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

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1 **MAJOR ARTICLE**

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

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46

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53 **Brief summary**

54 Invasive meningococcal strains isolated from patients with complement terminal pathway

55 deficiencies (TPD) present similar characteristics to those isolated from the nasopharynx of

56 asymptomatic carriers. This finding has implications in the management of patients with TPD.

57 **ABSTRACT**

58

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

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

64 **Results.** We included n=56 patients with C5 (n=8), C6 (n=20), C7 (n=18), C8 (n=9) or C9
65 (n=1) deficiency. Genetic study was performed in 47 patients and 30 pathogenic variants were
66 identified in the genes coding for C5 (n=4), C6 (n=5), C7 (n=12), C8 (n=7) and C9 (n=2). We
67 characterized 61 Nm strains responsible for IMD in the 56 patients with TPD. The most
68 frequent strains belonged to group Y (n=27; 44%), B (n=18; 30%) and W (n=8; 13%).
69 Hyperinvasive clonal complexes (cc) (cc11, cc32, cc41/44 or cc269) were responsible for
70 21% of IMD cases. The cc23 predominates and represented 26% of all invasive isolates.
71 Eleven out the 15 cc identified fit to 12 different cc belonging to carriage strains.

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

74

75 **Key words**

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

78

79 **INTRODUCTION**

80

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

91

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

102

103 The complement system is determinant in the immunity to Nm [8, 9]. Inborn errors in
104 components of the alternative pathway such as properdin or factor D and of the terminal
105 pathway (C5, C6, C7, C8 and C9) underlie susceptibility to meningococcal disease.
106 Autosomal recessive terminal complement pathway deficiencies (TPD) are primary immune
107 deficiencies [10]. The risk of IMD is 1 000 to 10 000-fold increased in patients with TPD
108 compared to the general population [9]. This increased risk has also been described in patients
109 with C9 deficiency [11], for which the prevalence is about 1/1000 in the Japanese population
110 [12, 13]. To date, TPD has been reported in more than 350 individuals but most frequently
111 without genetic study. TPD patients are more prone to be infected with Nm strains belonging
112 to minor or uncommon groups in the general population, such as groups W, Y or non-
113 groupable strains [9, 14]. However, the detailed characteristics of Nm strains infecting these
114 patients have been poorly described [15].

115

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

122

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

128

129 **METHODS**

130 **Patients' recruitment**

131 160 patients with TPD were diagnosed between 1980 and 2015 by the Complement
132 laboratory of the Broussais/European Georges-Pompidou Hospital from Paris. Among these
133 patients, we identified those in whom Nm isolates or available samples collected during IMD
134 were stored in the biobank of the French National Reference Center for Meningococci
135 (NRCM) (see **Supplementary Figure 1**). This retrospective study was conducted with
136 patients coming from 45 different French departments of pediatrics, internal medicine,
137 intensive care and infectious diseases. History of Nm episodes from TPD patients was
138 identified using both records of the Complement laboratory and the NRCM. Both documented
139 IMD and undocumented but clinically suspected IMD (i.e. meningitis, sepsis, feverish
140 *purpura*) were included in the description of patients' history.

141

142 **Complement exploration**

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

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

157

158 **Cultured and non-cultured meningococcal characterization**

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

175

176 **Ethics**

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

180

181 RESULTS

182 Demographics and infectious phenotype of the TPD patients' cohort

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

192

193 Characteristics of TPD

194 Genetic testing was performed in 47 of the 56 patients (84%). A total of 30 pathogenic
195 variants were identified in the coding exons or the flanking splice sites of *C5* (n=4), *C6* (n=5),
196 *C7* (n=12), *C8B* (n=7) and *C9* (n=2) genes (**Figure 2**). These variants were missense (n=6),
197 nonsense (n=8), small deletions (n=10) or variants in canonical splice sites (n=6). A total of
198 23 patients were identified with homozygous variants whereas 24 patients carried two (n=23)
199 or three (n=1, see **Supplementary Figure 2**) heterozygous variants in the same gene. Sixteen
200 variants have been previously reported in patients with TPD and 14 are newly described (see
201 **Supplementary Table 1**). Seven recurrent deleterious variants accounted for 59% of the
202 genetic defects: *C5* in-frame small deletion 960_962delCAA; *C6* three single-nucleotide
203 deletions c.821delA, c.1138delC and c.1879delG; *C7* missense variant c.1135G>C
204 (p.G379R), *C7* subtotal deficiency variant c.1561C>A (*C7SD*, p.R521S); and *C8B* nonsense
205 variant c.1282C>T (p.R428*). With the exception of 4 patients, the patients' complement

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

210

211 **Meningococcal isolates in TPD patients**

212 61 isolates from episodes of IMD in the 56 TPD patients were sent to NRCM for
213 confirmation, typing and antibiotic susceptibility testing. The infection was confirmed by
214 culturing isolates from sterile sites (n=39), by PCR assay (n=12) or by both analysis (n=10).
215 Using sero-agglutination or PCR, strains were assigned to group Y (n=27, 44%), group B
216 (n=18; 30%), group W (n=8; 13%), group E (n=3; 5%), group C (n=2; 3%) and non-
217 groupable (i.e. non-A, B, C, E, X, Y or W) (n=3; 5%) (**Table 1**). Using MLST, we determined
218 the genotypes of the isolates and characterized the clonal complexes for 53 of the total 61
219 cases of IMD (87%). The isolates from TPD patients belonged to 15 different clonal
220 complexes (**Figure 3**). Two isolates (3.7%) were not assigned to any known clonal complex.
221 The most frequent clonal complexes were cc23 (n=14; 26%), cc22 (n=6; 11%) and cc41/44
222 (n=5; 9%). Thirteen out of 14 strains from cc23 were from group Y isolates. Hyperinvasive
223 clonal complexes (i.e. cc11, cc32, cc41/44 and cc269) were responsible for 21% of the
224 invasive cases in TPD patients (**Table 1**). No correlation was found between a type of TPD
225 and a specific capsular group/clonal complex. The study of the variable regions (VR) of *porA*
226 genes showed 15 and 21 different VR1 and VR2 respectively. Sequencing of the variable
227 region of *fetA* gene also revealed high diversity with 20 different variable regions.

228

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

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

233

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

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

242

243 **Phenotypic and genotypic diversity of isolates from TPD patients compared to the** 244 **general population**

245 We compared the distribution of the IMD isolates from TPD patients with IMD
246 isolates from the French general population in the year 2008 and with carriage isolates from a
247 carriage study that was performed this same year [30]. The frequency of group B strains in
248 IMD was significantly lower in TPD patients than in the general French population (65.3% *vs*
249 30% respectively; $p < 0.0001$) and this percentage did not significantly differ from the
250 frequency of group B observed among carriage isolates (30% *vs* 24.5% respectively; $p = 0.43$)
251 (**Table 2**). The frequency of group Y in IMD was significantly higher in TPD patients when
252 compared to the general population. Half of the isolates from the carriage study were non-
253 groupable whereas in TPD patients with IMD, less than 5% of the isolates were non-
254 groupable. In the general population, the hyperinvasive lineages (cc11, cc32, cc41/44, and
255 cc269) are significantly overrepresented in collections of invasive isolates relative to

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

258

259 **DISCUSSION**

260

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

264

265 Nm is a human-specific bacterium carried by 10% of the population that can also be
266 responsible for life-threatening and fulminant invasive disease. A limited number of
267 hypervirulent lineages is responsible for the large majority of disease worldwide [2]. Patients
268 with hereditary TPD present a monogenic susceptibility to recurrent IMD [9]. Nm strains
269 infecting these patients are poorly documented limiting the improvement of disease
270 prevention. We show that the hyperinvasive lineages in TPD patients from our cohort
271 accounted for only 21% of IMD cases, which is similar to Nm carriage strains in upper
272 respiratory tract of healthy human carriers. The characteristics of invasive isolates from TPD
273 patients significantly differ from those of the general population. The 11 non-hyperinvasive
274 clonal complexes identified in the TPD patients such as cc35, cc167, cc213 or non-attributable
275 clonal complex are rarely associated with IMD in general population [2]. However, 95% of
276 the isolates from TPD patients are groupable suggesting that the isolates have the capacity to
277 express capsule and therefore display potential virulence. Nm Y:cc23 strains contribute to
278 26% of IMD in our TPD patients' cohort. In Italy as in several European countries, the
279 proportion of IMD cases due to group Y increased recently, ranging from 2% in 2007 to 17%
280 in 2013 [31]. High-resolution genetic analyses revealed extensive similarities between Nm Y
281 carriage-associated and disease associated organisms, indicating that all Nm Y and the cc23
282 circulating strains in the healthy population have the ability to cause the disease [32].
283 Therefore strains with low levels of virulence can be considered at risk for invasive disease in

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

289

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

306 A previous study including 21 patients with TPD showed no difference between the
307 distribution of strains infecting complement-deficient and complement-sufficient patients
308 [15]. However, this study has only used phenotypic or low discriminant genotypic techniques

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

311

312 It is recommended to vaccinate TPD patients with both tetravalent conjugate vaccines against
313 serogroups ACYW and with recombinant vaccine developed to target serogroup B isolates
314 [37]. However, in patients who lack the capacity to mediate meningococcal lysis by the MAC,
315 the adaptive humoral immunity is insufficient to provide full-effective protection [9, 38-41].
316 About half of TPD patients suffer from recurrent IMD with a variable interval between
317 episodes [9]. We illustrated a recurrence with the same strain after a 13 months interval and
318 an episode of IMD despite a high pre-existing serum bactericidal titer against the involved
319 strain. Among the 12 reported cases of IMD in 11 eculizumab-treated patients, two cases of
320 genuine vaccine failures (i.e. IMD with a group supposed to be covered by the previous
321 vaccination) have already been described [19, 21] (see **Supplementary Table 2**). Optimal
322 killing of Nm in blood depends on the MAC formation and effective IMD control in TPD
323 patients by vaccine remains controversial [42]. Chemoprophylaxis using penicillin V could
324 therefore be added to improve the protection of these patients. However, the proportion of
325 isolates with reduced susceptibility to penicillin represent 39%, which may eventually be
326 responsible for chemoprophylaxis failure. These observations underscore the need for
327 additional preventive measures among patients with primary or acquired TPD. Acquisition of
328 meningococci in the upper respiratory tract can be transient or lead to meningococcal carriage
329 but may also result in meningococcal disease. Carriage prevalence of Nm is generally higher
330 among household contacts of meningococcal patients [43] than in the general population.
331 Studies assessing the impact of household contacts screening for Nm isolates could open a
332 new strategy to reduce the risk of invasive infection in patients that lack functional terminal
333 complement pathway. In that sense, the large proportion of carriage-related strains observed

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

338

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

344

345 **NOTES**

346 **Potential conflicts of interest:** V.F-B. has received fees from Alexion Pharmaceuticals for
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355

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361

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464

465 **Table 1 – Demographic and clinical characteristics of TPD patients and of Nm strains**
 466 **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	19/49 (39)	4/9 (44)	3/15 (20)	9/18 (50)	2/6 (33)	1 (100)
G/Total number of tested strain (%)						

467 * data not available for one C7-deficient patient

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

	Disease isolates			Carriage isolates		
	TPD	General population		g1 vs g2	g1 vs g3	g2 vs g3
	g1	g2	g3			
	n	61	487			
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
N	5	0.6	48.9	0.01	<0.0001	<0.0001
G						
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

470

471 **Figure legend**

472

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

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

475

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

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

480

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

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

484

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

487 Data of invasive strains from the French general population and from carriage study
488 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
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

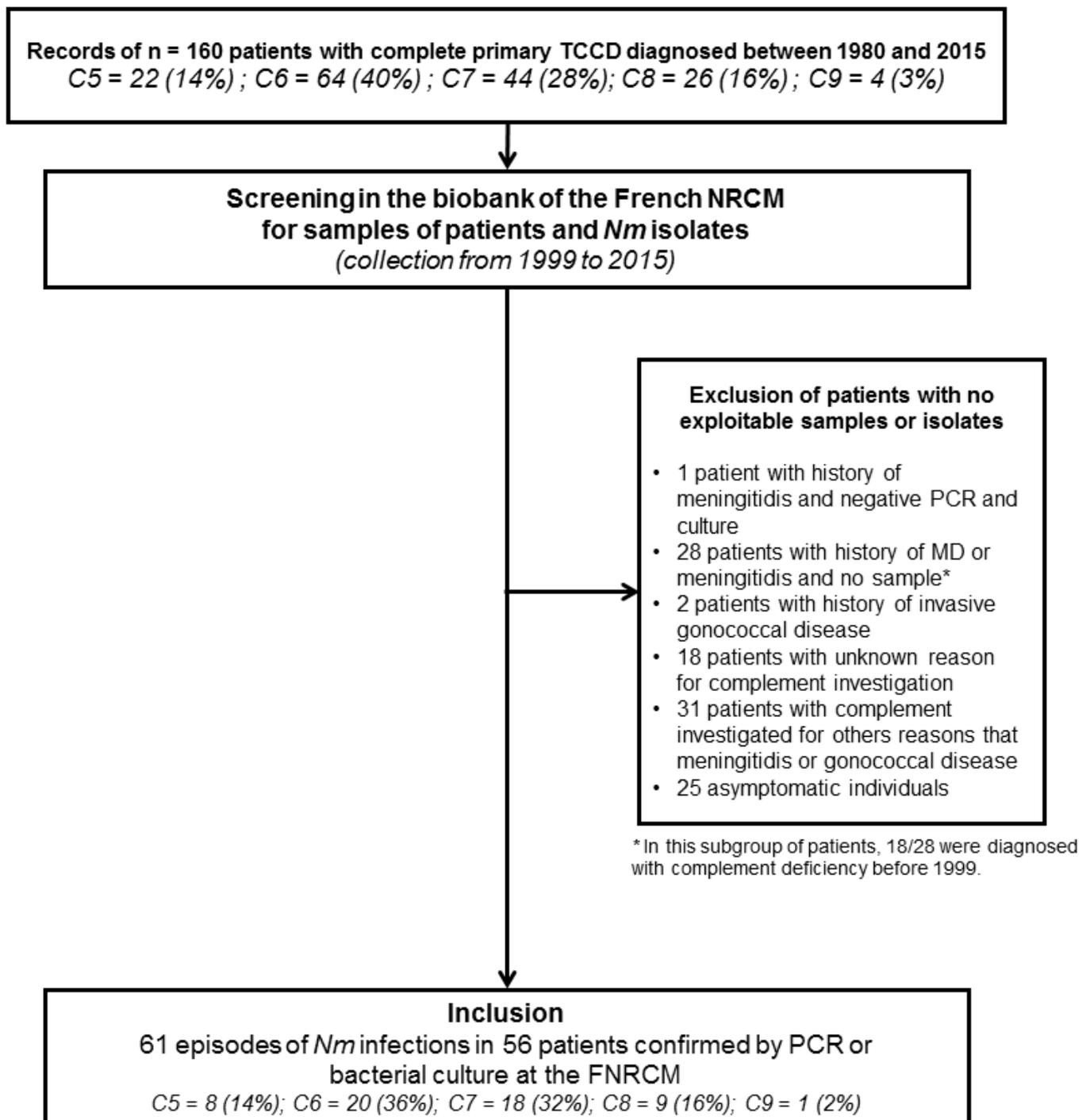
[§]One C7 deficient patient carried heterozygous c.1561C>A and c.2350 +2T>C variants within the same allele; [‡]retrieved from the Exome Aggregation Consortium [30]

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

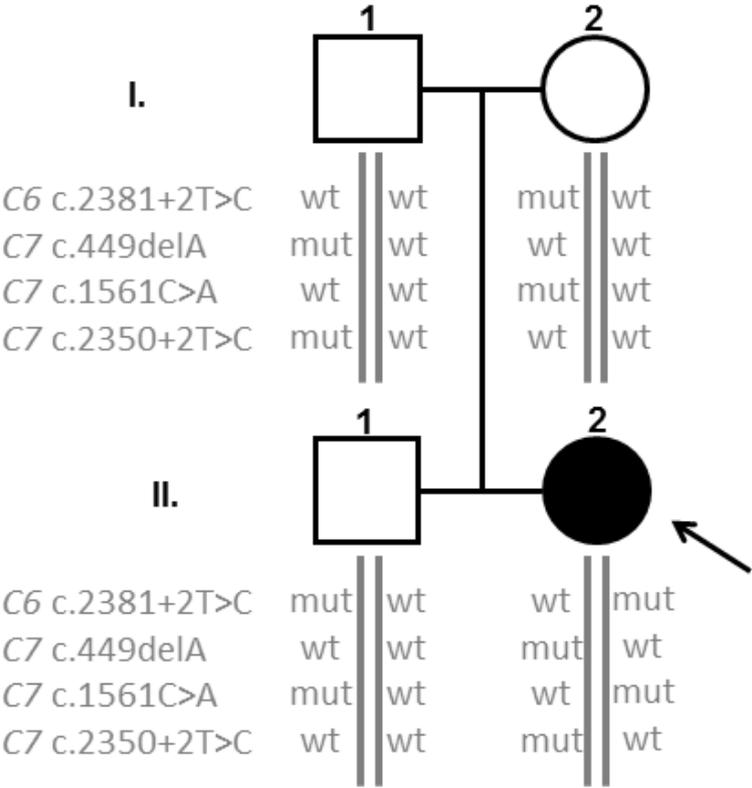
Indication	Age of IMD	Previous meningococcal vaccination	Serogroup	Outcome	Reference
PNH	24	ACYW*	B	Favorable	[31]
PNH	54	AC*	Y or W	Favorable	[31]
PNH	27	Unconjugated ACYW	X	Deceased	[32]
PNH	18 (first episode) 21 (second episode)	Conjugated ACYW Bexsero (2 doses)	B B	Favorable Favorable	[33]
PNH	41	Unconjugated ACYW	Undeterminate serogroup	NR	[34]
SLE	22	C* 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 = membranoproliferative glomerulonephritis; PNH = paroxysmal nocturnal hemoglobinuria; SLE = systemic lupus erythematosus ; NR = not reported

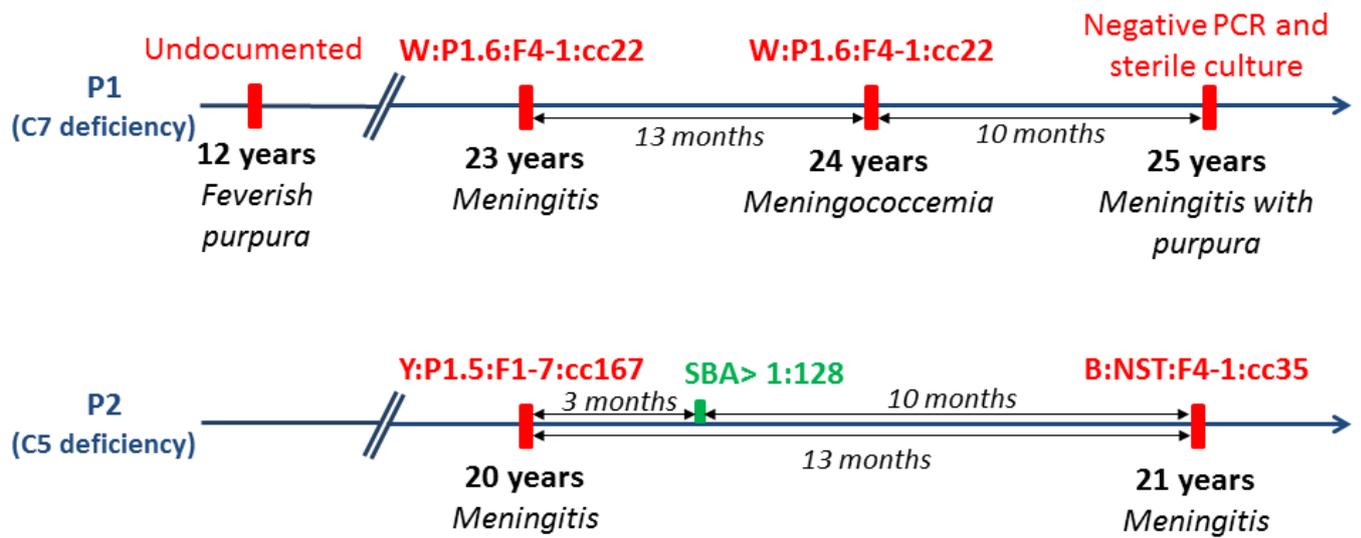
Supplementary Figure 1 - Flow-chart of the study



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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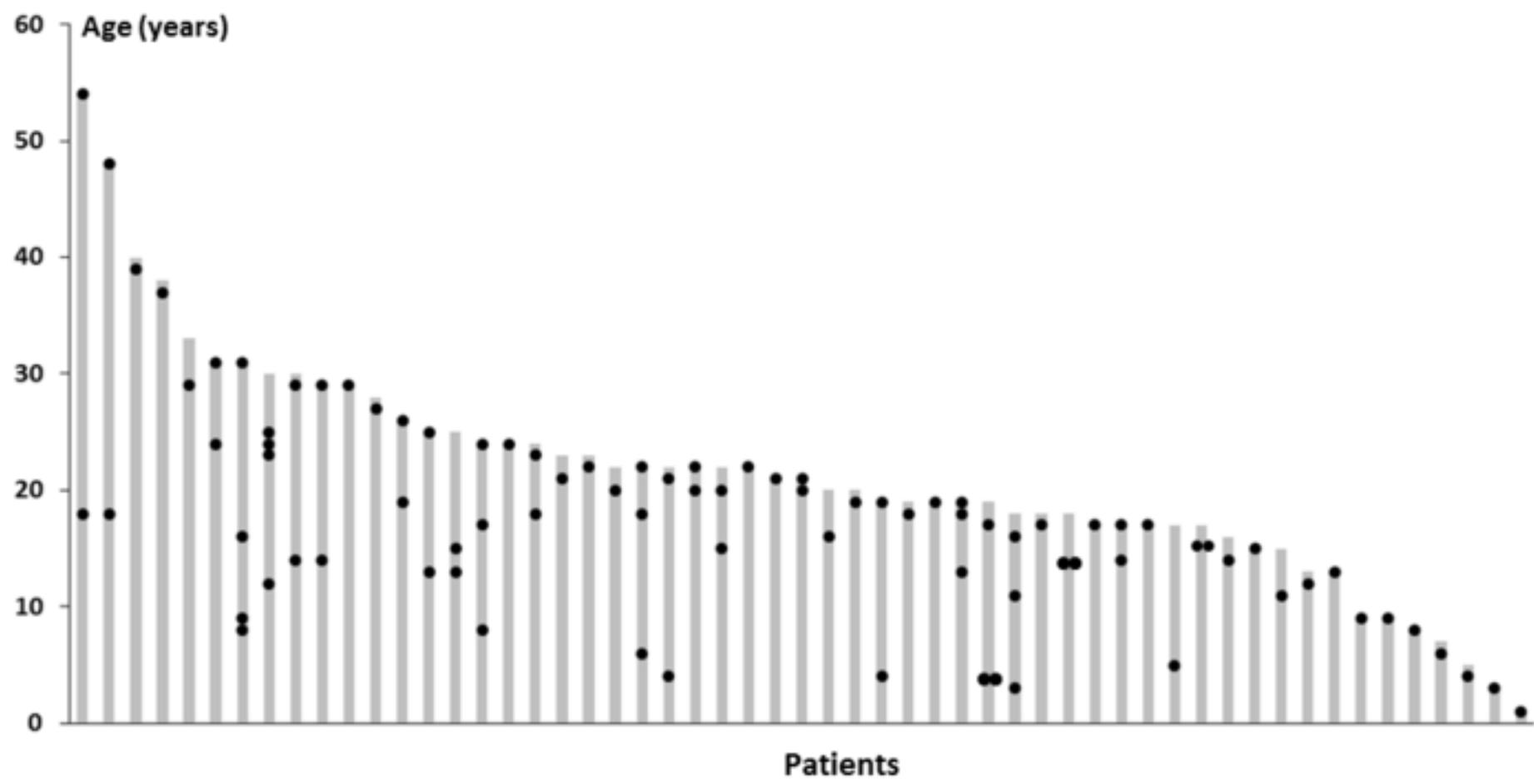


Figure2

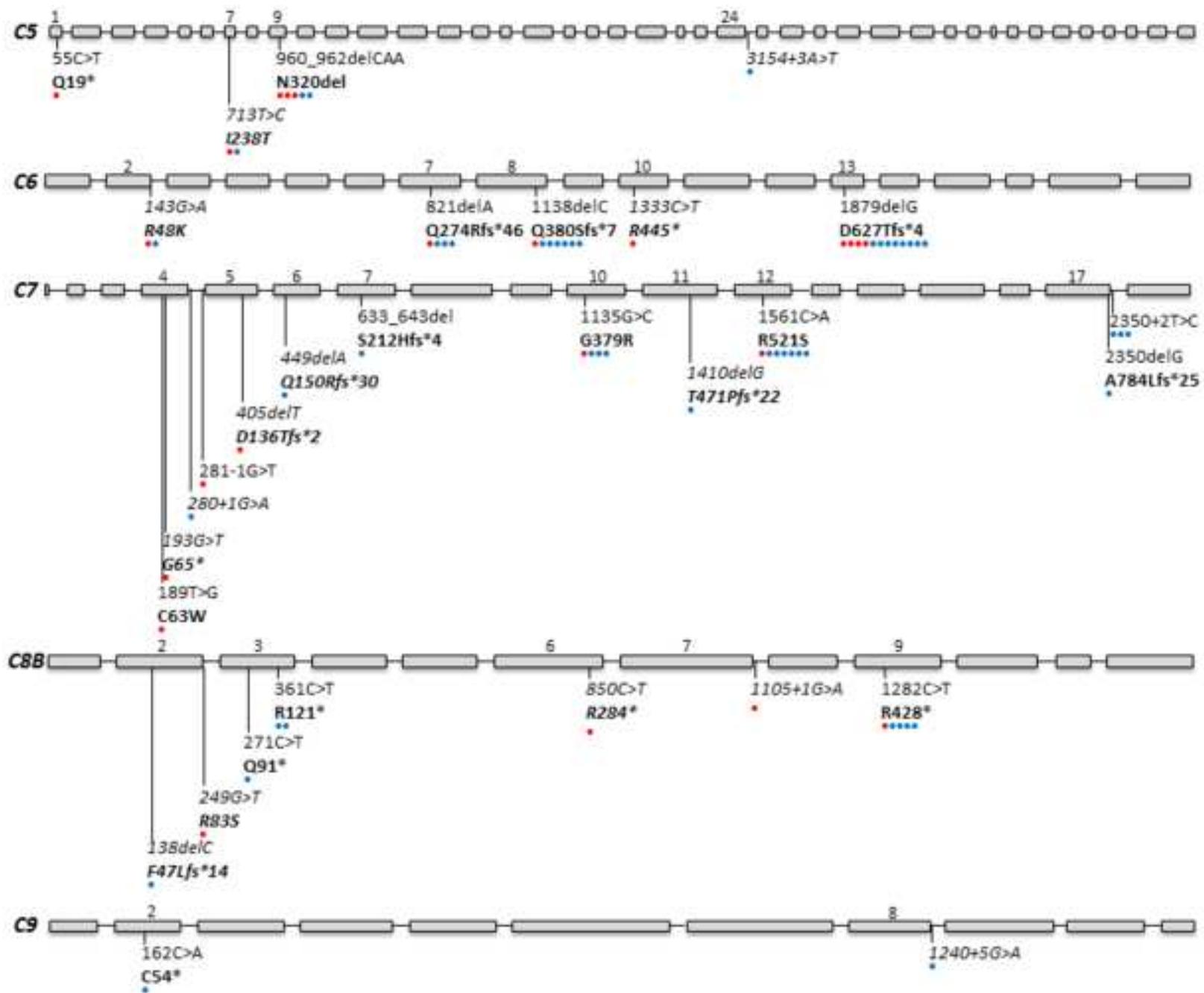


Figure3

