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TNFRSF13B/TACI Alterations in Greek Patients with Antibody Deficiencies

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Abstract *TNFRSF13B/TACI* defects have recently been associated with common variable immunodeficiency (CVID) pathogenesis. Considering that *TNFRSF13B/TACI* is very polymorphic and the frequency of its alterations may be different in various ethnic groups, we analyzed their prevalence in 47 Greek patients with antibody deficiencies, including CVID (16 patients), IgAD (16 patients), selective IgG4D (11 patients), and transient hypogammaglobulinemia of infancy (4 patients). A rather high frequency of *TNFRSF13B/TACI* defects was identified in patients with selective IgG4D (18.18%). Moreover, a patient with CVID was heterozygous in the common C104R mutation (6.25%). Both his children and a further healthy individual carried the same mutation, albeit without recurrent infections and/or hypogammaglobulinemia. The common polymorphisms V220A and P251L were identified in all disease subgroups, in an almost similar frequency with that observed in 259 healthy controls. Our data provide further evidence that *TNFRSF13B/TACI* alterations are not causative of CVID. Possibly, they predispose to humoral deficiencies and/or contribute to their phenotype when combined with other immune gene alterations.

Keywords *TNFRSF13B/TACI* · CVID · IgA deficiency · selective IgG subclass deficiency · transient hypogammaglobulinemia of infancy

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Introduction

Antibody deficiencies are the most common immunodeficiencies in humans. They are characterized by either low levels (hypogammaglobulinemia) or absence (agammaglobulinemia) of immunoglobulins in the peripheral blood, resulting in an increased susceptibility to infections by (mainly) encapsuled bacteria, as well as in an increased incidence of autoimmune diseases and lymphoproliferation [1, 2]. Nowadays, several genetic alterations leading to agammaglobulinemia, as well as class switch recombination (CSR) defects resulting in hypogammaglobulinemia of hyper-IgM syndromes, which occasionally mimic common variable immunodeficiency (CVID), have been demonstrated [3, 4]. On the contrary, the genetic alterations resulting in other common types of humoral immunodeficiencies remain unidentified. These types include CVID, selective IgA deficiency (sIgAD), subclass IgG deficiencies (selective or in combination with IgAD), specific antibody deficiency with normal Igs (SADNI), and transient hypogammaglobulinemia of the infancy (THI) [1, 2].

It is worth of note that the abovementioned antibody deficiencies display a large diversity considering their clinical and laboratory presentation. For example, sIgAD, the most common type of antibody deficiency in humans does not always result in overt disease, since only one third of the patients display an increased susceptibility to infections or

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autoimmunity [5, 6]. Likewise, the majority of the patients with selective subclass IgG deficiencies are asymptomatic, their diagnosis is usually made coincidently, and their clinical significance is still obscure [1, 2]. On the other hand, CVID is actually a term used to describe a heterogeneous group of disorders characterized by recurrent infections (mainly of the upper and lower respiratory tract) and a failure of antibody responses to vaccine antigens. Affected patients exhibit also a susceptibility to autoimmune diseases and cancer (especially, lymphoproliferative disorders and gastric cancer) [1, 7].

Recently, monoallelic (heterozygous) or biallelic (homozygous or compound heterozygous) alterations into *TNFRSF13B/TACI* gene have been demonstrated in approximately 10% of patients with CVID and sIgAD [8–10], and their presence seems to be correlated with an increased susceptibility to splenomegaly and autoimmunity [11, 12]. Indeed, in a recent large comprehensive analysis of 564 patients with CVID, Salzer et al. demonstrated that two alterations, namely C104R and A181E, seem to have a more profound effect and are more common in CVID patients compared to healthy controls [12]. However, *TNFRSF13B/TACI* alterations, including C104R and A181E, have also been detected in healthy individuals [11–14], indicating that *TNFRSF13B/TACI* variants may not be the causative, but rather represent a modifying factor influencing the severity and/or the phenotype of hypogammaglobulinemia. Moreover, considering that *TNFRSF13B/TACI* is very polymorphic, the incidence and the significance of *TNFRSF13B/TACI* alterations may be different in other ethnic groups, and such further studies may clarify its role in the pathogenesis of humoral deficiencies and/or their phenotype.

The aim of this study was to clarify, for the first time in the literature, the prevalence of *TNFRSF13B/TACI* alterations in Greek patients with antibody deficiencies in order to elucidate their role in disease pathogenesis and/or phenotype.

Methods

Patients

A cohort of 47 patients with antibody deficiencies was enrolled in the study. In particular, we evaluated 16 patients with CVID (male/female: 7/9, mean age at diagnosis: 26.4 years, range: 2–60), 16 with IgAD (12 with sIgAD and four accompanied by IgG4D; male/female: 7/9, mean age at diagnosis: 20.1 years, range: 4–79), 11 with sIgG4D (male/female: 5/9, mean age at diagnosis: 49.1 years, range: 4–80), and 4 with THI (male/female: 3/1, mean age at diagnosis: 9 months, range: 5–12). The majority of cases were sporadic, with the exception of three patients who exhibited a family history of immunodeficiency. Particularly, in the first family, two patients suffered from CVID (the proband and his niece) and three others from sIgG4D (a daughter and two nephews of the proband); in the second family, two patients suffered from CVID (the proband and her sister); and in the third, both twin brothers suffered from sIgAD. All family members with antibody deficiencies were also analyzed for *TNFRSF13B/TACI* alterations; thus, the total number of the analyzed patients was 53 (18 with CVID, 17 with IgAD, 14 with sIgG4D, and 4 with THI).

CVID diagnosed using standard criteria, including low levels of serum IgG, IgA, and/or IgM greater than 2 SD from the normal mean, absent or poor response to vaccines, and exclusion of other defined causes of hypogammaglobulinemia [15]. Nine out of the total 17 IgAD patients (52.9%) were diagnosed during evaluation of recurrent infections and/or autoimmunity and the rest 8 (47.1%) coincidently, including both twin brothers (third family) with sIgAD, who also suffered from COPD. Considering the 14 patients with sIgG4D, only 3 (21.4%) displayed recurrent infections, while the diagnosis for the rest (78.6%) was performed coincidently. Among them, a 71-year-old female displayed also sarcoidosis for 5 years, with pulmonary and ocular manifestations, and another 80-year-old male exhibited COPD. The diagnosis of sIgG4D for the above both patients was preformed coincidently. At the end, the diagnosis of THI was performed during evaluation of recurrent respiratory infections during infancy, and immunoglobulin levels were restored in all patients after the age of 2 years.

All CVID patients are subjected to immunoglobulin replacement therapy (intravenous or subcutaneous). With the exception of a patient with THI who needed replacement therapy, no other patient with IgAD, sIgG4, and THI received immunoglobulin treatment during follow-up. The clinical characteristics and the complications of the patients of the study are summarized in Table I.

A cohort of 259 individuals without history of recurrent infections and autoimmune disorders (male/female: 162/87; mean age: 69.2 years; range: 19–86) was served as healthy control group to detect the prevalence of *TNFRSF13B/TACI* variants in the general Greek population. Moreover, in healthy control group, the immunoglobulin levels, including IgG subclasses, were also measured in order to evaluate their possible correlations with common *TNFRSF13B/TACI* variants.

The study was approved by the institutional review board of the University Hospital of Larissa, and written informed consent was obtained from each individual or an accompanying relative, in the case of patients where consent was not legally applicable (e.g., with children).

Molecular Analyses

Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, UK), according

Table I Clinical characteristics and complications of the patients of the study

Clinical manifestations	CVID (*n*=18)	IgAD (*n*=17)	sIgG4D (*n*=14)	THI (*n*=4)
Recurrent infections				
Respiratory (*n*, %)	15 (83.3%)	5 (29.4%)	3 (21.4%)	4 (100%)
Gastrointestinal (*n*, %)	5 (27.7%)	1 (5.8%)		
Urinary (*n*, %)	3 (13.3%)	2 (11.5%)		
Splenomegaly (*n*, %)	5 (27.7%)			
Lymphadenopathy (*n*, %)	5 (27.7%)			
Granulomatous disease (*n*, %)	2 (11.1%)			
Autoimmunity (*n*, %)[a]	5 (27.7%)	5 (29.4%)		
Without symptoms (*n*, %)		8 (47.1%)	11 (78.6%)	
Complications				
Bronchiectasis	4 (22.2%)			
COPD	1 (5.6%)	2 (11.5%)		
CRPD	2 (11.1%)			
Hardness of hearing	1 (5.6%)			
Hypersplenism	1 (5.6%)			
Failure to thrive (*n*, %)		1 (5.8%)		
Neoplasia (*n*, %)[b]	2 (11.1%)			

COPD chronic obstructive pulmonary disease, *CRPD* chronic restrictive pulmonary disease, *CVID* common variable immunodeficiency, *IgAD* IgA deficiency (including 13 patients with selective IgAD and 4 with combination of IgA and IgG4 deficiencies), *sIgG4D* selective IgG4 deficiency, *THI* transient hypogammaglobulinemia of infancy

All family members were included (total number 54) since they displayed variable clinical phenotypes (especially those with CVID)

[a] The autoimmune manifestations of CVID patients were arthritis, recurrent idiopathic thrombocytopenic purpura/ITP, atopic dermatitis, diabetes mellitus type I, thyroiditis with hypothyroidism. Those of IgAD were recurrent ITP, sprue and thyroiditis, diabetes mellitus type I, Raynaud syndrome, and arthritis with uveitis and iridocyclitis

[b] Both patients developed Hodgkin disease 7 and 13 years after diagnosis

to manufacturer's instructions. Afterward, an amplification of all exons of *TNFRSF13B/TACI* gene was performed. The position and the sequences of the utilized primers, designed with the aid of the Oligo-6 software (NBI, Plymouth, MN, USA), as well as the conditions of PCR reactions, are shown in Table II. For each PCR reaction, a total of 100–200 ng of genomic DNA was amplified in a 30-µl reaction using 62.5 µM of each deoxynucleotide triphosphate, 20 pmol of each primer, 1.5 mM MgCl2, and 0.8 U Taq Polymerase (Invitrogen, UK) in a buffer supplied by the manufacturer. All PCR procedures were carried out in the PCR-engine apparatus PTC-200, MJ-Research (Watertown, Massachusetts), and the PCR products were analyzed in 2% TBE agarose gels, stained with ethidium bromide, and visualized under UV light.

Afterward, PCR products were purified by the QIAquick gel extraction kit (Qiagen) and directly sequenced using an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and a Big Dye Terminator DNA sequencing kit (Applied Biosystems).

Based on the later results, allele-specific PCR-RFLP (restriction fragment length polymorphism) protocols, for the rapid detection of *TNFRSF13B/TACI* mutations C104R, V220A, and P251L, were designed. In particular, for the detection of *TNFRSF13B/TACI*-C104R mutation in exon 3, the reverse primer (Table II) was modified at the 3′ end, creating a recognition site for the restriction enzyme BtgI, so that if a polymorphism was present, PCR-RFLP analysis would create digestion fragments, visible on agarose gels. Thus, a 337-bp fragment was amplified by PCR (Table II) and subjected to BtgI digestion (New England Biolabs, UK) for 4 h at 37°C. Undigested PCR products were indicative of wild-type samples, whereas the presence of the mutation resulted in the digestion of the PCR products to 316- and 21-bp fragments.

For the detection of *TNFRSF13B/TACI*-V220A and *TNFRSF13B/TACI*-P251L mutations in exon 5, the forward primer (Table II) was modified at the 3′ end, creating a recognition site for the restriction enzyme BveI. Thus, the 252-bp product amplified by PCR (Table II) was subjected to BveI (Fermentas, St. Leon-Rot, Germany) digestion for 16 h at 37°C. Due to the presence of another BveI restriction site, the wild-type PCR products digested to 192- and 60-bp fragments, while the presence of the V220A mutation resulted in the digestion of the product to 192-, 60-, 31-, and 29-bp fragments (heterozygous), or 192-, 31-, and 29-bp fragments (homozygous). At the end, the presence of the P251L polymorphism results in the

Table II Primers and PCR conditions used in this study

Exons	Primers	Sequence	PCR conditions	PCR product
1	1-forward 1-reverse	5′-TGCCTTTCATATAGCCATTG-3′ 5′-AGTCATTTTGCCTTTCATCTTT-3′	94°C for 2 min, followed by 31 cycles (94°C for 30 s, 62°C for 30 s, 72°C for 30 s) and a final elongation at 72°C for 5 min	340 bp
2	2-forward 2-reverse	5′-GCAACAGAGATGACTTTATGCATTGA-3′ 5′- CTGCATCTAAGTGAACTCCAA -3′	94°C for 2 min, followed by 30 cycles (94°C for 30 s, 64°C for 30 s, 72°C for 30 s) and a final elongation at 72°C for 5 min	319 bp
3	3-forward 3-reverse	5′-AAGCCCTTTCTATAAACCACATT-3′ 5′-TGTCTCCCTGTTGGTCAAA-3′	94°C for 2 min, followed by 31 cycles (94°C for 30 s, 62°C for 30 s, 72°C for 30 s) and a final elongation at 72°C for 5 min	635 bp
3[a]	3-reverse-CR	5′-GCTCCTGAGCTTGTTC(T)CCAC-3′	94°C for 2 min, followed by 32 cycles (94°C for 30 s, 55°C for 30 s, 72°C for 45 s) and a final elongation at 72°C for 5 min	337 bp
4	4-forward 4-reverse	5′-TGGGGGATATTCTTTTTGGTC-3′ 5′-TCTAGAATTAGGCCTTGGAGATGTT-3′	94°C for 2 min, followed by 35 cycles (94°C for 30 s, 60°C for 30 s, 72°C for 30 s) and a final elongation at 72°C for 5 min	540 bp
5	5-forward 5-reverse	5′-GTGTATGAAAGGCAATGGAGAGG-3′ 5′-AGAATGCTGGCCCATTAAAGATGAT-3′	94°C for 2 min, followed by 30 cycles (94°C for 30 s, 55°C for 30 s, 72°C for 30 s) and a final elongation at 72°C for 5 min	717 bp
5[b]	5-forward-VP 5-reverse-VP	5′-ACGCGATGGAAGCCGGCAG(C)ACCTG-3′ 5′-CATTTATGCACCTGGGCCCCCCTC-3′	94°C for 2 min, followed by 30 cycles (94°C for 30 s, 68°C for 30 s) and a final elongation at 72°C for 5 min (2-step PCR)	252 bp

[a] The primers 3-reverse-CR and 3-forward were used for the detection of C104R mutation by PCR-RFLP as described in "Methods" section

[b] The primers for this subregion of exon 5 were used for the detection of V220A and P251L polymorphisms by PCR-RFLP as described in "Methods" section. The nucleotides in the brackets of the primers 3-reverse-CR and 5-forward-VP were modified and changed to the underlined ones

creation of a DNA sequence recognized by the restriction enzyme MnlI (New England Biolabs). After the digestion of PCR products at 37°C for 16 h, the undigested ones were indicative of wild-type samples, whereas the presence of the polymorphism resulted in the digestion to 134- and 118-bp fragments.

Statistical Analysis

Chi-square ($\chi 2$) test with Yates' correction was used to compare the allele and genotype frequencies of C104R, V220A, and P251L mutations between patients and healthy controls, while Fisher's exact test was performed when needed. Linear correlation studies were performed using the nonparametric Spearman's correlation coefficient (r) when appropriate. A variable was considered significant when $p < 0.05$. The above tests were performed using the Statistical Package for Social Sciences (SPSS version 10, Chicago, IL, USA).

Results

Prevalence of *TNFRSF13B/TACI* Mutations in Patients with Antibody Deficiencies and Healthy Controls

Four missense mutations, an insertion, three silent polymorphisms, and three intronic alterations, already described in the literature, were detected in the patients of the study (Table III, Fig. 1). In particular, a patient with CVID displayed the C104R mutation (rs34557412), another with sIgG4D carried the A181E mutation (rs72553883), and a third with sIgG4D the frameshift alteration c.204insA (p. L69TfsX12) (rs72553875). Moreover, another patient with CVID carried the polymorphic variant V220A (rs56063729), while the other common variant P251L (rs34562254) was present in 11 patients (3 with CVID, 5 with sIgAD, 2 with sIgG4D, and 1 with THI). All patients were heterozygotes for the above alterations, with the exception of a patient with sIgAD who was homozygous in the P251L mutation. The allele frequency of the silent mutations 27T (rs8072293), 97P (rs35062843), and 277S (rs11078355) and the intronic polymorphisms 24625A>C (rs2274892), 33402T>G (rs11652843), and 33482T>C (rs11652811) are presented in Table III.

Pedigrees of the three families with antibody deficiencies and the molecular defects identified in the affected members are presented in Fig. 2. Among these patients, the proband of the first family and both the proband and her sister of the second family were heterozygotes for the P251L polymorphism (Fig. 1). Moreover, in the first family, a 6-year-old patient with sIgG4D carried the R202H mutation (rs104894649), which was absent from the other

Table III Allele frequency (percentage) of *TNFRSF13B/TACI* alterations in the patients of the study

	Number	Functional polymorphisms					Synonymous polymorphisms			Intronic alterations[a]		
		C104R	A181E	p.L69TfsX12	V220A	P251L	27T	97P	277S	24625A > C rs2274892	33402T > G rs11652843	33482T > C rs11652811
CVID	16	3.13	0	0	3.13	9.38	65.63	0	31.25	43.75	40.63	37.50
IgAD	16	0	0	0	0	18.75	56.25	0	43.75	43.75	53.13	53.13
sIgG4D	11	0	4.55	4.55	0	9.09	72.73	4.55	31.82	40.91	40.91	40.91
THI	4	0	0	0	0	12.50	75.00	0	50.00	50.00	25.00	25.00
Total	47	1.06	1.06	1.06	1.06	12.77	64.89	1.06	37.23	43.62	43.62	42.55

CVID common variable immunodeficiency, *IgAD* IgA deficiency (including 13 patients with selective IgAD and 4 patients with IgAD and IgG4 subclass deficiency), *sIgG4D* selective IgG4 deficiency, *THI* transient hypogammaglobulinemia

[a] The *TNFRSF13B/TACI gene* numbering is according to GenBank accession number AB22299

family members, since it was inherited from his asymptomatic father (Fig. 2).

Afterward, PCR-RFLP analyses demonstrated the prevalence of the C104R, V220A, and P251L mutations in a healthy Greek population (allele frequencies: 0.19%, 4.63%, and 16.41%, respectively). In particular, among the 259 analyzed healthy individuals, 6 were homozygotes for the P251L, 1 was homozygous for the V220A, and 5 compound heterozygotes for both polymorphisms. Interestingly, a 75-year-old healthy individual was heterozygous for the C104R mutation, and he did not have any medical history indicative for immunodeficiency. At the end, no significant differences were identified regarding the prevalence of the abovementioned polymorphisms between patients with antibody deficiencies and healthy controls ($p=0.284$ for the C104R, $p=0.283$ for the V220A, and $p=0.610$ for the P251L).

Examples of direct sequencing and PCR-RFLP analyses showing the presence of the abovementioned *TNFRSF13B/TACI* alterations are presented in Fig. 1.

Clinical Correlations of *TNFRSF13B/TACI* Mutations

Both sIgG4D patients (71 and 69 years old, respectively) who carried the *TNFRSF13B/TACI* mutations A181E and p.L69TfsX12 were diagnosed coincidently. They did not have any history of recurrent infections or autoimmunity, while their family history was not indicative for immunodeficiency. Moreover, the R202H mutation observed in a patient with sIgG4D and a family history of humoral immunodeficiency was absent from the other family members (with CVID or sIgG4D) and was inherited to him from his asymptomatic father.

A patient carrying the C104R mutation was diagnosed with CVID 5 years after the onset of his symptoms (at the age of 38 years). At diagnosis, he also displayed granulomatous disease and splenomegaly, and 2 years later, he developed Hodgkin disease. Interestingly, both his children (analyzed at the age of 22 and 14 years old, respectively) carry the same mutation. None of them, however, has a history of recurrent infections, while the younger displays a slight hypogammaglobulinemia (IgG levels: 808 mg/dl).

Furthermore, the presence of any *TNFRSF13B/TACI* variant was not correlated with the type of recurrent infections, the presence of splenomegaly or lymphadenopathy at diagnosis, the presence of autoimmune manifestations or granulomatous disease, and the development of neoplasia ($p>0.05$ in all cases). Further analysis in patients with antibody deficiencies (as a whole, but also in subgroups) did not reveal any significant correlation of the variants V220A, P251L, rs8072293, rs35062843, rs2274892, rs11652843, and rs11652811 with the absolute number of leucocytes, T cells (and their subpopulations), B

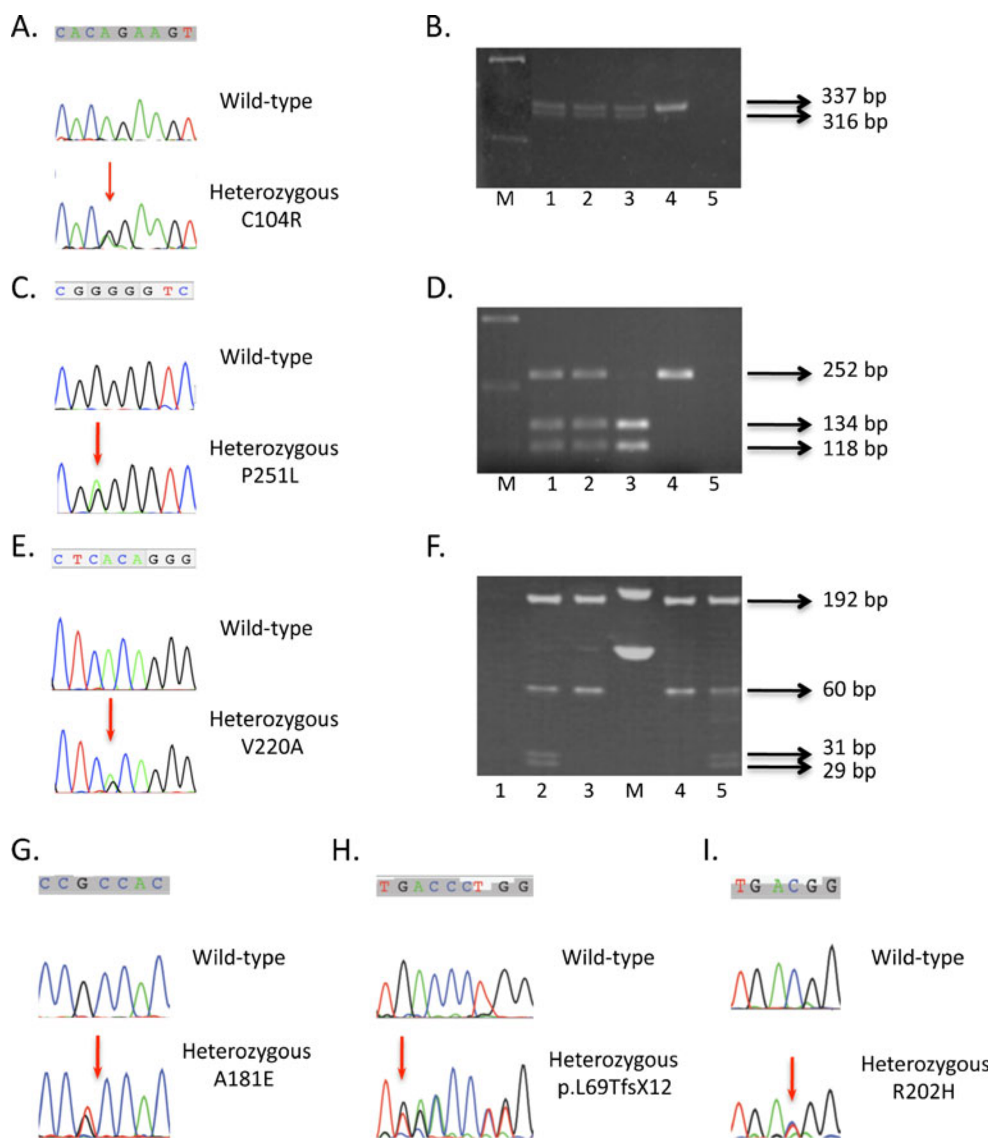

Fig. 1 The detected frameshift alteration and missense mutations of *TNFRSF13B/TACI* gene in Greek patients with antibody deficiencies. **a** Sequencing analysis of a patient with the C104R mutation. **b** Representative digestion showing the C104R mutation. *M* 200-bp ladder molecular weight marker (Invitrogen, UK). *Lanes 1–3* heterozygotes for the C104R mutation, *lane 4* sample without the mutation (*wild-type*), *lane 5* negative PCR control. The samples were run on 2% agarose gel, and the 21-bp fragments were not visible. **c** Sequencing analysis of a patient with the P251L mutation. **d** Representative digestion showing the P251L mutation. *M* 200-bp ladder molecular weight marker (Invitrogen, UK). *Lanes 1* and *2* heterozygotes for the P251L mutation, *lane 3* sample homozygous, *lane 4* wild-type sample, *lane 5* negative PCR control. The samples were run on 2% agarose gel. **e** Sequencing analysis of a patient with the V220A mutation. **f** Representative digestion showing the V220A mutation. *M* 80-bp ladder molecular weight marker (Invitrogen, UK). *Lane 1* negative PCR control, *lane 1* and *5* samples heterozygous for the V220A mutation, *lanes 3* and *4* wild-type samples. The samples were run on 6% acrylamide gel. **g** Sequencing analysis of the patient with the A181E mutation. **h** Sequencing analysis of the patient with the c.204insA (p.L69TfsX12) frameshift alteration. **i** Sequencing analysis of the patient with the R202H mutation

cells (and their subpopulations), NK cells, monocytes, and immunoglobulin levels in the peripheral blood at diagnosis.

In healthy control group, the immunoglobulin levels, including IgG subclasses, were also evaluated (IgG mean± SD: 1,112.3±249.8, range: 553.0−1,700.0 mg/dl; IgM: 94.2± 77.8, 20.2–330.0 mg/dl; IgA: 249.6±120.7, 81.6–482.0 mg/dl; IgG1: 662.9±168.5, 316.0–1,570.0 mg/dl; IgG2: 351.5± 122.9, 92.5–822.0 mg/dl; IgG3: 53.2±26.1, 11.4–156.0 mg/dl; IgG4: 79.1±73.0, 5.2–417.0 mg/dl), but no correlation between them and the presence of the common polymorphisms V220A and P251L was observed.

Finally, the presence of the 277S (rs11078355) polymorphism was found negatively correlated with the percentage and the absolute number of the peripheral $IgD^{+}CD27^{+}$ B cells (r=−0.591, p=0.001), most strongly among CVID patients (r=−0.841, p=0.001). In CVID

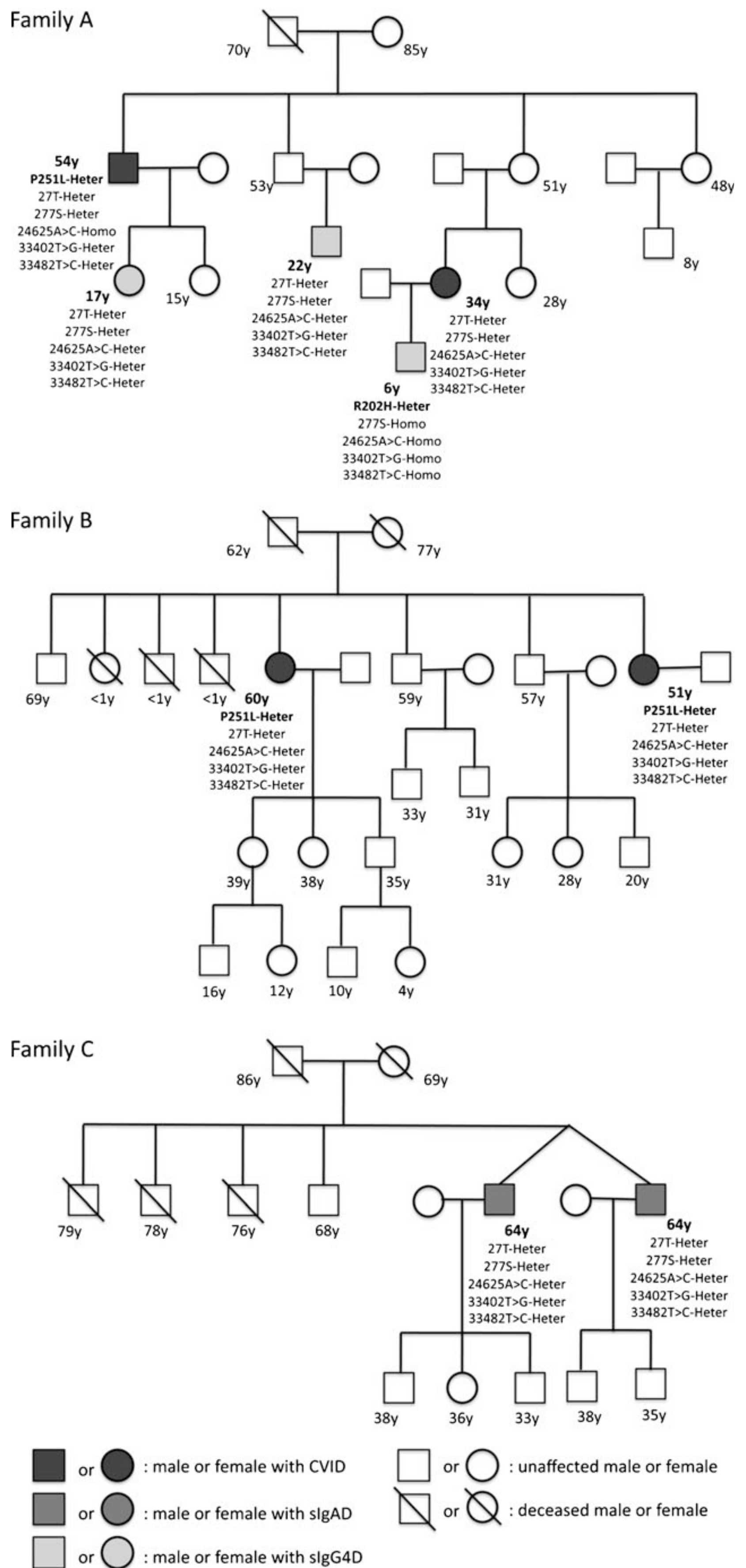

Fig. 2 Pedigrees of the families with antibody deficiencies and the molecular defects of *TNFRSF13B/TACI* gene identified in the affected members. *CVID* common variable immunodeficiency, *sIgAD* selective IgA deficiency, *sIgG4D* selective IgG4 deficiency

patients, the above polymorphism was also negatively correlated with the absolute B cell number ($r=-0.586$, $p=0.028$) and the IgA levels ($r=-0.600$, $p=0.014$). At the end, we observed that IgAD patients presenting with recurrent infections displayed a higher prevalence of P251L and rs2274892 SNPs, compared with those diagnosed coincidently (Table IV); however, the number of the analyzed patients was very low (5 and 7 patients, respectively) to perform statistical analysis.

Discussion

For the first study in the literature, we studied the prevalence of *TNFRSF13B/TACI* alterations in Greek patients with antibody deficiencies. It was revealed that Greek patients display several of the already described common alterations of *TNFRSF13B/TACI* gene. Their prevalence in CVID patients was found somewhat lower (one out of 16 patients, 6.25%) but comparable to that observed in other Caucasian populations. Pathogenic *TNFRSF13B/TACI* alterations were absent in patients with IgAD (selective or in combination with other IgG subclass deficiencies) and THI, while their prevalence in patients with sIgG4D was rather high (18.18%). It is worth of note that this is the second study analyzing the prevalence of *TNFRSF13B/TACI* alterations in patients with subclass IgG deficiencies (in particular, sIgG4D), considering that in the first one no clear data for their prevalence were provided [12]. Moreover, we demonstrated that the variants V220A and P251L were common both in patients with antibody deficiencies and healthy controls, similarly with previous studies [8, 9, 12]. At the end, the increased frequency of recurrent infections observed in our patients with sIgAD carrying the P251L and rs2274892 SNPs needs further elucidation.

Previous studies have demonstrated that some *TNFRSF13B/TACI* alterations have a direct effect on B cell function in vitro [16], but do not always results in immunodeficiency in vivo. Moreover, the clinical severity and the B cell phenotype of patients carrying *TNFRSF13B/TACI* alterations, especially of those with CVID, are quite variable [11–14]. Salzer et al. have suggested that heterozygous mutations in *TNFRSF13B/TACI* represent susceptibility factors for CVID, while biallelic alterations predictably cause disease [12]. However, some individuals with *TNFRSF13B/TACI* alterations on both alleles have only mildly decreased serum immunoglobulins, without overt disease [3]. In this context, we consider that our data provide further evidence that *TNFRSF13B/TACI* alterations are indeed not causative of CVID, but they can be considered as susceptibility or modifying factors. Coincident alterations in other immune genes might modify their effect resulting occasionally in the emergence of a pathologic condition or affecting its phenotype and/or severity [3]. In support of this hypothesis are the detection of C104R mutation in healthy individuals (relatives of a proband or not) without any clinical or laboratory sign of immunodeficiency, the presence of R202H mutation in a patient with sIgG4D that was absent in his mother suffering from CVID (Fig. 2), and finally the presence of alterations that could lead to a severe immune defect (e.g., the p.L69TfsX12 defect, which results in the premature truncation of TACI) but coincidently were identified in patients with sIgG4D.

Table IV *TNFRSF13B/TACI* alterations and clinical manifestations of 16 patients with IgAD

	Number (%)	Functional polymorphisms allele frequency (%), (hetero-, homozygotes)	Silent polymorphisms allele frequency (%), (hetero-, homozygotes)		Intronic alterations allele frequency (%), (hetero-, homozygotes)		
		P251L rs34562254	27 T rs8072293	277 T rs11078355	24625A > C rs2274892	33402 T > G rs11652843	33482 T > C rs11652811
Recurrent infections	5 (31.3)	40.0 (2, 1)	60.0 (4, 1)	40.0 (2, 1)	70.0 (1, 3)	60.0 (2, 2)	60.0 (2, 2)
Respiratory	5 (31.3)	40.0 (2, 1)	60.0 (4, 1)	40.0 (2, 1)	70.0 (1, 3)	60.0 (2, 2)	60.0 (2, 2)
Urinary[b]	2 (12.5)	25.0 (1, 0)	50.0 (2, 0)	25.0 (1, 0)	100.0 (0, 2)	75.0 (1, 1)	75.0 (1, 1)
Gastrointestinal[b]	1 (6.3)	50.0 (1, 0)	50.0 (1, 0)	50.0 (1, 0)	100.0 (0, 1)	100.0 (0, 1)	100.0 (0, 1)
Autoimmunity[c]	5 (31.3)	12.5 (1, 0)	40.0 (4, 0)	40.0 (2, 1)	50.0 (1, 2)	50.0 (3, 1)	50.0 (3, 1)
Failure to thrive[b]	1 (6.3)	50.0 (1, 0)	50.0 (1, 0)	100.0 (0, 1)	0 (0, 0)	100.0 (0, 1)	100.0 (0, 1)
Without symptoms[a]	7 (43.8)	7.1 (1, 0)	64.2 (3, 3)	42.8 (4, 1)	21.4 (3, 0)	50.0 (3, 2)	50.0 (3, 2)
Total	16	18.8 (4, 1)	56.3 (10, 4)	43.8 (8, 3)	40.6 (5, 4)	53.1 (7, 5)	53.1 (7, 5)

[a] For the allele frequencies of *TNFRSF13B/TACI* alterations, only one from the twin brothers with sIgAD was evaluated

[b] These patients displayed also recurrent respiratory infections

[c] The autoimmune manifestations of IgAD patients were presented in Table I (only one patient with combined IgAD and IgG4D displayed both recurrent respiratory and urinary infections along with autoimmunity—Raynaud syndrome)

As mentioned above, we demonstrated a rather high frequency (18.18% for the initial 11 and 21.43% for the total 14 analyzed) of *TNFRSF13B/TACI* alterations in sIgG4D patients. IgG subclass deficiencies can be established if one or more IgG subclasses are 2 SDs below the normal mean for age [1, 17]. They are considered rather benign immune defects, since the affected patients are usually asymptomatic [1, 17]. Symptomatic patients usually suffer from recurrent sinopulmonary bacterial infections, while an association with atopy and autoimmune diseases has also been reported, similar to IgA deficiencies [1]. There are limited data in the literature about their frequency and pathogenesis, since only immunoglobulin heavy-chain deletions have been implicated for their emergence [17, 18]. It is noteworthy that the role of *TNFRSF13B/TACI* alterations in their pathogenesis and/or phenotype is still unknown. Interestingly enough, we demonstrated that *TNFRSF13B/TACI* defects are rather common in sIgG4D and higher than CVID. Indeed, the type of some alterations were indicative of a severe protein dysfunction, since the p. L69TfsX12 results in a premature truncation, while the A181E mutation has been attributed as a severe risk factor for the development of CVID [12, 13]. It is worth of note that TACI-deficient mice displayed a reduction of all IgG subclass responses after vaccination with a thymus-independent antigen [19]. Moreover, Bacchelli et al. demonstrated a reduction of IgG subclass levels, especially those of IgG2b and IgG1, in a TACI knock-in (C76R, equivalent of human C104R) animal model [20], indicating that *TNFRSF13B/TACI* defects in vivo may result mainly in subclass deficiencies, strengthening the findings of our study. In any case, our data might be in accordance to the Moreli's old assumption that "IgG subclass deficiencies represent an indicator of more basic immunologic abnormalities" [21].

Conclusions

TNFRSF13B/TACI alterations were observed in Greek patients with CVID in a rather similar frequency as in other Caucasian populations. However, the presence of *TNFRSF13B/TACI* defects in healthy individuals, as well as their rather high frequency in patients with IgG4 subclass deficiency, indicates that possibly, they represent genetic modifiers predisposing, when combined with other (genetic or environmental) factors, to the development and/or the phenotype of overt immunodeficiencies.

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