with a pool of plasma-EDTA of healthy donors [22-24]. TPD was defined by a decreased CH50 activity associated with a low level in one of the terminal complement components (i.e. <10% for C5, C6, C7 and C9 components and <50% for C8 component). The addition of normal plasma to the patients' plasma restored its ability to sustain total hemolytic activity whereas the addition of plasma depleted of the deficient component did not.

Genetic testing was performed on DNA extracted from whole-blood EDTA. Exons and flanking splice sites were amplified using specific primers (available on request) and sequenced using Sanger method.

**Cultured and non-cultured meningococcal characterization**

As part of the mandatory reporting system of IMD in France, Nm isolates from IMD are systematically sent to the NRCM for full characterization and typing. Serogroups were determined by agglutination using specific in-house rabbit antibodies to Nm. Antibiotic susceptibility testing for penicillin G was performed using E-test as previously described [25]. Isolates showing reduced susceptibility to penicillin G (intermediate isolates) were defined as those with a minimal inhibitory concentration (MIC) to penicillin G equal or superior to 0.125 mg/L [26]. Cases with negative culture were confirmed by PCR-based detection and genotype-grouped as previously described [27]. Cultured isolates as well as PCR-confirmed cases were genotyped by MLST in addition to sequencing of *porA* and *penA* [28]. Alleles, sequence types and clonal complexes (cc) were assigned using the *Neisseria* MLST database (available on http://neisseria.org). The level of expression of fHbp was quantified by ELISA using anti-fHbp antibodies as previously described [29]. The characteristics of Nm invasive strains, all isolated from TPD patients between 1999 and 2015, were compared to those of carriage and invasive strains from the general French population, using the data of a carriage study conducted in 2008 in Normandy by the NRCM and those of all invasive strains of the general population in 2008 [30].

## Ethics

This study was approved by an ethic committee ("Comité de Protection des Personnes Ile de France 5", ref. a-11-15), allowing the retrospective crossing of the data from the Complement laboratory and from the NRCM.

## RESULTS

### Demographics and infectious phenotype of the TPD patients' cohort

We enrolled in this study 56 patients (from 56 unrelated kindreds) with C5 deficiency (n=8), C6 deficiency (n=20), C7 deficiency (n=18), C8 deficiency (n=9) or C9 deficiency (n=1). The clinical and biological characteristics of these patients are shown in **Table 1**. The sex-ratio (M/F) was 2.1. Median age of first IMD episode was 15 years (range 14 months – 39 years). Median follow-up was 21 years. Detailed histories were available for 55 patients (**Figure 1**). Among the 56 patients with suspected or confirmed IMD episodes, 25 (45%) had more than one IMD episodes. Eight of the 25 patients experiencing recurrences had at least three episodes. The median interval between invasive episodes in patients with recurrences was 6 years (mean 8 years; range 5 months – 36 years).

### Characteristics of TPD

Genetic testing was performed in 47 of the 56 patients (84%). A total of 30 pathogenic variants were identified in the coding exons or the flanking splice sites of *C5* (n=4), *C6* (n=5), *C7* (n=12), *C8B* (n=7) and *C9* (n=2) genes (**Figure 2**). These variants were missense (n=6), nonsense (n=8), small deletions (n=10) or variants in canonical splice sites (n=6). A total of 23 patients were identified with homozygous variants whereas 24 patients carried two (n=23) or three (n=1, see **Supplementary Figure 2)** heterozygous variants in the same gene. Sixteen variants have been previously reported in patients with TPD and 14 are newly described (see **Supplementary Table 1**). Seven recurrent deleterious variants accounted for 59% of the genetic defects: *C5* in-frame small deletion 960_962delCAA; *C6* three single-nucleotide deletions c.821delA, c.1138delC and c.1879delG; *C7* missense variant c.1135G>C (p.G379R), *C7* subtotal deficiency variant c.1561C>A (C7SD, p.R521S); and *C8B* nonsense variant c.1282C>T (p.R428*). With the exception of 4 patients, the patients' complement

profile was characterized by the lack of detectable CH50 activity (defined by CH50<10% of normal values). Among the four patients with detectable CH50 activity, three of them carried C7SD allele (p.R521S) and one presented C9 deficiency. The highest CH50 detectable value was 38% and was found in the C9 deficient patient.

**Meningococcal isolates in TPD patients**

61 isolates from episodes of IMD in the 56 TPD patients were sent to NRCM for confirmation, typing and antibiotic susceptibility testing. The infection was confirmed by culturing isolates from sterile sites (n=39), by PCR assay (n=12) or by both analysis (n=10). Using sero-agglutination or PCR, strains were assigned to group Y (n=27, 44%), group B (n=18; 30%), group W (n=8; 13%), group E (n=3; 5%), group C (n=2; 3%) and non-groupable (i.e. non-A, B, C, E, X, Y or W) (n=3; 5%) (**Table 1**). Using MLST, we determined the genotypes of the isolates and characterized the clonal complexes for 53 of the total 61 cases of IMD (87%). The isolates from TPD patients belonged to 15 different clonal complexes (**Figure 3**). Two isolates (3.7%) were not assigned to any known clonal complex. The most frequent clonal complexes were cc23 (n=14; 26%), cc22 (n=6; 11%) and cc41/44 (n=5; 9%). Thirteen out of 14 strains from cc23 were from group Y isolates. Hyperinvasive clonal complexes (i.e. cc11, cc32, cc41/44 and cc269) were responsible for 21% of the invasive cases in TPD patients (**Table 1**). No correlation was found between a type of TPD and a specific capsular group/clonal complex. The study of the variable regions (VR) of *porA* genes showed 15 and 21 different VR1 and VR2 respectively. Sequencing of the variable region of *fetA* gene also revealed high diversity with 20 different variable regions.

MIC to penicillin G of 49 meningococcal cultured isolates was also determined and 39% of these isolates exhibited reduced susceptibility which was confirmed by the presence of altered

*penA* gene (data not shown). All tested isolates (n=41; 84% of the cultured isolates) expressed factor H binding protein.

**Nm strains from IMD in TPD patients with multiple documented infections**

Among patients with recurrent invasive disease, the isolates from at least two episodes were available for four TPD patients. The isolates involved in multiple episodes from same patient were different for three patients. The isolates were undistinguishable for two episodes separated by 13 months (W/cc22) for one patient with C7 deficiency (see **Supplementary Figure 3**). One C5-deficient patient developed a second episode of IMD despite presenting 10 months before this second episode a high level of serum bactericidal antibody against the responsible isolate (see **Supplementary Figure 3**).

**Phenotypic and genotypic diversity of isolates from TPD patients compared to the general population**

We compared the distribution of the IMD isolates from TPD patients with IMD isolates from the French general population in the year 2008 and with carriage isolates from a carriage study that was performed this same year [30]. The frequency of group B strains in IMD was significantly lower in TPD patients than in the general French population (65.3% *vs* 30% respectively; $p<0.0001$) and this percentage did not significantly differ from the frequency of group B observed among carriage isolates (30% *vs* 24.5% respectively; $p=0.43$) (**Table 2**). The frequency of group Y in IMD was significantly higher in TPD patients when compared to the general population. Half of the isolates from the carriage study were non groupable whereas in TPD patients with IMD, less than 5% of the isolates were non-groupable. In the general population, the hyperinvasive lineages (cc11, cc32, cc41/44, and cc269) are significantly overrepresented in collections of invasive isolates relative to

collections of asymptomatic carriage isolates and of invasive isolates from the TPD patients (**Figure 4** and **Table 2**).

## DISCUSSION

We took advantage of a large cohort of 56 confirmed-TPD patients to demonstrate that Nm isolated from these patients present highly diverse genotypes and belong to clonal complexes rarely associated with invasive strains in the general population.

Nm is a human-specific bacterium carried by 10% of the population that can also be responsible for life-threatening and fulminant invasive disease. A limited number of hypervirulent lineages is responsible for the large majority of disease worldwide [2]. Patients with hereditary TPD present a monogenic susceptibility to recurrent IMD [9]. Nm strains infecting these patients are poorly documented limiting the improvement of disease prevention. We show that the hyperinvasive lineages in TPD patients from our cohort accounted for only 21% of IMD cases, which is similar to Nm carriage strains in upper respiratory tract of healthy human carriers. The characteristics of invasive isolates from TPD patients significantly differ from those of the general population. The 11 non-hyperinvasive clonal complexes identified in the TPD patients such as cc35, cc167, cc213 or non-attribuable clonal complex are rarely associated with IMD in general population [2]. However, 95% of the isolates from TPD patients are groupable suggesting that the isolates have the capacity to express capsule and therefore display potential virulence. Nm Y:cc23 strains contribute to 26% of IMD in our TPD patients' cohort. In Italy as in several European countries, the proportion of IMD cases due to group Y increased recently, ranging from 2% in 2007 to 17% in 2013 [31]. High–resolution genetic analyses revealed extensive similarities between Nm Y carriage–associated and disease associated organisms, indicating that all Nm Y and the cc23 circulating strains in the healthy population have the ability to cause the disease [32]. Therefore strains with low levels of virulence can be considered at risk for invasive disease in

patients with acquired or genetic defects in terminal complement pathway. These findings are consistent with the role of meningococcal carriage in the nasopharynx in the invasive disease in TPD patients. Those patients are vulnerable to disease caused by less invasive meningococci as previously demonstrated in very young or relatively old (age<1 or >65 years) [33].

Screening of TPD can be made using the reference hemolytic method or using functional ELISA [34]. Our results confirm that detectable but decreased CH50 activity can be associated with TPD and can underlie C9 deficiency or subtotal TPD [13, 23, 24, 35]. In clinical practice, immunochemical assays for individual TP components must be performed to investigate unexplained reduced but detectable CH50 activity. Our study incorporates genetic data proving the permanent complement deficiency. We report the genetic defects of the largest series of patients with a lack of functional terminal pathway. We identified causal pathogenic variants in each tested patient. The variants are missense, frameshift, splicing or nonsense. We report 14 new variants. Several pathogenic variants are present at very low frequency in the general population and have already been reported in patients with TPD. Seven patients from our cohort carry C7 subtotal variant (p. R521S), which is associated with the production of a small quantity of C7 [23]. Our result confirms previous reports showing that patients with C7 subtotal deficiency are also susceptible to recurrent IMD, contrary to the patients with subtotal C6 deficiency caused by *C6* c.2350+2T>C variant. In subjects homozygous for this variant, the C6 level is about 1–5% of normal but retains hemolytic and bactericide properties [35, 36].

A previous study including 21 patients with TPD showed no difference between the distribution of strains infecting complement-deficient and complement-sufficient patients [15]. However, this study has only used phenotypic or low discriminant genotypic techniques

for strain characterization and also included non-TPD patients such as patients with deficiencies of the alternative pathway or patients with C3 nephritic factor.

It is recommended to vaccinate TPD patients with both tetravalent conjugate vaccines against serogroups ACYW and with recombinant vaccine developed to target serogroup B isolates [37]. However, in patients who lack the capacity to mediate meningococcal lysis by the MAC, the adaptive humoral immunity is insufficient to provide full-effective protection [9, 38-41]. About half of TPD patients suffer from recurrent IMD with a variable interval between episodes [9]. We illustrated a recurrence with the same strain after a 13 months interval and an episode of IMD despite a high pre-existing serum bactericidal titer against the involved strain. Among the 12 reported cases of IMD in 11 eculizumab-treated patients, two cases of genuine vaccine failures (i.e. IMD with a group supposed to be covered by the previous vaccination) have already been described [19, 21] (see **Supplementary Table 2**). Optimal killing of Nm in blood depends on the MAC formation and effective IMD control in TPD patients by vaccine remains controversial [42]. Chemoprophylaxis using penicillin V could therefore be added to improve the protection of these patients. However, the proportion of isolates with reduced susceptibility to penicillin represent 39%, which may eventually be responsible for chemoprophylaxis failure. These observations underscore the need for additional preventive measures among patients with primary or acquired TPD. Acquisition of meningococci in the upper respiratory tract can be transient or lead to meningococcal carriage but may also result in meningococcal disease. Carriage prevalence of Nm is generally higher among household contacts of meningococcal patients [43] than in the general population. Studies assessing the impact of household contacts screening for Nm isolates could open a new strategy to reduce the risk of invasive infection in patients that lack functional terminal complement pathway. In that sense, the large proportion of carriage-related strains observed

in TPD patients of our cohort argues for a possible contamination from close contacts. Vaccination of these close contacts of TPD patients may thus be advocated to improve IMD prevention, as ACYW conjugated vaccines and serogroup B vaccines have been proven to reduce carriage [44].

In summary, our data show that carriage strains with low virulence capacity can be responsible for IMD in patients with TPD. In addition to the currently recommended prophylaxis based on vaccination, antibiotic and education [37], our study promotes a new prophylactic strategy based on the eradication of nasopharyngeal carriage in patients and their household contacts.

## NOTES

**Potential conflicts of interest**: V.F-B. has received fees from Alexion Pharmaceuticals for invited lectures and is member of an expert board supported by Alexion Pharmaceuticals. D.L. has received grants from Octapharm and CSL Behring. All others authors have no conflict of interest to declare.

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464

**Table 1 – Demographic and clinical characteristics of TPD patients and of Nm strains involved in IMD**

| | All | C5 | C6 | C7 | C8 | C9 |
|---|---|---|---|---|---|---|
| ***Characteristics of patients*** | | | | | | |
| Number | 56 | 8 | 20 | 18 | 9 | 1 |
| Male/Female ratio | 2.1 | 1.7 | 3.0 | 2.0 | 2.0 | NA |
| Median age of first IMD (year) | 15* | 9 | 18 | 14* | 14 | 12 |
| Median age of IMD (year) | 17* | 18 | 19 | 17* | 16 | 12 |
| Mean number of IMD episodes per patient | 1.7 | 1.8 | 1.3 | 2.1 | 1.6 | 1 |
| Follow-up (Median, year) | 21 | 22 | 22 | 20 | 17 | 13 |
| Patients with more than one episode of IMD (n) *(%)* | 25 *(45)* | 5 *(63)* | 5 *(25)* | 10 *(56)* | 5 *(56)* | 0 |
| ***IMD episodes*** | | | | | | |
| Episodes (n) | 61 | 9 | 20 | 22 | 9 | 1 |
| Median age of included episodes (year) | 20 | 21 | 21 | 19 | 14 | 12 |
| Mean number of included episodes | 1.6 | 1.7 | 1.3 | 1.8 | 1.4 | 1 |
| **Nm group** | | | | | | |
| Y (n) *(%)* | 27 *(44)* | 4 *(44)* | 10 *(50)* | 9 *(41)* | 3 *(33)* | 1 *(100)* |
| B (n) *(%)* | 18 *(30)* | 2 *(22)* | 6 *(30)* | 7 *(32)* | 3 *(33)* | 0 |
| W (n) *(%)* | 8 *(13)* | 0 | 3 *(15)* | 4 *(18)* | 1 *(11)* | 0 |
| C (n) *(%)* | 2 *(3)* | 1 *(11)* | 0 | 0 | 1 *(11)* | 0 |
| E (n) *(%)* | 3 *(5)* | 1 *(11)* | 0 | 2 *(9)* | 0 | 0 |
| NG (n) *(%)* | 3 *(5)* | 1 *(11)* | 1 *(5)* | 0 | 1 *(11)* | 0 |
| **Clonal complexes (n = 53 tested)** | | | | | | |
| Hyperinvasive clonal complexes (n) *(%)* | 11 *(21)* | 2 *(22)* | 4 *(21)* | 4 *(22)* | 1 *(17)* | 0 |
| cc23 (n) *(%)* | 14 *(26)* | 3 *(33)* | 5 *(26)* | 4 *(22)* | 2 *(33)* | 0 |
| Others (n) *(%)* | 28 *(53)* | 4 *(44)* | 10 *(53)* | 10 *(56)* | 3 *(50)* | 1 *(100)* |
| **Strains with reduced susceptibility to penicillin G/Total number of tested strain *(%)*** | 19/49 *(39)* | 4/9 *(44)* | 3/15 *(20)* | 9/18 *(50)* | 2/6 *(33)* | 1 *(100)* |

* data not available for one C7-deficient patient

**Table 2 – Repartition of Nm group in the cohort of TPD patients compared to the those of invasive and carriage strains in the general population in France**

| | | Disease isolates | | Carriage isolates | | | |
|---|---|---|---|---|---|---|---|
| | | TPD | General population | | | | |
| | | g1 | g2 | g3 | g1 vs g2 | g1 vs g3 | g2 vs g3 |
| | n | 61 | 487 | 188 | | | |
| Serogroups (%) | Y | 44 | 6.2 | 8.5 | <0.0001 | <0.0001 | 0.28 |
| | B | 30 | 65.3 | 24.5 | <0.0001 | 0.43 | <0.0001 |
| | W | 13 | 4.3 | 3.2 | 0.006 | 0.0068 | 0.5 |
| | C | 3 | 23.6 | 8.5 | 0.0024 | 0.187 | <0.0001 |
| | NG | 5 | 0.6 | 48.9 | 0.01 | <0.0001 | <0.0001 |
| Hyperinvasive clonal complexes (%) | | 21 | 77.8 | 33.7 | <0.0001 | 0.075 | <0.0001 |
| Susceptibility for penicillin G (%) | | 61 | 78 | 66 | 0.0033 | <0.0001 | 0.001 |

## Figure legend

**Figure 1 – History of IMD episodes in patients with TPD**

Follow-up of 55 TPD patients (grey), and age at episode of IMD (black circle).

**Figure 2 - Pathogenic variants identified in patients with hereditary TPD**

Genetic study was performed in n = 47 patients. Homozygous variants are numbered with a red circle and heterozygous variants are numbered with a blue circle. Genes and introns are not at scale. In italic are new variants.

**Figure 3 - Distribution of clonal complexes among IMD isolates from the TPD patients cohort.**

n = 53 strains isolated from 48 patients with TPD. NA = non attributable clonal complex

**Figure 4 - Distribution of clonal complexes among IMD strains from the general population and TPD patients and from a carriage study**

Data of invasive strains from the French general population and from carriage study conducted in Normandy were obtained in the year 2008 [30]

## Supplementary Table 1 – Characteristics of variants found in the 56 TCCD patients

| Gene | Variant | SNP reference | Proteic impact | Allele count in the study population | MAF[£] | Previously reported |
|---|---|---|---|---|---|---|
| ***C5*** NM_001735.2 | c.55C>T | rs121909587 | p.Gln19* | 2 | 1.E-04 | Yes [1-3] |
| | c.713T>C | rs567288479 | p.Ile238Thr | 3 | 9.E-05 | No |
| | c.960_962delCAA | NA | p.Asn320del | 8 | <1.E-04 | Yes [4] |
| | c.3154+3A>T | NA | ND | 1 | private | No |
| ***C6*** NM_000065.3 | c.143G>A | rs145422926 | p.Arg48Lys | 3 | 2.E-04 | No |
| | c.821delA | rs557023458 | p.Gln274Argfs*46 | 5 | 5.E-04 | Yes [5-8] |
| | c.1138delC | rs375762365 | p.Gln380Serfs*7 | 8 | 7.E-04 | Yes [5, 7-11] |
| | c.1333C>T | NA | p.Arg445* | 2 | 3.E-05 | No |
| | c.1879delG | rs61469168 | p.Asp627Thrfs*4 | 16 | 9.E-04 | Yes [5, 7, 8, 10, 12] |
| ***C7*** NM_000587.2 | c.189T>G | NA | p.Cys63Trp | 2 | 8.E-06 | Patient previously reported [13] |
| | c.193G>T | NA | p.Gly65* | 2 | private | No |
| | c.280+1G>A | NA | ND | 1 | 2.E-05 | No |
| | c.281-1G>T | rs531103546 | ND | 2 | 1.E-04 | Yes [14, 15] |
| | c.405delT | rs139491301 | p.Asn136Thrfs*2 | 2 | 2.E-04 | No |
| | c.449delA[$] | NA | p.Gln150Argfs*30 | 1 | 8.E-06 | No |
| | c.633_643del | NA | p.Ser212Hisfs*4 | 1 | <1.E-04 | Yes [16] |
| | c.1135G>C | rs121964921 | p.Gly379Arg | 5 | 1.E-04 | Yes [13, 16-20] |
| | c.1410delG | NA | p.Thr471Profs*22 | 1 | private | No |
| | c.1561C>A | rs121964920 | p.Arg521Ser | 8 | 3.E-05 | Yes [13, 16, 17, 20, 21] |
| | c.2350delG | NA | p.Ala784Leufs*25 | 1 | 9.E-06 | Yes [17, 20] |
| | c.2350+2T>C[$] | rs201240159 | ND | 3 | 3.E-04 | Yes [13, 17, 20, 22] |
| ***C8B*** NM_000066.3 | c.138delC | NA | p.Phe47Leufs*14 | 1 | 8.E-06 | No |
| | c.249G>T | rs567145070 | p.Arg83Ser | 2 | 6.E-05 | No |
| | c.271C>T | rs146187042 | p.Gln91* | 1 | 7.E-05 | Yes [23-25] |
| | c.361C>T | NA | p.Arg121* | 2 | 1.E-04 | Yes [23, 24] |
| | c.850C>T | rs374155702 | p.Arg284* | 2 | 8.E-06 | No |
| | c.1105+1G>A | NA | ND | 2 | private | No |
| | c.1282C>T | rs41286844 | p.Arg428* | 6 | 1.E-04 | Yes [23, 24, 26, 27] |
| ***C9*** NM_001737.3 | c.162C>A | rs34000044 | p.Cys54* | 1 | 1.E-03 | Yes [28, 29] |
| | c.1240+5G>A | NA | ND | 1 | private | No |

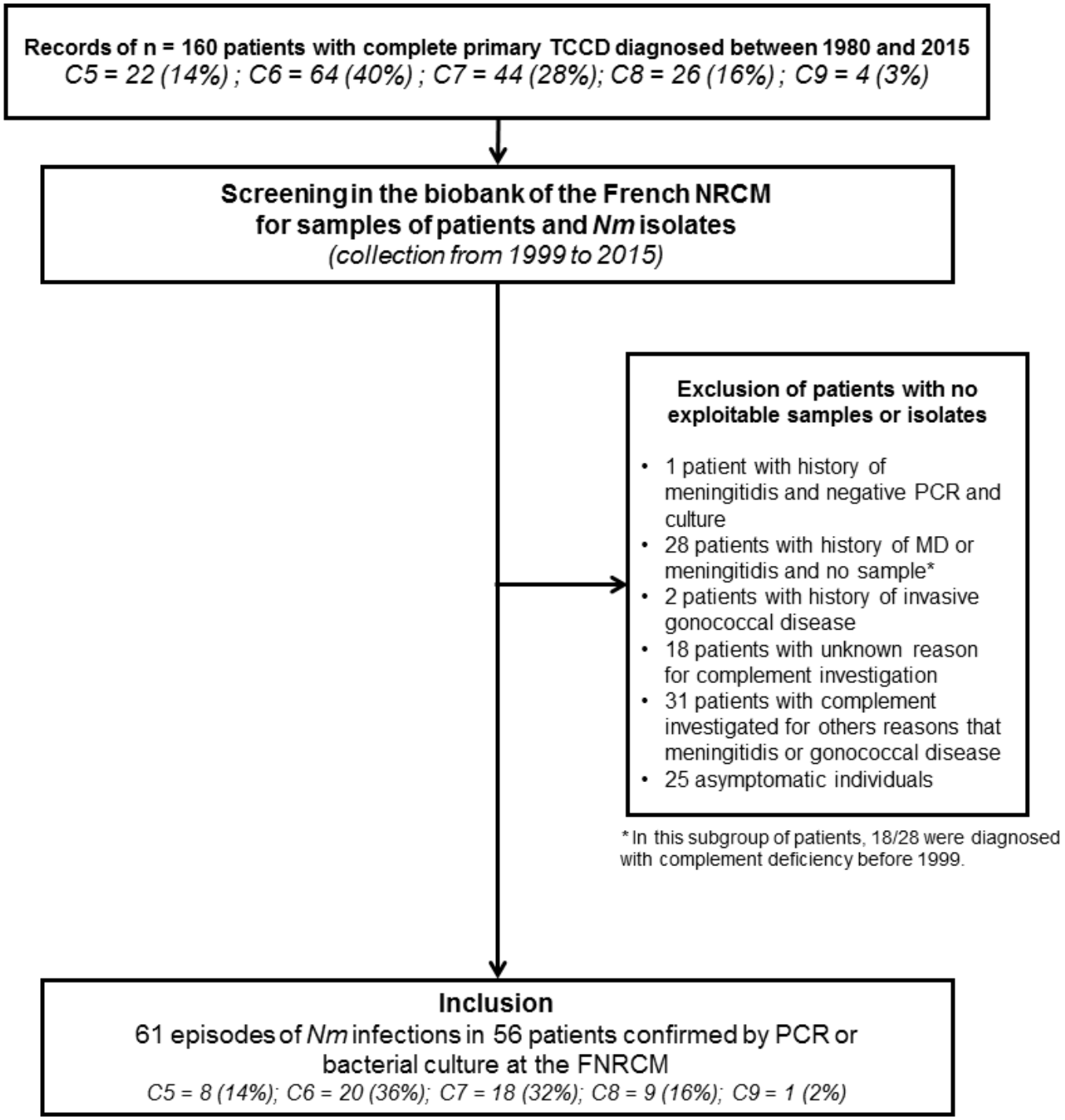
[$]One C7 deficient patient carried heterozygous c.1561C>A and c.2350 +2T>C variants within the same allele; [£]retrieved from the Exome Aggregation Consortium [30]

**Supplementary Table 2 – Reported IMD in eculizumab-treated patients.** The two cases of vaccine failures are depicted in bold.

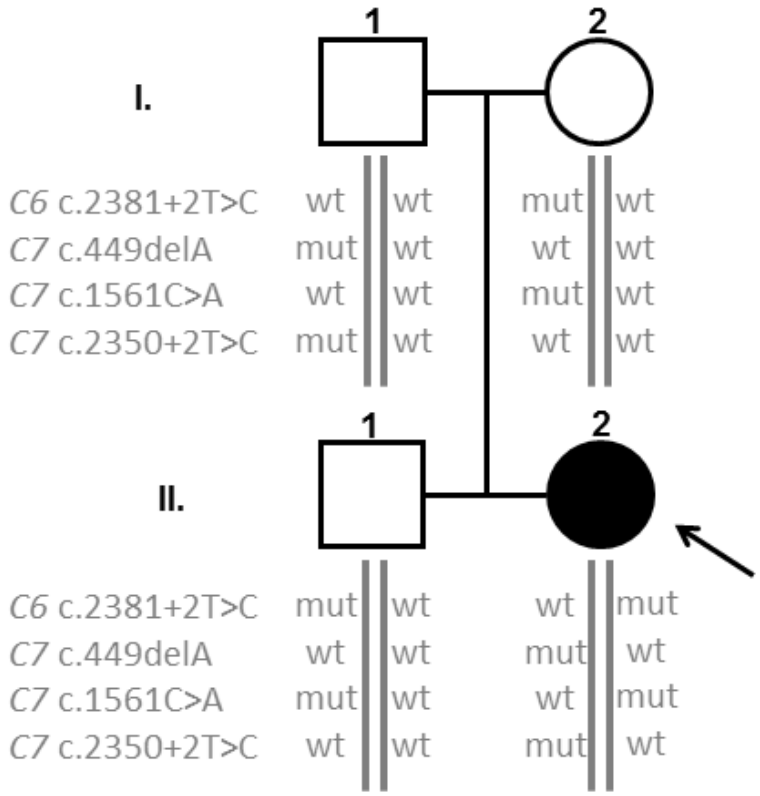
| Indication | Age of IMD | Previous meningococcal vacination | Serogroup | Outcome | Reference |
|---|---|---|---|---|---|
| PNH | 24 | ACYW* | B | Favorable | [31] |
| PNH | 54 | AC* | Y or W | Favorable | [31] |
| PNH | 27 | Unconjugated ACYW | X | Deceased | [32] |
| PNH | 18 (first episode)<br>21 (second episode) | Conjugated ACYW<br>**Bexsero (2 doses)** | B<br>**B** | Favorable<br>Favorable | [33] |
| PNH | 41 | Unconjugated ACYW | Undeterminate serogroup | NR | [34] |
| SLE | 22 | C*<br>Anti-B vaccine | W | Favorable | [35] |
| NO | 19 | ACYW* | Non groupable | Favorable | [36] |
| MPGN | NR | Non vaccinated | NR | NR | [37] |
| aHUS | 29 | Conjugated ACYW | Unknown | Favorable | [38] |
| aHUS | 24 | Unconjugated ACYW | B | Favorable | [38] |
| aHUS | ~22 | **Unconjugated ACYW** | **W** | Favorable | [39] |

* not precised if unconjugated or conjugated capsular vaccine; aHUS = atypical hemolytic and uremic syndrome; NO = neuromyelitis optica; MPNG = membanoproliferative glomerulonephritis; PNH = paroxysmal nocturnal hemoglobinuria; SLE = systemic lupus erythematosus ; NR = not reported

**Supplementary Figure 1 -** Flow-chart of the study



**Supplementary Figure 2 – Pedigree of a C7-deficient kindred where the index case carries three heterozygous pathogenic variants located on the two different *C7* alleles (II.2).** To note, the index case, her mother (I.2) and her brother (II.1) also carry heterozygous subtotal variant in C6 (*C6* c.2381+2T>C) previously described to be in linkage disequilibrium with the C7 subtotal deficiency associated-variant (*C7* c.1561C>A) [21]. wt = wild-type allele; mut = mutated allele.



**Supplementary Figure 3 – Detailed histories of two patients with recurrent documented IMD.** In P2, plasma sample was stored between the second episode and exhibited high serum bactericidal antibody (SBA) titre against the strain responsible for the second episode. SBA was determined using baby-rabbit serum.

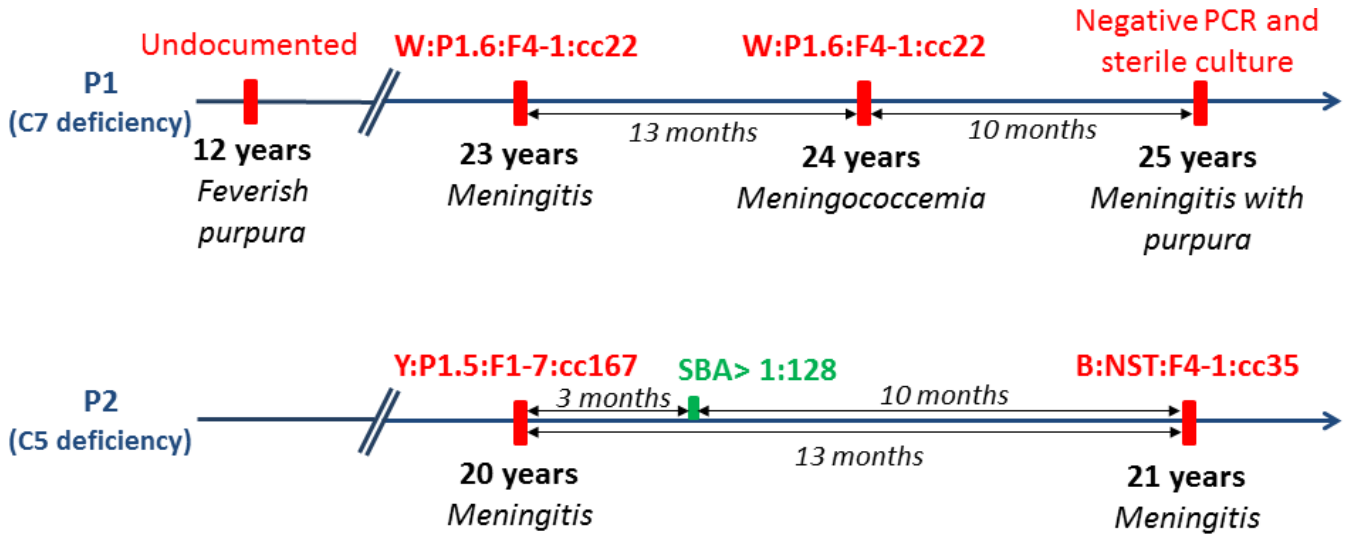


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Figure1

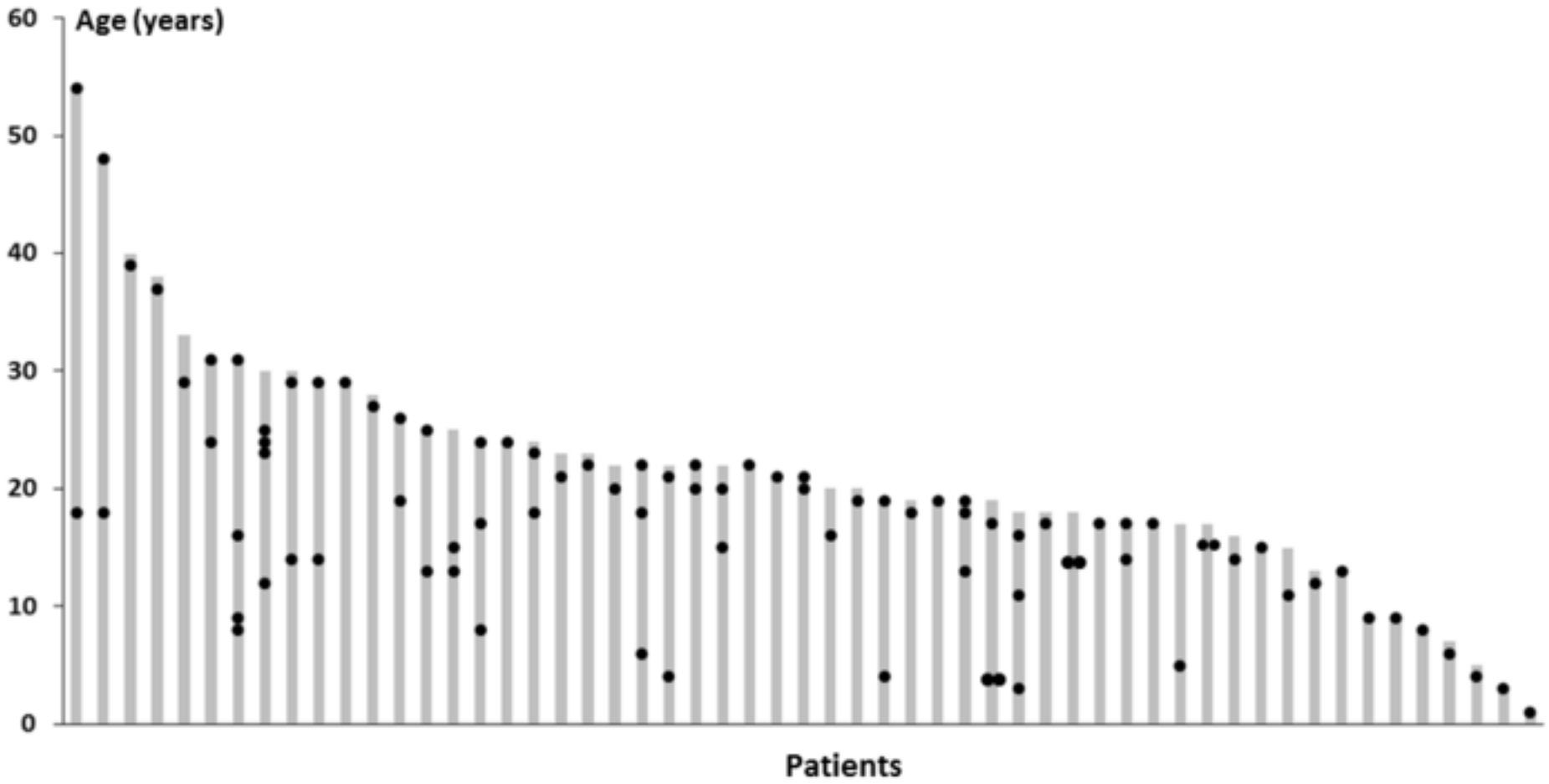

Figure2

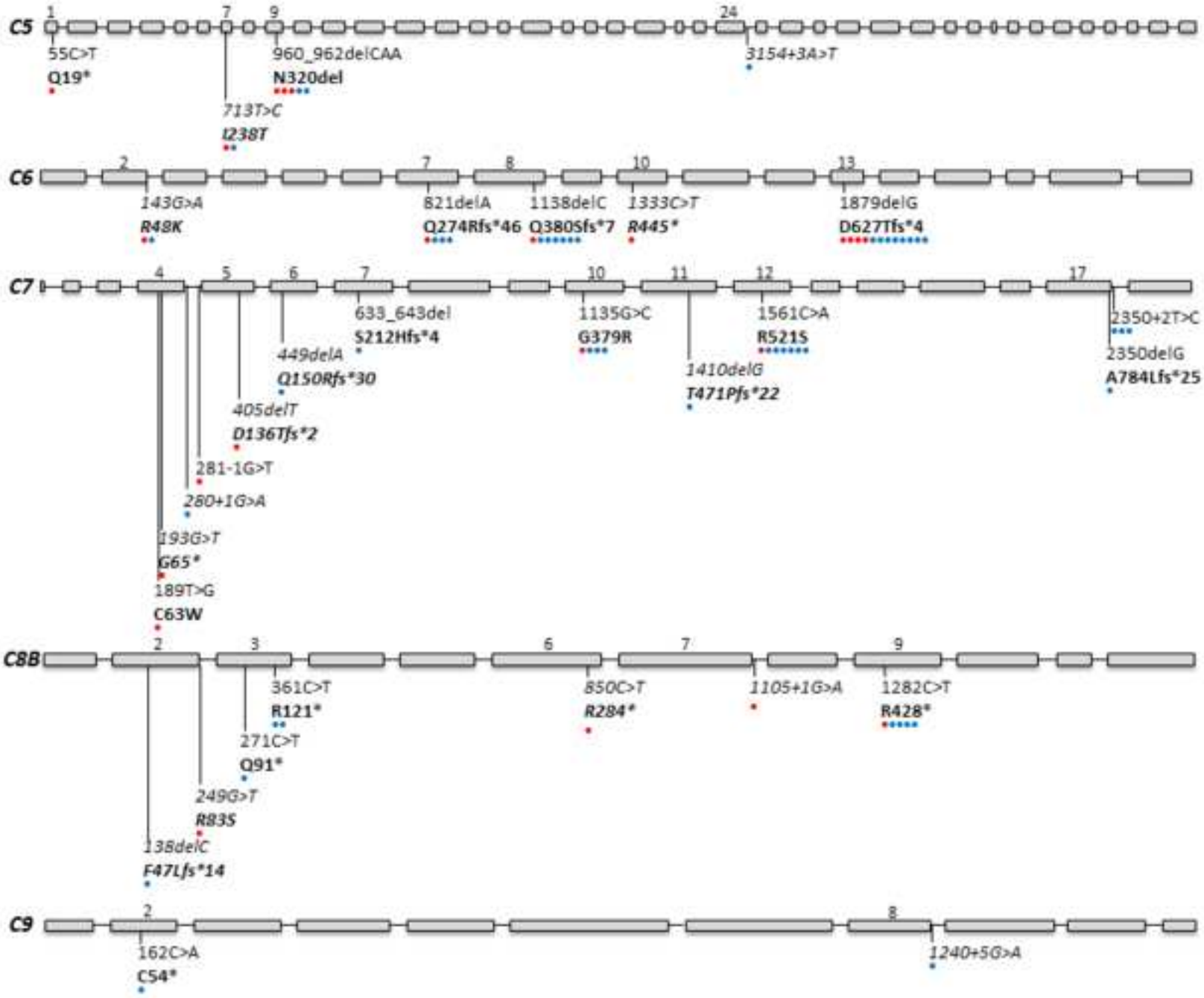
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Figure3

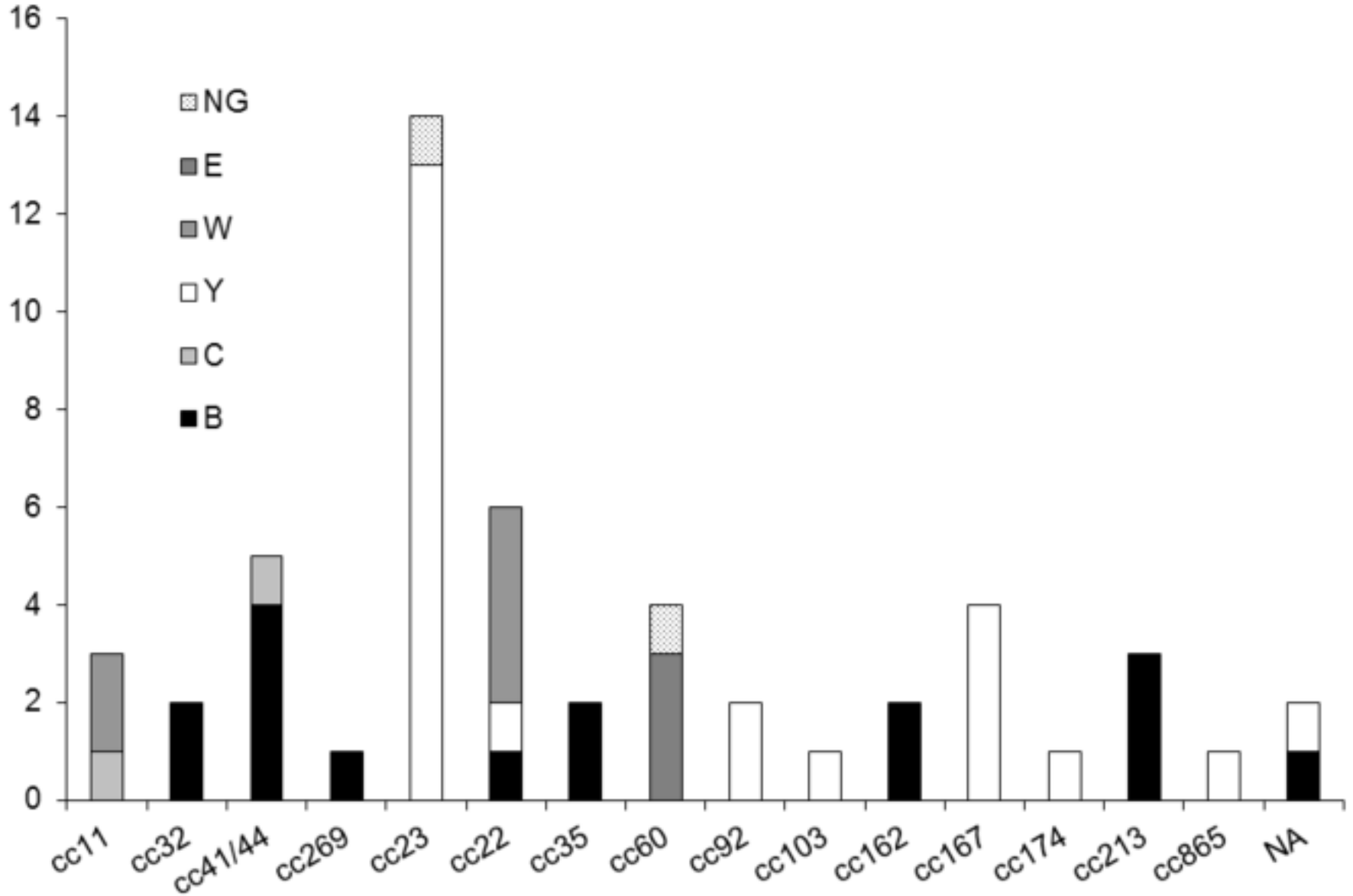
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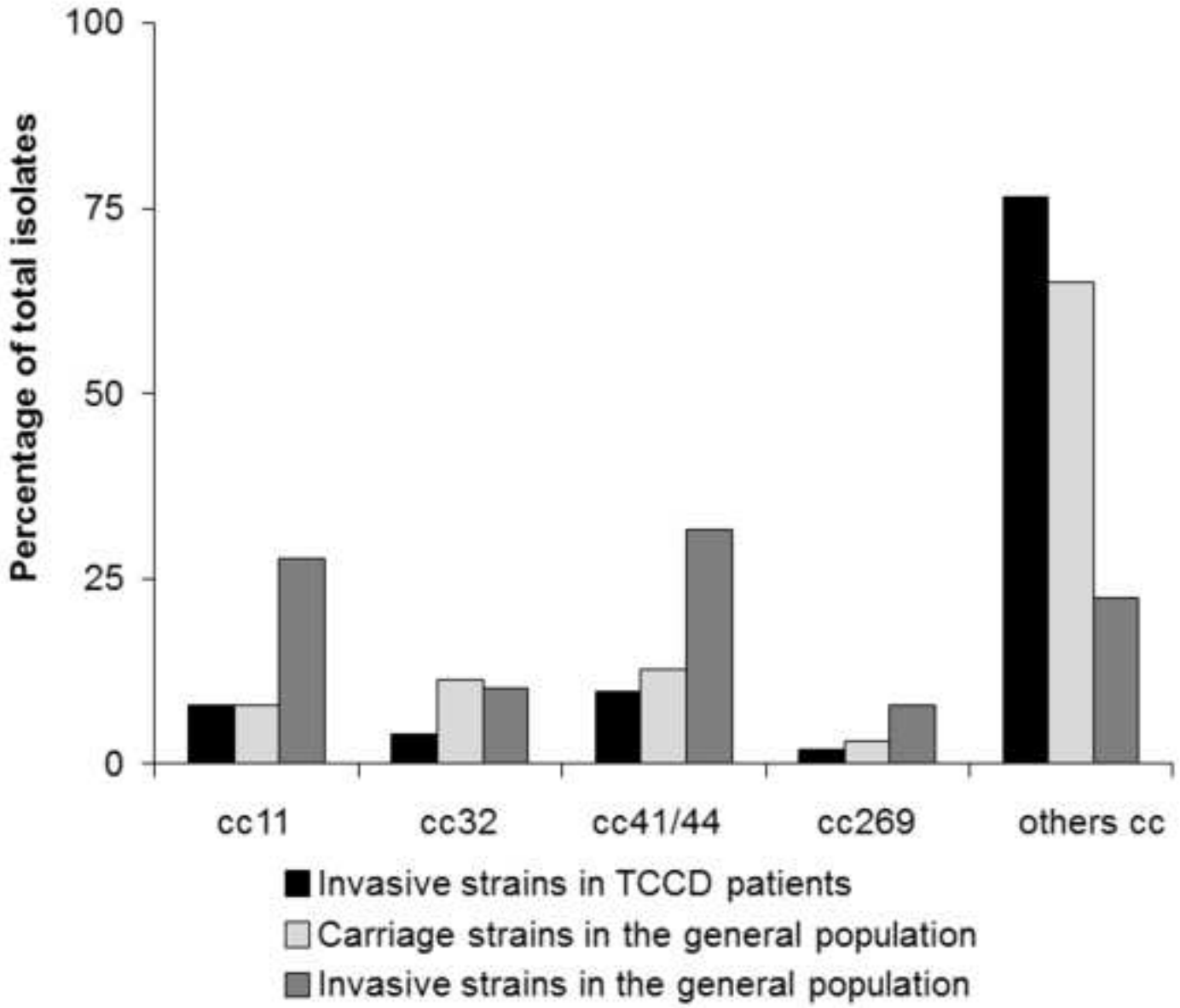
Figure4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Carriage strains in the general population
Invasive strains in the general population