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Phenotype, penetrance, and treatment of 133 CTLA-4-insufficient individuals

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Abstract:	Background Cytotoxic-T-lymphocyte-antigen-4 (CTLA-4) is a negative immune regulator. Heterozygous CTLA4 germline mutations can cause a complex immune dysregulation syndrome in humans. Objective To characterize the penetrance, the clinical features and the best treatment options in 133 CTLA4 mutation carriers. Methods Genetics, clinical features, laboratory values, and outcome of treatment options were assessed in a worldwide cohort of CTLA4 mutation carriers. Results We identified 133 individuals from 54 unrelated families carrying 45 different heterozygous CTLA4 mutations, including 28 previously undescribed mutations. Ninety mutation carriers were considered affected, suggesting the clinical penetrance of at least 67%; median age of onset was 11 years, and mortality rate within affected mutation carriers was 16% (n=15). Main clinical manifestations included hypogammaglobulinemia (84%), lymphoproliferation (73%), autoimmune cytopenia (62%), respiratory- (68%), gastrointestinal- (59%), or neurological features (29%). Eight affected mutation carriers developed lymphoma, three gastric cancer. An EBV association was found in six malignancies. CTLA4 mutations were associated with lymphopenia and decreased T-, B-, and NK-cell counts. Successful targeted therapies included the application of CTLA-4-fusion-proteins, mTOR-inhibitors, and hematopoietic stem cell transplantation. EBV reactivation occurred in two affected mutation carriers under immunosuppression. Conclusions Affected mutation carriers with CTLA-4 insufficiency may present in any medical specialty. Family members should be counseled, as disease manifestation may occur as late as age 50. EBV- and CMV-associated complications must be closely monitored. Treatment interventions should be coordinated in clinical trials.

Responses to comments (lines mentioned in the responses correspond to unmarked manuscript)

EDITOR'S SPECIFIC COMMENTS:

The authors have significantly improved the manuscript. Two minor comments need to be addressed.

Please modify:

1. line 454. State the number of patients that had elevated Tregs. Please present Treg data similar to the other T cell subsets with a normal range as shaded area. The authors might not conclude that this high Tregs percentage is an attribute of this cohort unless many measurements in the same individual establish as a characteristic rather than a transient finding, as stated in the discussion line 590.

Response: We included a sentence, saying that eight mutation carriers had elevated Tregs (Line 456-457). We changed the layout in Figure S2 as suggested. We inserted into the discussion, that the elevation of regulatory T cells could be a transient or permanent finding (Line 590-592).

2. line 457. Please state double negative T cells are TCRalpha beta or gamma delta. If no information, consider omit this data, or discuss in light that this is a parameter that brings up proposed ALPS diagnostic criteria.

Response: We inserted a comment and a reference into the discussion (Line 594-595).

COMMENTS FROM THE EDITORIAL OFFICE:

1. Please note that the supplemental Tables have a grey or other color element to them. All tables must appear in strictly black and white. Please remove any other color elements from these.

Response: We removed colors from all supplemental tables.

2. Please collect and submit separate Conflict of Interest Disclosure statements for each author who is listed on the title page, using the form found on the Journal's submissions site. You can download the form directly from http://ees.elsevier.com/jaci/img/forms.html. Please send all the COI statements in a zipped file to mmweist@gmail.com. Please do not include any other files in this zipped file.

Response: We are collecting the Conflict of interest Disclosure statements from each author and will submit them as soon as possible.

Phenotype, penetrance, and treatment of 133 CTLA-4-insufficient individuals

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Abstract

Background

Cytotoxic-T-lymphocyte-antigen-4 (CTLA-4) is a negative immune regulator. Heterozygous *CTLA4* germline mutations can cause a complex immune dysregulation syndrome in humans.

Objective

To characterize the penetrance, the clinical features and the best treatment options in 133 *CTLA4* mutation carriers.

Methods

Genetics, clinical features, laboratory values, and outcome of treatment options were assessed in a worldwide cohort of *CTLA4* mutation carriers.

Results

We identified 133 individuals from 54 unrelated families carrying 45 different heterozygous *CTLA4* mutations, including 28 previously undescribed mutations. Ninety mutation carriers were considered affected, suggesting the clinical penetrance of at least 67%; median age of onset was 11 years, and mortality rate within affected mutation carriers was 16% (n=15).

Main clinical manifestations included hypogammaglobulinemia (84%), lymphoproliferation (73%), autoimmune cytopenia (62%), respiratory- (68%), gastrointestinal- (59%), or neurological features (29%). Eight affected mutation carriers developed lymphoma, three gastric cancer. An EBV association was found in six malignancies. *CTLA4* mutations were associated with lymphopenia and decreased T-, B-, and NK-cell counts. Successful targeted therapies included the application of CTLA-4-fusion-proteins, mTOR-inhibitors, and hematopoietic stem cell transplantation. EBV reactivation occurred in two affected mutation carriers under immunosuppression.

Conclusions

Affected mutation carriers with CTLA-4 insufficiency may present in any medical specialty. Family members should be counseled, as disease manifestation may occur as late as age 50. EBV- and CMV-associated complications must be closely monitored. Treatment interventions should be coordinated in clinical trials.

Clinical Implication

This large cohort of affected *CTLA4* mutation carriers gives first insights into different possible treatment options and presents available clinical information on treatment response and survival. With this knowledge, affected mutation carriers will benefit from an individualized management.

Capsule summary

We present the clinical spectrum, new mutations, and possible modifiers of the world-wide largest cohort of *CTLA4* mutation carriers. We encourage physicians to consider mutations in genes such as *CTLA4* as a monogenetic cause for complex disease presentations.

Key words

Cytotoxic T lymphocyte antigen 4, primary immunodeficiency, autoimmunity, hypogammaglobulinemia, hematopoietic stem cell transplantation, abatacept, sirolimus, immune dysregulation, common variable immunodeficiency

Abbreviations

alloHSCT, allogeneic hematopoietic stem cell transplantation

APC, antigen-presenting cells

CMV, cytomegalovirus

CTLA-4, cytotoxic T lymphocyte antigen 4

CVID, common variable immunodeficiency

EBV, Epstein-Barr virus

GLILD, granulomatous-lymphocytic interstitial lung disease

GvHD, graft-versus-host disease

PRCA, pure red cell aplasia

Treg, regulatory T cell

Introduction

Heterozygous germline mutations in cytotoxic T lymphocyte antigen 4 (*CTLA4*) can lead to haploinsufficiency, impaired CTLA-4 dimerization, or impaired ligand binding, and can cause an autosomal dominant immune dysregulation syndrome and immunodeficiency in humans.(1-3) CTLA-4 is a negative immune regulator essential for the function of regulatory T cells (Tregs), which are responsible for maintaining self-tolerance and immune homeostasis through the suppression of T cell proliferation and differentiation.(4-9) CTLA-4 competes with the costimulatory receptor CD28 for its ligands CD80 and CD86, expressed on antigen-presenting cells (APCs).(10, 11) CTLA-4 binds these ligands with a higher affinity and avidity than CD28 and removes them from the surface of APCs *via* transendocytosis, resulting in a reduction of APC-mediated activation of conventional T cells.(12, 13) *CTLA4* encodes for four exons; exon 1 encodes the signal peptide, exon 2 the ligand binding and dimerization domains, exon 3 the transmembrane domain, and exon 4 the cytoplasmic tail.(14)

The clinical diagnosis of CTLA-4 insufficiency is complicated by a highly variable phenotype including various organ-specific autoimmune diseases, hypogammaglobulinemia, recurrent infections, and malignancies; the natural history of this condition is largely unknown.(1, 3, 15-19) CTLA-4 insufficiency in humans was associated with incomplete penetrance.

Here, we describe the largest known cohort of *CTLA4* mutation carriers including 133 individuals to aid diagnosis in similar cases and give guidance for their treatment.

Methods

See Supplements.

Results

Age distribution and origin

We identified 133 individuals of 54 unrelated families (66 female, 67 male) from Europe (n=87), Asia (n=26), South America (n=7), and North America (n=13) (Table 1, Figure 1). Median age of onset was 11 (<1 to 59) years, median age at evaluation was 23 years in affected mutation carriers, and 46 years in unaffected carriers (Figure 2). Three-fourths of affected mutation carriers were under the age of 18 years when showing first symptoms; there was no significant difference in the age of onset between women and men.

Genetics and protein function

We identified 45 unique heterozygous *CTLA4* germline mutations including 28 missense mutations, ten deletions or insertions, and seven nonsense mutations (Table 1, Figure 3). Mutations in seven affected carriers had occurred *de novo.* Twenty-eight mutations were novel and seventeen have previously been described.(1, 3, 15, 17-21) Eight mutations were located in exon 1, 31 in exon 2 and six within exon 3. Mutations at seven loci were identified in multiple families (Table 2). CTLA-4 expression within stimulated Tregs was reduced in all tested *CTLA4* mutation carriers. CD4+ T cells were co-cultured with CD80-GFP-expressing CHO cells and GFP-uptake was measured within CTLA-4 positive cells to estimate the ability of cells to perform transendocytosis, which was reduced in all tested mutation carriers (Table 1, Figure S1).(13) An association between genotype and onset, penetrance, or disease phenotype was not observed. So far 115 exonic variants have been described within *CTLA4*; all but two variants have a minor allele frequency (MAF) <0.01, seven variants have been described to be disease causing or are part of our cohort (Table S3). (1, 2, 19)

Symptoms and signs at presentation

First symptoms included autoimmune cytopenia (33%), respiratory manifestations (21%), enteropathy (17%), type 1 diabetes (8%), neurological symptoms (seizures, headache, nausea) (6%),

thyroid disease (5%), arthritis (3%), growth retardation, fever or night sweats, atopic dermatitis, alopecia (2% each), and primary biliary cirrhosis, Addison's disease, or a wound healing disorder, in one affected mutation carrier each.

Main diagnoses

At the time of data collection, affected mutation carriers had diverse main diagnoses: Twenty-six (29%) had a diagnosis of cytopenia and 23 (26%) had common variable immunodeficiency (CVID). CVID was diagnosed according to the revised European society of immune deficiencies (ESID) registry.(22) Twenty affected mutation carrier (22%) suffered mainly from severe gastrointestinal symptoms such as enteropathy or inflammatory bowel disease (IBD) and ten (11%) from respiratory disease including infections (n=9), granulomatous lymphoproliferative interstitial lung disease (GLILD, n=9), bronchiectasis (n=9), and asthma (n=2). In seven affected mutation carriers (8%) lymphoma was the leading diagnosis, five (6%) had mainly endocrinopathies, and four (4%) had inflammatory CNS disease. Individual affected mutation carriers had widespread lymphadenopathy (n=3, 3%), an autoimmune lymphoproliferative syndrome (ALPS)-like phenotype (n=2, 2%), an immune dysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX)-like phenotype (n=1, 1%), a primary biliary cirrhosis (n=1, 1%), liver cirrhosis of unknown etiology (n=1, 1%), rheumatoid arthritis (n=1, 1%), and psoriatic arthritis (n=1, 1%). Ten affected mutation carriers (11%) had several main diagnoses (Table S1). At the time of data collection 65 affected mutation carriers were under immunosuppression. Forty-three mutation carriers were considered unaffected (Table S1).

Clinical spectrum of CTLA-4 insufficiency

While *CTLA4* mutations were associated with autoimmunity and immune dysregulation in all affected mutation carriers, the affected organ systems varied substantially: hypogammaglobulinemia (84%), lymphoproliferation (73%), respiratory involvement (68%), gastrointestinal features (59%), autoimmune cytopenia (62%), dermatological involvement (56%, mainly atopic dermatitis), endocrinopathy (33%), and neurological features (29%) were often observed. Arthritis (14%), growth

retardation (14%), renal (12%) or liver (12%) involvement were less frequent (Figure 4, Table S1). One affected mutation carrier had severe psoriatic arthritis (T.II.1). In total, ninety of the 133 *CTLA4* mutation carriers (67.6%) were considered affected, as they had sought medical attention for disease-related symptoms. Case reports can be found in the Supplements.

Non-malignant lymphoproliferation

Sixty-two affected mutation carriers (73%) had non-malignant lymphoproliferation, including splenomegaly (n=51, Figure 3, Figure 5 Panel A), chronic lymphadenopathy (n=43), and hepatomegaly (n=17). Thirteen affected mutation carriers underwent splenectomy for severe cytopenia. Forty-three affected mutation carriers (50%) had lymphocytic infiltrations into lung (n=27), gastrointestinal tract (n=17), brain (n=12), bone marrow (n=6, Figure 6 Panel E), kidney (n=6), or retroperitoneal tissue (n=4). Upon biopsy, 21 affected mutation carriers had T cell infiltrations, both CD4+ (n=9) and CD8+ (n=8) infiltrations were observed. Twelve predominately had B cell infiltrations, four of them in the lung tissue as part of their GLILD. Ten out of 29 biopsied affected mutation carriers with non-malignant lymphoproliferation also had granulomas in at least two different organ systems upon biopsy; eight in the lung, two in the lymph nodes, and one each in kidney, brain, or gastrointestinal tract.

Respiratory tract involvement

Respiratory tract involvement was common (68%; 61/90; Figure 5 Panels C, D, E; Figure 6 Panel A, B) including recurrent lower (n=48) and upper (n=41) respiratory tract infections, granulomatous-lymphocytic interstitial lung disease (GLILD) (n=32), and bronchiectasis (n=20). Two affected mutation carriers underwent lung transplantation due to idiopathic lung fibrosis (B.III.2) or common variable immunodeficiency (CVID) (23) with recurrent infections, emphysema, and parenchymal lung damage (A.II.9); both died 12 and 15 months, respectively, after transplantation due to pulmonary demise following a relapse of disease.

Pathogens and infections

Sixty-one percent of affected mutation carriers (55/90) had respiratory tract infections including pneumonia, sinusitis, and otitis media. Isolated pathogens were *Haemophilus influenzae* (n=6) and *Streptococcus pneumoniae* (n=4). The most common enteritis pathogen was *Salmonella enteritidis* (6/7). *Staphylococcus aureus* was detected in various organs of eleven affected mutation carriers. Twenty-seven affected mutation carriers reactivated a Herpes virus infection: Epstein-Barr virus (EBV) led to clinically apparent infections in sixteen affected mutation carriers (Figure 6 Panel C), including EBV-induced hemophagocytic lymphohistiocytosis (B.II.3). Two affected mutation carriers developed EBV-associated lymphoid granulomatosis in lung or brain (H.II.2, N.III.2). Cytomegalovirus (CMV) reactivation was found in nine affected mutation carriers including CMV-associated diarrhea or gastritis (D.II.1, M.II.3, NN.II.1), chronic active CMV infection (LL.II.1), CMV lymphadenitis (K.II.1), bilateral parotid hypertrophy (O.II.1), and respiratory CMV infection (R.II.5); eight of them were on immunosuppressive treatment. *Mycobacterium tuberculosis* polymerase chain reaction was positive in four affected mutation carriers, with two of them developing pulmonary or esophageal tuberculosis (A.II.8, A.II.9).

Fungal infections were present in 15 affected mutation carriers with either *Candida species pluralis* infections (n=13) or *Aspergillus species pluralis* pneumonia (n=2); thirteen of them received immunosuppressive treatment at the time of data collection. Ten affected mutation carriers, of whom eight were immunosuppressed, developed sepsis due to bacterial or fungal pathogens leading to death in five. In one affected mutation carrier sepsis followed a perforation of the small bowel, and in one *Salmonella enteritidis* sepsis was the first manifestation of CTLA-4 insufficiency at the age of three months (UU.IV.12).

Gastrointestinal involvement

Gastrointestinal involvement across our cohort was frequent (59%; 53/90) and often severe. Nine of the 15 deceased affected mutation carriers had severe gastrointestinal features prior to their death. Diarrhea was frequent (n=51), ranging from mild to severe diarrhea with weight loss, wasting, and

total parenteral nutrition-dependency. Pathogens were rarely identified. Crohn disease (n=7), atrophic gastritis (n=8) (Figure 6 Panel D), coeliac disease (JJ.II.1), acute pancreatitis (M.II.3), and pancreatic insufficiency (N.III.2, QQ.II.1) were observed. In three affected mutation carriers, severe long-lasting CVID-gastroenteropathy preceded gastric cancer (B.II.4, G.III.2, M.II.3). Macroscopic findings ranged from normal appearing mucosa albeit histologically proven deep T cell infiltrations in the submucosa, to superficial ulcerative lesions or deep-seated inflammatory changes as seen in severe Crohn's disease. Despite decreased serum immunoglobulin levels, histology revealed increased numbers of plasma cells in the gastric (B.II.4, QQ.II.1), intestinal, and colonic (QQ.II.1) lamina propria. Further histology changes included severe lymphocytic infiltrates, and EBV-positive gastric cancer (Figure 6 Panel C). Median age of onset of gastrointestinal features was 15 (<1 to 51) years.

Cytopenia

Autoimmune cytopenia was often severe, life-threatening, and treatment-resistant and formed the main indication for allogeneic hematopoietic stem cell transplantation (alloHSCT) (7/12).

Sixty-two percent of affected mutation carriers (55/89) had autoimmune cytopenia, including immune thrombocytopenia (n=41), autoimmune hemolytic anemia (n=37), pure red cell aplasia (PRCA) (n=2) or autoimmune neutropenia (n=16). In 32 affected mutation carriers cytopenia affected more than one cell lineage, nineteen of those were diagnosed with Evans syndrome, and nine had a trilineage cytopenia. Median age of onset of cytopenia was 12 (1.3 to 48) years.

Neurological involvement

Twenty-eight percent of affected mutation carriers (28/90) presented with a broad spectrum of neurological features (Figure 5 Panel F, G, H, Figure 6 Panel F). Three had autoimmune encephalitis or encephalomyelitis with cerebral perivascular lymphocytic infiltrations leading to vomiting, headache or paraplegia with bladder dysfunction (N.III.2, P.II.2, GG.II.1). In four affected mutation carriers neurological features were attributed to cerebral infiltrations that were not biopsied: nausea

and headache (A.III.1), facial nerve paralysis (H.II.1), aphasia and paresis of the left arm (K.II.1), or a patchy inflammatory demyelinating process with twitching episodes of hands with normal electroencephalography (WW.II.1). Three affected mutation carriers had neurological features secondary to hematological causes: hemiplegia following brain ischemia during AIHA (DD.II.1), hemiparesis and aphasia due to cerebral arterial thrombosis (H.II.2), hemiparesis following cerebral bleeding due to thrombocytopenia (GG.II.1). In two affected mutation carriers clinical and radiological investigation could not identify an underlying cause for tonic-clonic seizures, or recurrent transient paralysis of the left leg respectively (A.II.8, EE.II.1). One affected mutation carrier had life-threatening HLH with increased cerebral pressure leading to cerebral herniation and seizures (J.II.1). Other diagnoses were stiff person syndrome (H.I.2), West-syndrome and developmental delay (UU.V.1), progressive memory loss starting age 57 years (UU.III.7), and chronic hydrocephalus (UU.III.4). Two affected mutation carriers suffered from optic neuritis (TT.II.4) and retinal tear due to lymphocytic infiltrations into the retina (SS.II.1). One had gliosis (ZZ.II.1) and one developed cognitive dysfunction, chorea, ataxia, and mood instability; biopsies revealed inflammation, lymphocytic infiltrations, and a demyelinating-like transformation, which was clinically responsive to steroid treatment (G.III.1). One affected mutation carrier was diagnosed with tuberous sclerosis with tonic-clonic seizures, right-sided hemiparesis, mental retardation, angiofibromas, angiomyolipomas, and a concurrent TSCA2 mutation (LL.II.1).

Malignancies

Eleven affected mutation carriers (12%) developed malignancies. Out of eight with lymphoma, EBV-positivity was found in five. Lymphoma in five affected mutation carriers was classified as Hodgkin lymphoma; one developed a relapsing EBV-associated diffuse large B cell lymphoma (K.II.1) and one a Burkitt lymphoma (FF.II.1) (Figure 6 Panel G, H). Four affected mutation carriers died due to complications of their lymphoma, two underwent successful alloHSCT.

Three affected mutation carriers developed a gastric adenocarcinoma, including one EBV-associated carcinoma (B.II.4, Figure 6 Panel C), and one CMV-associated carcinoma (M.II.3). Two affected

mutation carriers subsequently underwent total gastrectomy, one of whom died following bacterial sepsis (M.II.3) while the other one is alive and well (G.III.2).

Fatal Outcome

Sixteen percent of affected mutation carriers (15/90) died due to their clinical manifestations or resulting complications at a median age of 23 (14 to 60) years. Four died of sepsis on a background of wasting enteropathy, Evans syndrome, or CVID with infections (M.II.3, G.III.1, C.II.3, L.I.2). Three died due to complications of Non-Hodgkin lymphoma (K.II.1, FF.II.1, UU.III.3), one died during chemotherapy of his Hodgkin lymphoma due to septic multi-organ failure (H.II.1), and two following lung transplantation and relapse of disease (A.II.9, B.III.2). One affected mutation carrier died of acute liver failure following many years of gastrointestinal disease (B.II.2). Wasting enteropathy, respiratory insufficiency, and neurological features led to death in one affected mutation carrier (A.II.8). Another one suffered from severe enteropathy and cytopenia, and died following colectomy (F.II.1). Three affected mutation carriers died following alloHSCT due to GvHD (Q.II.1, LL.II.1) or due to diabetic ketoacidosis (S.II.1). There was a significant difference of the age of death between affected and the unaffected *CTLA4* mutation carriers (Figure 2 B).

Immunological phenotype

Thirty-nine percent (26/66) of affected mutation carriers with available immunological data had lymphopenia of which twenty-four were under immunosuppressive treatment at the time of data collection. The absolute CD3+ T cell count was reduced in 36% (16/44) of affected mutation carriers. The absolute CD3+CD4+ helper T cell count was reduced in 20% (13/62) of affected mutation carriers especially due to the noteworthy reduction of naïve CD4+ T cells. An elevated percentage of the activation marker HLA-DR+ was seen in one third of tested affected mutation carriers (11/31). Percentage of CD4+FoxP3+ Tregs was significantly increased in mutation carriers in comparison to healthy controls (p=0.0034). There was no significant difference in the Treg percentage between affected and unaffected mutation carriers (p=0.3882). Eight mutation carriers had Treg numbers

457 above the normal range. Absolute CD3+CD8+ cytotoxic T cell count was normal in 60% (35/58) of
458 affected mutation carriers. Double-negative T cells were elevated up to 5.3% (median 2.2%; norm:
459 0.3-2.0%) in 53% of tested affected mutation carriers (9/17). Absolute CD19+ B cell counts were
460 reduced in 41% (26/58) of affected mutation carriers. B cell subsets showed a decrease in switched
461 memory B cells (23/30) and consecutively a relative increase in naïve B cells (14/29). CD21-low B cells
462 were elevated in all affected mutation carriers tested. Five affected mutation carriers with no history
463 of rituximab therapy had no measurable B cells. Hypogammaglobulinemia was present in 84%
464 (65/77), with low IgM in 30, low IgG in 42, and low IgA in 53 affected mutation carriers (Figure 4).
465 Absolute CD16+CD56+ NK cell counts were reduced in 52% (32/61). The percentage of CD3+ and
466 CD3+CD4+ was increased in the majority of affected mutation carriers, as the overall lymphopenia
467 affected CD3+CD8+, B, and NK cells more than the CD4 compartment (Figure S2). Antinuclear
468 autoantibodies (ANA) and anti-neutrophil cytoplasmic antibodies (ANCA) were the most commonly
469 measured autoantibodies; however, they were negative in most affected mutation carriers (ANA
470 (4/51), ANCA (3/42)).
471
472 ***Treatment***
473 *CTLA-4 fusion proteins and mTOR inhibitors*
474 CTLA-4 replacement by CTLA-4-Fc, or inhibition of the CD28 signaling pathway through mTOR
475 inhibitors are potential targeted therapies to inhibit the underlying hyper-active signaling in *CTLA4*
476 mutation carriers.
477 In total, fourteen affected mutation carriers received the CTLA-4 fusion proteins abatacept or
478 belatacept; eleven of whom responded with an improvement of their clinical symptoms. In six of
479 them enteropathy improved, leading to normal stool frequency and weight gain within three months
480 (B.II.4, D.II.1, L.II.2, HH.II.1, SS.II.1, VV.II.1, Figure S3). In two affected mutation carriers primarily
481 presenting with GLILD (RR.II.1, SS.II.1), CTLA4-Fc led to resolution of lymphoproliferation in the lung
482 (SS.II.1), cough and sputum production decreased, and sIL2R concentration dropped from 1228 U/ml
483 to 750 U/ml within five months (RR.II.1). Other observations were an improvement of

lymphadenopathy (G.III.2), stabilization of platelet counts, resolution of bleeding episodes, and regression of optic neuritis (TT.II.4). In two affected mutation carriers, additional systemic immunosuppressive medication could be reduced, as abatacept treatment led to inhibition of the disease progression (J.II.1) or to improvement of lung function and diarrhea (PP.II.1). In six affected mutation carriers treatment was discontinued: three underwent alloHSCT (L.II.2, VV.II.1, GG.II.1), two had an EBV reactivation (B.II.3, B.II.4), and one developed severe respiratory infections, neutropenia, and agranulocytosis (TT.II.4).

Thirteen affected mutation carriers were treated with the mTOR inhibitor sirolimus with a good response in eight (D.II.1, E.II.3, L.II.2, O.II.1, P.II.2, Z.III.1, TT.II.5, WW.II.1). Improvement of clinical features included resolution of transfusion-dependent PRCA (Z.III.1), regression of lymphadenopathy and splenomegaly, reduced IG consumption, and improved CMV viral load (O.II.1). Enteropathy improved in three affected mutation carriers following combination of sirolimus with either prednisolone (D.II.1), belatacept (L.II.2), or rituximab and steroids (WW.II.1). In one affected mutation carrier cytopenia stabilized on co-medication with rituximab, but neurological features and severe aphthae occurred (P.II.2). Sirolimus led to reduced spleen size (volume decreased from 5l to 2.8l) in one affected mutation carrier, who developed arthritis and erythema nodosum during the treatment (E.II.3). In two affected mutation carriers sirolimus treatment was discontinued due to ineffectiveness for cytopenia (GG.II.1), or due to increased blood pressure on the background of a renal impairment (B.II.4). In one affected mutation carrier CMV copies rose under sirolimus treatment in combination with methylprednisolone (DD.II.1), in one lymphopenia worsened (O.II.1), one died due to sepsis during sirolimus treatment (G.III.1), and in one sirolimus treatment was stopped due to serious respiratory infections (SS.II.1). Daily dosage ranged from 2 mg to 2.64 mg (n=5); trough levels were available for two affected mutation carriers (6,2 ng/ml and 8 ng/ml), for three affected mutation carriers target blood values were available (8-12 ng/ml (n=2); 12-15 ng/ml (n=1)).

Hematopoietic stem cell transplantation

Twelve affected mutation carriers underwent alloHSCT between 10 and 50 years of age.(15) Main indications for transplantation included treatment-resistant cytopenia, enteropathy, and Hodgkin lymphoma; often combined with other autoimmune manifestations, lymphoproliferation or severe infections. Nine of these affected mutation carriers are alive, of whom three are more than five years post-HSCT and currently well off all medication (L.II.1, T.II.1, Y.II.1), and six are between 100 days and 12 months post-transplant (B.II.3, L.II.2, P.II.2, W.II.2, GG.II.1, VV.II.1) (Table S2). In half of the affected mutation carriers the *CTLA4* mutation was known prior to transplantation (6/12), the other half was transplanted due to the severity of their symptoms and the *CTLA4* mutation was only identified after transplantation.

Immunoglobulin substitution

Sixty-three percent of affected mutation carriers (55/88) received immunoglobulin substitution either due to hypogammaglobulinemia or due to cytopenia. Twenty-eight affected mutation carriers had both diagnoses at the time of data collection and received immunoglobulin substitution due to both.

Additional treatment options can be found in the Supplements.

Chromosome 2 contiguous gene deletion involving CTLA4

Two unrelated individuals have a heterozygous 2q33.2-2q33.3 deletion involving *CTLA4* and present a CTLA-4 insufficiency-like phenotype, which is possibly influenced by the deletion of additional genes including *CD28* and *ICOS* (Supplements).

Mutation carriers who did not seek medical attention

We identified 43 unaffected family members carrying the same *CTLA4* mutation as their affected relatives. The treating physician of the affected mutation carrier classified family members as unaffected if they did not repeatedly seek medical care, were not under a long-term drug regimen

due to CTLA-4 insufficiency-related symptoms, or if they were not restricted in their health-related quality of life due to their symptoms. Their median age at evaluation was 46 (6 to 87) years, hence in most cases beyond the median age of manifestation. Upon thorough questioning and clinical investigation, seven carriers had diarrhea without weight loss, two had atrophic gastritis or pernicious anemia, and one had coeliac disease. Three carriers had respiratory infections and in one clinically unapparent pulmonary nodules were detected on a routine scan. Nine had dermatological involvement (psoriasis, eczema, vitiligo), and two hypothyroidism. One carrier developed colon cancer aged 78, which was successfully treated by surgery but is otherwise healthy at currently 87 years of age (A.I.2). Four carriers (without recurrent infections) had IgA-deficiency, one each had low IgG or IgM, and one had low IgA and IgM, possibly contributing to respiratory infections (R.III.1). Twenty-six carriers were reported to be clinically completely healthy.

Their immunological phenotyping revealed similarities to affected mutation carriers, including a decrease in NK and CD19+ B cells, but also differences, including significantly higher CD4+ T cells counts, and a higher percentage of switched memory B cells. There was no significant difference with regard to the Treg percentages in affected mutation carriers (Figure S2).

Discussion

In our work, we estimate the clinical penetrance of CTLA-4 insufficiency to be around 67%; however, as genetic analysis could not be performed in all healthy first degree family members, ascertainment was incomplete. Once symptoms have occurred, the clinical course can be severe and was fatal in 15 affected mutation carriers (16%).

The clinical phenotype was characterized by infections, autoimmunity, and lymphoproliferation, affecting various organ systems. Affected mutation carriers have an elevated risk to develop malignancies and for EBV reactivation highlighting the importance of monitoring EBV and possibly CMV viral load, especially under immunosuppressive treatment. Cytopenia and enteropathy were the most life-threatening and treatment-resistant manifestations. This is evidenced by the fact that cytopenia was one of the main indications for alloHSCT (7/12), and half of the deceased affected mutation carriers died following a history of enteropathy and associated complications. Initial symptoms were diverse, emphasizing the importance of raising awareness of this immunodeficiency not only among immunologists but also other specialists including hematologists, neurologists, gastroenterologists, pathologists, dermatologists, and chest physicians. As the age of onset in 75% of affected mutation carriers is under the age of 18 years, CTLA-4 insufficiency should be considered in children with severe immune dysregulation of unknown origin. Also in individuals being evaluated for IBD, CVID, and ALPS, CTLA-4 insufficiency should be considered.

To diagnose CTLA-4 insufficiency, we recommend sequencing the four exons of *CTLA4* and then testing the effect of identified mutations on the protein by measuring CTLA-4 expression or CTLA-4-mediated transendocytosis.(24) Both were reduced in all analyzed mutation carriers, but because this is also seen in individuals with mutations in other genes such as *LRBA*(25), these functional tests cannot be used as the only diagnostic tool to screen for *CTLA4* mutations. In addition to the clinical presentation, an autosomal dominant family history can hint towards CTLA-4 insufficiency.

589 The immunological phenotype revealed perturbed T and B cell homeostasis and significantly
590 increased Treg percentages within the CD4+ T cell compartment. The latter may be a transient or
591 permanent compensatory mechanism of the CTLA-4-deficient immune system to counteract the
592 immune-activation. The expanded and activated effector T cells may produce a cytokine profile
593 leading to an increased Treg cell polarization in order to counterbalance the accelerated immune
594 activation. Elevated double negative T cell counts in *CTLA4* mutation carriers should prompt
595 investigators to evaluate proposed ALPS diagnostic criteria. (26)

596 We present first insights into targeted therapeutic strategies: Out of thirteen affected mutation
597 carriers treated with CTLA-4-Fc, eleven responded favorably, especially enteropathy improved.
598 Further clinical studies are necessary to determine the effectiveness and safety of CTLA-4-Fc
599 treatment for individual clinical manifestations. Out of twelve affected mutation carriers undergoing
600 alloHSCT, nine are alive and well (15); although long-term survival still has to be determined,
601 alloHSCT should be considered as a treatment option in carefully selected affected mutation carriers.

602 In individuals presenting with immunodeficiency, autoimmunity, and lymphoproliferation with
603 impaired Treg development or function, besides CTLA-4 insufficiency, also mutations in *FoxP3, LRBA,*
604 *IL2RA*, *FAS-L, FAS, PI3K, NFKB1 and 2, STAT3,* and *STAT5b* should be considered as a differential
605 diagnosis.(6) (25, 27-34) Mutations in *FOXP3* lead to a loss of Treg cells and cause IPEX which is an X-
606 linked condition and characterized by enteropathy, immune dysregulation, and polyendocrinopathy,
607 but has an earlier onset, and complete penetrance.(27, 34) Immunological findings in IPEX-syndrome
608 include normal lymphocyte counts and immunoglobulin levels in contrast to CTLA-4 insufficiency. In
609 LRBA deficiency lysosomal CTLA-4 degradation is accelerated and CTLA-4 trafficking to the cell
610 surface is disturbed; hence the inhibitory function of Treg cells is impaired. (25) Biallelic *LRBA*
611 mutations most often lead to complete absence of the LRBA protein; affected mutation carriers
612 present with a phenotype very similar to CTLA-4 insufficiency, characterized by various autoimmune
613 features, lymphoproliferation with dysregulated Treg function, and a defect in production cell
614 homeostasis,(28, 31, 32, 35-44) albeit with an earlier onset, complete penetrance and an autosomal

recessive inheritance. In addition, germline gain-of-function mutations in *STAT3* lead to a broad range of autoimmune disorders such as autoimmune cytopenias and multiorgan autoimmunity (lung, gastrointestinal, hepatic, and endocrine), in combination with an increased susceptibility to infections and a short stature. Further, *STAT3* gain-of-function mutations lead to secondary defects in STAT5 and STAT1 phosphorylation and impair the Treg compartment.(29, 33)

As our results were collected retrospectively, several limiting factors should be considered: affected mutation carriers were treated and evaluated by different physicians and medical departments worldwide. This can lead to an incomplete picture of the clinical phenotype. Also, data was collected at one time point, which often makes it difficult to reconstruct whether symptoms or the immunological phenotype are due to immunosuppressive treatment or the natural course of this immunodeficiency.

In LRBA deficiency, sIL2R, a biomarker for T cell-mediated inflammation, decreases on abatacept treatment.(25) In our cohort sIL2R was only sporadically measured; in one affected mutation carrier sIL2R levels dropped while being on abatacept treatment. Systematical measurement of sIL2R should be considered in all mutation carriers to see whether it indicates disease activity. Affected and unaffected mutation carriers both show impaired *in vitro* CTLA-4 function, indicating the presence of additional factors influencing the clinical phenotype and penetrance such as environmental, genetic, or epigenetic differences. Ethnicity and origin of the mutation carriers could influence age of onset, penetrance, and severity of disease-related symptoms. We cannot assess this, as the world-wide distribution in our study is not equal and the diverse countries of origin varied in diagnostic procedures and standards. In general, there could either be one single modifier, or multiple interacting factors influencing the clinical phenotype. The latter could explain the highly variable expressivity of the phenotype. Another hypothesis suggests an internal threshold within the immune system of CTLA-4-insufficient individuals. Once it is exhausted, immune dysregulation cannot be contained by the organism and individuals develop symptoms; this could explain why healthy mutation carriers may develop life-threatening symptoms late in life (e.g. patient B.II.3 developed

641 hemophagocytic lymphohistiocytosis and Hodgkin lymphoma at the age of 50 years). These cases
642 teach us to carefully monitor all first-degree relatives for CTLA-4-associated disease activity, while
643 the search for modifying factors in CTLA-4 insufficiency continues.

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Declaration of interest

The authors declare no competing financial or personal interests.

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822 **Figure legends**

823 **Figure 1. Pedigrees of families with CTLA-4 insufficiency**

824 Pedigrees of all families with more than one *CTLA4* mutation carrier. Squares, male subjects; circles,
825 female subjects; black filled symbols, mutation carriers classified as affected; gray filled symbols,
826 mutation carriers classified as unaffected; slashed symbols, deceased subjects; *, sequencing of
827 *CTLA4* was performed; §, genotype inferred from clinical symptoms.

828

829 **Figure 2. Age of onset and age of death in CTLA-4 insufficient individuals**

830 A. Kaplan Meier curve of age of onset of *CTLA4* mutation carriers (n=85).
831 B. Age of death in affected (n=86) versus unaffected mutation carriers (n=39).

832

833 **Figure 3. Heterozygous germline mutations within the *CTLA4* gene are distributed throughout**
834 **exon 1-3.**

835 Figure 3 shows the distribution of the heterozygous germline mutations throughout the *CTLA4* gene.
836 Eight mutations are located in exon 1, 31 are located in exon 2, and six are located in exon 3. §,
837 mutation was functionally tested by transendocytosis assay.

838

839 **Figure 4. Main clinical findings in CTLA-4 insufficiency**

840 Percentage distribution of clinical manifestations within affected mutation carriers. Clinical data was
841 available for 71 to 90 affected mutation carriers.

842

843 **Figure 5. Exemplary findings upon CT and MRI in CTLA-4-insufficient individuals**

844 Panel A: splenomegaly (17.5 cm in diameter) and lymphadenopathy in A.III.3. Panel B: large
845 pneumatocele following necrotizing pneumonia in PP.II.1. Panel C: CT scan of ZZ.II.1 showing
846 peripheral bronchiectasis with inflammatory nodules in all lobes of the lung. Panel D: bronchiectasis
847 with peribronchial ground glass nodules in keeping with bronchiolitis in XX.II.1. Panel E: multiple

inflammatory nodules in O.II.1. Panel F: signal change in the right temporal lobe and cerebellum consistent with inflammation in KK.II.1. Panel G: enhancement in the thoracic cord in keeping with inflammation in KK.II.1. Panel H: signal change and swelling in the cerebellum in keeping with inflammation in P.II.2.

Figure 6. Lymphocytic infiltrations and loss of EBV control define the spectrum of inflammatory and neoplastic lesions

Panel A and B: lung samples of PP.II.1 and KK.II.1 with follicular bronchitis/ bronchiolitis, respectively. Lymphoid follicles are marked by asterisks. In Panel A, the follicle contains a germinal center. Panel C: EBV-coded small RNAs (EBER) positive nuclei (dark blue staining) of an early invasive gastric adenocarcinoma of B.II.4. Panel D: autoimmune gastritis with severely atrophic mucosa of the stomach, antral metaplasia and numerous intraepithelial CD8+ T cells (brown staining) of B.II.4. Panel E: nodular T cell lymphocytosis (brown staining) in the bone marrow of Z.II.2. Panel F: perivascular lymphocytes in the brain tissue of KK.II.1 (arteriolar wall highlighted by arrowhead, lumen marked by asterisk). Panel G and H: Hodgkin lymphoma in a lymph node excision sample of MM.II.1. Reed-Sternberg cell is highlighted by an arrowhead (G) or CD30 immunohistochemistry (red staining in H). Nuclei of Hodgkin cells and Reed-Sternberg cells were positive for EBER (dark blue staining, inlet H).

Phenotype, penetrance, and treatment of 133 CTLA-4-insufficient individuals

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Abstract

Background

Cytotoxic-T-lymphocyte-antigen-4 (CTLA-4) is a negative immune regulator. Heterozygous *CTLA4* germline mutations can cause a complex immune dysregulation syndrome in humans.

Objective

To characterize the penetrance, the clinical features and the best treatment options in 133 *CTLA4* mutation carriers.

Methods

Genetics, clinical features, laboratory values, and outcome of treatment options were assessed in a worldwide cohort of *CTLA4* mutation carriers.

Results

We identified 133 individuals from 54 unrelated families carrying 45 different heterozygous *CTLA4* mutations, including 28 previously undescribed mutations. Ninety mutation carriers were considered affected, suggesting the clinical penetrance of at least 67%; median age of onset was 11 years, and mortality rate within affected mutation carriers was 16% (n=15).

Main clinical manifestations included hypogammaglobulinemia (84%), lymphoproliferation (73%), autoimmune cytopenia (62%), respiratory- (68%), gastrointestinal- (59%), or neurological features (29%). Eight affected mutation carriers developed lymphoma, three gastric cancer. An EBV association was found in six malignancies. *CTLA4* mutations were associated with lymphopenia and decreased T-, B-, and NK-cell counts. Successful targeted therapies included the application of CTLA-4-fusion-proteins, mTOR-inhibitors, and hematopoietic stem cell transplantation. EBV reactivation occurred in two affected mutation carriers under immunosuppression.

Conclusions

Affected mutation carriers with CTLA-4 insufficiency may present in any medical specialty. Family members should be counseled, as disease manifestation may occur as late as age 50. EBV- and CMV-associated complications must be closely monitored. Treatment interventions should be coordinated in clinical trials.

Clinical Implication

This large cohort of affected *CTLA4* mutation carriers gives first insights into different possible treatment options and presents available clinical information on treatment response and survival. With this knowledge, affected mutation carriers will benefit from an individualized management.

Capsule summary

We present the clinical spectrum, new mutations, and possible modifiers of the world-wide largest cohort of *CTLA4* mutation carriers. We encourage physicians to consider mutations in genes such as *CTLA4* as a monogenetic cause for complex disease presentations.

Key words

Cytotoxic T lymphocyte antigen 4, primary immunodeficiency, autoimmunity, hypogammaglobulinemia, hematopoietic stem cell transplantation, abatacept, sirolimus, immune dysregulation, common variable immunodeficiency

Abbreviations

alloHSCT, allogeneic hematopoietic stem cell transplantation

APC, antigen-presenting cells

CMV, cytomegalovirus

CTLA-4, cytotoxic T lymphocyte antigen 4

CVID, common variable immunodeficiency

EBV, Epstein-Barr virus

GLILD, granulomatous-lymphocytic interstitial lung disease

GvHD, graft-versus-host disease

PRCA, pure red cell aplasia

Treg, regulatory T cell

Introduction

Heterozygous germline mutations in cytotoxic T lymphocyte antigen 4 (*CTLA4*) can lead to haploinsufficiency, impaired CTLA-4 dimerization, or impaired ligand binding, and can cause an autosomal dominant immune dysregulation syndrome and immunodeficiency in humans.(1-3) CTLA-4 is a negative immune regulator essential for the function of regulatory T cells (Tregs), which are responsible for maintaining self-tolerance and immune homeostasis through the suppression of T cell proliferation and differentiation.(4-9) CTLA-4 competes with the costimulatory receptor CD28 for its ligands CD80 and CD86, expressed on antigen-presenting cells (APCs).(10, 11) CTLA-4 binds these ligands with a higher affinity and avidity than CD28 and removes them from the surface of APCs *via* transendocytosis, resulting in a reduction of APC-mediated activation of conventional T cells.(12, 13) *CTLA4* encodes for four exons; exon 1 encodes the signal peptide, exon 2 the ligand binding and dimerization domains, exon 3 the transmembrane domain, and exon 4 the cytoplasmic tail.(14)

The clinical diagnosis of CTLA-4 insufficiency is complicated by a highly variable phenotype including various organ-specific autoimmune diseases, hypogammaglobulinemia, recurrent infections, and malignancies; the natural history of this condition is largely unknown.(1, 3, 15-19) CTLA-4 insufficiency in humans was associated with incomplete penetrance.

Here, we describe the largest known cohort of *CTLA4* mutation carriers including 133 individuals to aid diagnosis in similar cases and give guidance for their treatment.

Methods

See Supplements.

Results

Age distribution and origin

We identified 133 individuals of 54 unrelated families (66 female, 67 male) from Europe (n=87), Asia (n=26), South America (n=7), and North America (n=13) (Table 1, Figure 1). Median age of onset was 11 (<1 to 59) years, median age at evaluation was 23 years in affected mutation carriers, and 46 years in unaffected carriers (Figure 2). Three-fourths of affected mutation carriers were under the age of 18 years when showing first symptoms; there was no significant difference in the age of onset between women and men.

Genetics and protein function

We identified 45 unique heterozygous *CTLA4* germline mutations including 28 missense mutations, ten deletions or insertions, and seven nonsense mutations (Table 1, Figure 3). Mutations in seven affected carriers had occurred *de novo.* Twenty-eight mutations were novel and seventeen have previously been described.(1, 3, 15, 17-21) Eight mutations were located in exon 1, 31 in exon 2 and six within exon 3. Mutations at seven loci were identified in multiple families (Table 2). CTLA-4 expression within stimulated Tregs was reduced in all tested *CTLA4* mutation carriers. CD4+ T cells were co-cultured with CD80-GFP-expressing CHO cells and GFP-uptake was measured within CTLA-4 positive cells to estimate the ability of cells to perform transendocytosis, which was reduced in all tested mutation carriers (Table 1, Figure S1).(13) An association between genotype and onset, penetrance, or disease phenotype was not observed. So far 115 exonic variants have been described within *CTLA4*; all but two variants have a minor allele frequency (MAF) <0.01, seven variants have been described to be disease causing or are part of our cohort (Table S3). (1, 2, 19)

Symptoms and signs at presentation

First symptoms included autoimmune cytopenia (33%), respiratory manifestations (21%), enteropathy (17%), type 1 diabetes (8%), neurological symptoms (seizures, headache, nausea) (6%),

thyroid disease (5%), arthritis (3%), growth retardation, fever or night sweats, atopic dermatitis, alopecia (2% each), and primary biliary cirrhosis, Addison's disease, or a wound healing disorder, in one affected mutation carrier each.

Main diagnoses

At the time of data collection, affected mutation carriers had diverse main diagnoses: Twenty-six (29%) had a diagnosis of cytopenia and 23 (26%) had common variable immunodeficiency (CVID). CVID was diagnosed according to the revised European society of immune deficiencies (ESID) registry.(22) Twenty affected mutation carrier (22%) suffered mainly from severe gastrointestinal symptoms such as enteropathy or inflammatory bowel disease (IBD) and ten (11%) from respiratory disease including infections (n=9), granulomatous lymphoproliferative interstitial lung disease (GLILD, n=9), bronchiectasis (n=9), and asthma (n=2). In seven affected mutation carriers (8%) lymphoma was the leading diagnosis, five (6%) had mainly endocrinopathies, and four (4%) had inflammatory CNS disease. Individual affected mutation carriers had widespread lymphadenopathy (n=3, 3%), an autoimmune lymphoproliferative syndrome (ALPS)-like phenotype (n=2, 2%), an immune dysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX)-like phenotype (n=1, 1%), a primary biliary cirrhosis (n=1, 1%), liver cirrhosis of unknown etiology (n=1, 1%), rheumatoid arthritis (n=1, 1%), and psoriatic arthritis (n=1, 1%). Ten affected mutation carriers (11%) had several main diagnoses (Table S1). At the time of data collection 65 affected mutation carriers were under immunosuppression. Forty-three mutation carriers were considered unaffected (Table S1).

Clinical spectrum of CTLA-4 insufficiency

While *CTLA4* mutations were associated with autoimmunity and immune dysregulation in all affected mutation carriers, the affected organ systems varied substantially: hypogammaglobulinemia (84%), lymphoproliferation (73%), respiratory involvement (68%), gastrointestinal features (59%), autoimmune cytopenia (62%), dermatological involvement (56%, mainly atopic dermatitis), endocrinopathy (33%), and neurological features (29%) were often observed. Arthritis (14%), growth

retardation (14%), renal (12%) or liver (12%) involvement were less frequent (Figure 4, Table S1). One affected mutation carrier had severe psoriatic arthritis (T.II.1). In total, ninety of the 133 *CTLA4* mutation carriers (67.6%) were considered affected, as they had sought medical attention for disease-related symptoms. Case reports can be found in the Supplements.

Non-malignant lymphoproliferation

Sixty-two affected mutation carriers (73%) had non-malignant lymphoproliferation, including splenomegaly (n=51, Figure 3, Figure 5 Panel A), chronic lymphadenopathy (n=43), and hepatomegaly (n=17). Thirteen affected mutation carriers underwent splenectomy for severe cytopenia. Forty-three affected mutation carriers (50%) had lymphocytic infiltrations into lung (n=27), gastrointestinal tract (n=17), brain (n=12), bone marrow (n=6, Figure 6 Panel E), kidney (n=6), or retroperitoneal tissue (n=4). Upon biopsy, 21 affected mutation carriers had T cell infiltrations, both CD4+ (n=9) and CD8+ (n=8) infiltrations were observed. Twelve predominately had B cell infiltrations, four of them in the lung tissue as part of their GLILD. Ten out of 29 biopsied affected mutation carriers with non-malignant lymphoproliferation also had granulomas in at least two different organ systems upon biopsy; eight in the lung, two in the lymph nodes, and one each in kidney, brain, or gastrointestinal tract.

Respiratory tract involvement

Respiratory tract involvement was common (68%; 61/90; Figure 5 Panels C, D, E; Figure 6 Panel A, B) including recurrent lower (n=48) and upper (n=41) respiratory tract infections, granulomatous-lymphocytic interstitial lung disease (GLILD) (n=32), and bronchiectasis (n=20). Two affected mutation carriers underwent lung transplantation due to idiopathic lung fibrosis (B.III.2) or common variable immunodeficiency (CVID) (23) with recurrent infections, emphysema, and parenchymal lung damage (A.II.9); both died 12 and 15 months, respectively, after transplantation due to pulmonary demise following a relapse of disease.

Pathogens and infections

Sixty-one percent of affected mutation carriers (55/90) had respiratory tract infections including pneumonia, sinusitis, and otitis media. Isolated pathogens were *Haemophilus influenzae* (n=6) and *Streptococcus pneumoniae* (n=4). The most common enteritis pathogen was *Salmonella enteritidis* (6/7). *Staphylococcus aureus* was detected in various organs of eleven affected mutation carriers. Twenty-seven affected mutation carriers reactivated a Herpes virus infection: Epstein-Barr virus (EBV) led to clinically apparent infections in sixteen affected mutation carriers (Figure 6 Panel C), including EBV-induced hemophagocytic lymphohistiocytosis (B.II.3). Two affected mutation carriers developed EBV-associated lymphoid granulomatosis in lung or brain (H.II.2, N.III.2). Cytomegalovirus (CMV) reactivation was found in nine affected mutation carriers including CMV-associated diarrhea or gastritis (D.II.1, M.II.3, NN.II.1), chronic active CMV infection (LL.II.1), CMV lymphadenitis (K.II.1), bilateral parotid hypertrophy (O.II.1), and respiratory CMV infection (R.II.5); eight of them were on immunosuppressive treatment. *Mycobacterium tuberculosis* polymerase chain reaction was positive in four affected mutation carriers, with two of them developing pulmonary or esophageal tuberculosis (A.II.8, A.II.9).

Fungal infections were present in 15 affected mutation carriers with either *Candida species pluralis* infections (n=13) or *Aspergillus species pluralis* pneumonia (n=2); thirteen of them received immunosuppressive treatment at the time of data collection. Ten affected mutation carriers, of whom eight were immunosuppressed, developed sepsis due to bacterial or fungal pathogens leading to death in five. In one affected mutation carrier sepsis followed a perforation of the small bowel, and in one *Salmonella enteritidis* sepsis was the first manifestation of CTLA-4 insufficiency at the age of three months (UU.IV.12).

Gastrointestinal involvement

Gastrointestinal involvement across our cohort was frequent (59%; 53/90) and often severe. Nine of the 15 deceased affected mutation carriers had severe gastrointestinal features prior to their death. Diarrhea was frequent (n=51), ranging from mild to severe diarrhea with weight loss, wasting, and

total parenteral nutrition-dependency. Pathogens were rarely identified. Crohn disease (n=7), atrophic gastritis (n=8) (Figure 6 Panel D), coeliac disease (JJ.II.1), acute pancreatitis (M.II.3), and pancreatic insufficiency (N.III.2, QQ.II.1) were observed. In three affected mutation carriers, severe long-lasting CVID-gastroenteropathy preceded gastric cancer (B.II.4, G.III.2, M.II.3). Macroscopic findings ranged from normal appearing mucosa albeit histologically proven deep T cell infiltrations in the submucosa, to superficial ulcerative lesions or deep-seated inflammatory changes as seen in severe Crohn's disease. Despite decreased serum immunoglobulin levels, histology revealed increased numbers of plasma cells in the gastric (B.II.4, QQ.II.1), intestinal, and colonic (QQ.II.1) lamina propria. Further histology changes included severe lymphocytic infiltrates, and EBV-positive gastric cancer (Figure 6 Panel C). Median age of onset of gastrointestinal features was 15 (<1 to 51) years.

Cytopenia

Autoimmune cytopenia was often severe, life-threatening, and treatment-resistant and formed the main indication for allogeneic hematopoietic stem cell transplantation (alloHSCT) (7/12).

Sixty-two percent of affected mutation carriers (55/89) had autoimmune cytopenia, including immune thrombocytopenia (n=41), autoimmune hemolytic anemia (n=37), pure red cell aplasia (PRCA) (n=2) or autoimmune neutropenia (n=16). In 32 affected mutation carriers cytopenia affected more than one cell lineage, nineteen of those were diagnosed with Evans syndrome, and nine had a trilineage cytopenia. Median age of onset of cytopenia was 12 (1.3 to 48) years.

Neurological involvement

Twenty-eight percent of affected mutation carriers (28/90) presented with a broad spectrum of neurological features (Figure 5 Panel F, G, H, Figure 6 Panel F). Three had autoimmune encephalitis or encephalomyelitis with cerebral perivascular lymphocytic infiltrations leading to vomiting, headache or paraplegia with bladder dysfunction (N.III.2, P.II.2, GG.II.1). In four affected mutation carriers neurological features were attributed to cerebral infiltrations that were not biopsied: nausea

and headache (A.III.1), facial nerve paralysis (H.II.1), aphasia and paresis of the left arm (K.II.1), or a patchy inflammatory demyelinating process with twitching episodes of hands with normal electroencephalography (WW.II.1). Three affected mutation carriers had neurological features secondary to hematological causes: hemiplegia following brain ischemia during AIHA (DD.II.1), hemiparesis and aphasia due to cerebral arterial thrombosis (H.II.2), hemiparesis following cerebral bleeding due to thrombocytopenia (GG.II.1). In two affected mutation carriers clinical and radiological investigation could not identify an underlying cause for tonic-clonic seizures, or recurrent transient paralysis of the left leg respectively (A.II.8, EE.II.1). One affected mutation carrier had life-threatening HLH with increased cerebral pressure leading to cerebral herniation and seizures (J.II.1). Other diagnoses were stiff person syndrome (H.I.2), West-syndrome and developmental delay (UU.V.1), progressive memory loss starting age 57 years (UU.III.7), and chronic hydrocephalus (UU.III.4). Two affected mutation carriers suffered from optic neuritis (TT.II.4) and retinal tear due to lymphocytic infiltrations into the retina (SS.II.1). One had gliosis (ZZ.II.1) and one developed cognitive dysfunction, chorea, ataxia, and mood instability; biopsies revealed inflammation, lymphocytic infiltrations, and a demyelinating-like transformation, which was clinically responsive to steroid treatment (G.III.1). One affected mutation carrier was diagnosed with tuberous sclerosis with tonic-clonic seizures, right-sided hemiparesis, mental retardation, angiofibromas, angiomyolipomas, and a concurrent TSCA2 mutation (LL.II.1).

Malignancies

Eleven affected mutation carriers (12%) developed malignancies. Out of eight with lymphoma, EBV-positivity was found in five. Lymphoma in five affected mutation carriers was classified as Hodgkin lymphoma; one developed a relapsing EBV-associated diffuse large B cell lymphoma (K.II.1) and one a Burkitt lymphoma (FF.II.1) (Figure 6 Panel G, H). Four affected mutation carriers died due to complications of their lymphoma, two underwent successful alloHSCT.

Three affected mutation carriers developed a gastric adenocarcinoma, including one EBV-associated carcinoma (B.II.4, Figure 6 Panel C), and one CMV-associated carcinoma (M.II.3). Two affected

mutation carriers subsequently underwent total gastrectomy, one of whom died following bacterial sepsis (M.II.3) while the other one is alive and well (G.III.2).

Fatal Outcome

Sixteen percent of affected mutation carriers (15/90) died due to their clinical manifestations or resulting complications at a median age of 23 (14 to 60) years. Four died of sepsis on a background of wasting enteropathy, Evans syndrome, or CVID with infections (M.II.3, G.III.1, C.II.3, L.I.2). Three died due to complications of Non-Hodgkin lymphoma (K.II.1, FF.II.1, UU.III.3), one died during chemotherapy of his Hodgkin lymphoma due to septic multi-organ failure (H.II.1), and two following lung transplantation and relapse of disease (A.II.9, B.III.2). One affected mutation carrier died of acute liver failure following many years of gastrointestinal disease (B.II.2). Wasting enteropathy, respiratory insufficiency, and neurological features led to death in one affected mutation carrier (A.II.8). Another one suffered from severe enteropathy and cytopenia, and died following colectomy (F.II.1). Three affected mutation carriers died following alloHSCT due to GvHD (Q.II.1, LL.II.1) or due to diabetic ketoacidosis (S.II.1). There was a significant difference of the age of death between affected and the unaffected *CTLA4* mutation carriers (Figure 2 B).

Immunological phenotype

Thirty-nine percent (26/66) of affected mutation carriers with available immunological data had lymphopenia of which twenty-four were under immunosuppressive treatment at the time of data collection. The absolute CD3+ T cell count was reduced in 36% (16/44) of affected mutation carriers. The absolute CD3+CD4+ helper T cell count was reduced in 20% (13/62) of affected mutation carriers especially due to the noteworthy reduction of naïve CD4+ T cells. An elevated percentage of the activation marker HLA-DR+ was seen in one third of tested affected mutation carriers (11/31). Percentage of CD4+FoxP3+ Tregs was significantly increased in mutation carriers in comparison to healthy controls (p=0.0034). There was no significant difference in the Treg percentage between affected and unaffected mutation carriers (p=0.3882). Eight mutation carriers had Treg numbers

457 above the normal range. Absolute CD3+CD8+ cytotoxic T cell count was normal in 60% (35/58) of
458 affected mutation carriers. Double-negative T cells were elevated up to 5.3% (median 2.2%; norm:
459 0.3-2.0%) in 53% of tested affected mutation carriers (9/17). Absolute CD19+ B cell counts were
460 reduced in 41% (26/58) of affected mutation carriers. B cell subsets showed a decrease in switched
461 memory B cells (23/30) and consecutively a relative increase in naïve B cells (14/29). CD21-low B cells
462 were elevated in all affected mutation carriers tested. Five affected mutation carriers with no history
463 of rituximab therapy had no measurable B cells. Hypogammaglobulinemia was present in 84%
464 (65/77), with low IgM in 30, low IgG in 42, and low IgA in 53 affected mutation carriers (Figure 4).
465 Absolute CD16+CD56+ NK cell counts were reduced in 52% (32/61). The percentage of CD3+ and
466 CD3+CD4+ was increased in the majority of affected mutation carriers, as the overall lymphopenia
467 affected CD3+CD8+, B, and NK cells more than the CD4 compartment (Figure S2). Antinuclear
468 autoantibodies (ANA) and anti-neutrophil cytoplasmic antibodies (ANCA) were the most commonly
469 measured autoantibodies; however, they were negative in most affected mutation carriers (ANA
470 (4/51), ANCA (3/42)).
471
472 ***Treatment***
473 *CTLA-4 fusion proteins and mTOR inhibitors*
474 CTLA-4 replacement by CTLA-4-Fc, or inhibition of the CD28 signaling pathway through mTOR
475 inhibitors are potential targeted therapies to inhibit the underlying hyper-active signaling in *CTLA4*
476 mutation carriers.
477 In total, fourteen affected mutation carriers received the CTLA-4 fusion proteins abatacept or
478 belatacept; eleven of whom responded with an improvement of their clinical symptoms. In six of
479 them enteropathy improved, leading to normal stool frequency and weight gain within three months
480 (B.II.4, D.II.1, L.II.2, HH.II.1, SS.II.1, VV.II.1, Figure S3). In two affected mutation carriers primarily
481 presenting with GLILD (RR.II.1, SS.II.1), CTLA4-Fc led to resolution of lymphoproliferation in the lung
482 (SS.II.1), cough and sputum production decreased, and sIL2R concentration dropped from 1228 U/ml
483 to 750 U/ml within five months (RR.II.1). Other observations were an improvement of

lymphadenopathy (G.III.2), stabilization of platelet counts, resolution of bleeding episodes, and regression of optic neuritis (TT.II.4). In two affected mutation carriers, additional systemic immunosuppressive medication could be reduced, as abatacept treatment led to inhibition of the disease progression (J.II.1) or to improvement of lung function and diarrhea (PP.II.1). In six affected mutation carriers treatment was discontinued: three underwent alloHSCT (L.II.2, VV.II.1, GG.II.1), two had an EBV reactivation (B.II.3, B.II.4), and one developed severe respiratory infections, neutropenia, and agranulocytosis (TT.II.4).

Thirteen affected mutation carriers were treated with the mTOR inhibitor sirolimus with a good response in eight (D.II.1, E.II.3, L.II.2, O.II.1, P.II.2, Z.III.1, TT.II.5, WW.II.1). Improvement of clinical features included resolution of transfusion-dependent PRCA (Z.III.1), regression of lymphadenopathy and splenomegaly, reduced IG consumption, and improved CMV viral load (O.II.1). Enteropathy improved in three affected mutation carriers following combination of sirolimus with either prednisolone (D.II.1), belatacept (L.II.2), or rituximab and steroids (WW.II.1). In one affected mutation carrier cytopenia stabilized on co-medication with rituximab, but neurological features and severe aphthae occurred (P.II.2). Sirolimus led to reduced spleen size (volume decreased from 5l to 2.8l) in one affected mutation carrier, who developed arthritis and erythema nodosum during the treatment (E.II.3). In two affected mutation carriers sirolimus treatment was discontinued due to ineffectiveness for cytopenia (GG.II.1), or due to increased blood pressure on the background of a renal impairment (B.II.4). In one affected mutation carrier CMV copies rose under sirolimus treatment in combination with methylprednisolone (DD.II.1), in one lymphopenia worsened (O.II.1), one died due to sepsis during sirolimus treatment (G.III.1), and in one sirolimus treatment was stopped due to serious respiratory infections (SS.II.1). Daily dosage ranged from 2 mg to 2.64 mg (n=5); trough levels were available for two affected mutation carriers (6,2 ng/ml and 8 ng/ml), for three affected mutation carriers target blood values were available (8-12 ng/ml (n=2); 12-15 ng/ml (n=1)).

Hematopoietic stem cell transplantation

Twelve affected mutation carriers underwent alloHSCT between 10 and 50 years of age.(15) Main indications for transplantation included treatment-resistant cytopenia, enteropathy, and Hodgkin lymphoma; often combined with other autoimmune manifestations, lymphoproliferation or severe infections. Nine of these affected mutation carriers are alive, of whom three are more than five years post-HSCT and currently well off all medication (L.II.1, T.II.1, Y.II.1), and six are between 100 days and 12 months post-transplant (B.II.3, L.II.2, P.II.2, W.II.2, GG.II.1, VV.II.1) (Table S2). In half of the affected mutation carriers the *CTLA4* mutation was known prior to transplantation (6/12), the other half was transplanted due to the severity of their symptoms and the *CTLA4* mutation was only identified after transplantation.

Immunoglobulin substitution

Sixty-three percent of affected mutation carriers (55/88) received immunoglobulin substitution either due to hypogammaglobulinemia or due to cytopenia. Twenty-eight affected mutation carriers had both diagnoses at the time of data collection and received immunoglobulin substitution due to both.

Additional treatment options can be found in the Supplements.

Chromosome 2 contiguous gene deletion involving CTLA4

Two unrelated individuals have a heterozygous 2q33.2-2q33.3 deletion involving *CTLA4* and present a CTLA-4 insufficiency-like phenotype, which is possibly influenced by the deletion of additional genes including *CD28* and *ICOS* (Supplements).

Mutation carriers who did not seek medical attention

We identified 43 unaffected family members carrying the same *CTLA4* mutation as their affected relatives. The treating physician of the affected mutation carrier classified family members as unaffected if they did not repeatedly seek medical care, were not under a long-term drug regimen

due to CTLA-4 insufficiency-related symptoms, or if they were not restricted in their health-related quality of life due to their symptoms. Their median age at evaluation was 46 (6 to 87) years, hence in most cases beyond the median age of manifestation. Upon thorough questioning and clinical investigation, seven carriers had diarrhea without weight loss, two had atrophic gastritis or pernicious anemia, and one had coeliac disease. Three carriers had respiratory infections and in one clinically unapparent pulmonary nodules were detected on a routine scan. Nine had dermatological involvement (psoriasis, eczema, vitiligo), and two hypothyroidism. One carrier developed colon cancer aged 78, which was successfully treated by surgery but is otherwise healthy at currently 87 years of age (A.I.2). Four carriers (without recurrent infections) had IgA-deficiency, one each had low IgG or IgM, and one had low IgA and IgM, possibly contributing to respiratory infections (R.III.1). Twenty-six carriers were reported to be clinically completely healthy.

Their immunological phenotyping revealed similarities to affected mutation carriers, including a decrease in NK and CD19+ B cells, but also differences, including significantly higher CD4+ T cells counts, and a higher percentage of switched memory B cells. There was no significant difference with regard to the Treg percentages in affected mutation carriers (Figure S2).

Discussion

In our work, we estimate the clinical penetrance of CTLA-4 insufficiency to be around 67%; however, as genetic analysis could not be performed in all healthy first degree family members, ascertainment was incomplete. Once symptoms have occurred, the clinical course can be severe and was fatal in 15 affected mutation carriers (16%).

The clinical phenotype was characterized by infections, autoimmunity, and lymphoproliferation, affecting various organ systems. Affected mutation carriers have an elevated risk to develop malignancies and for EBV reactivation highlighting the importance of monitoring EBV and possibly CMV viral load, especially under immunosuppressive treatment. Cytopenia and enteropathy were the most life-threatening and treatment-resistant manifestations. This is evidenced by the fact that cytopenia was one of the main indications for alloHSCT (7/12), and half of the deceased affected mutation carriers died following a history of enteropathy and associated complications. Initial symptoms were diverse, emphasizing the importance of raising awareness of this immunodeficiency not only among immunologists but also other specialists including hematologists, neurologists, gastroenterologists, pathologists, dermatologists, and chest physicians. As the age of onset in 75% of affected mutation carriers is under the age of 18 years, CTLA-4 insufficiency should be considered in children with severe immune dysregulation of unknown origin. Also in individuals being evaluated for IBD, CVID, and ALPS, CTLA-4 insufficiency should be considered.

To diagnose CTLA-4 insufficiency, we recommend sequencing the four exons of *CTLA4* and then testing the effect of identified mutations on the protein by measuring CTLA-4 expression or CTLA-4-mediated transendocytosis.(24) Both were reduced in all analyzed mutation carriers, but because this is also seen in individuals with mutations in other genes such as *LRBA*(25), these functional tests cannot be used as the only diagnostic tool to screen for *CTLA4* mutations. In addition to the clinical presentation, an autosomal dominant family history can hint towards CTLA-4 insufficiency.

The immunological phenotype revealed perturbed T and B cell homeostasis and significantly increased Treg percentages within the CD4+ T cell compartment. The latter may be a transient or permanent compensatory mechanism of the CTLA-4-deficient immune system to counteract the immune-activation. The expanded and activated effector T cells may produce a cytokine profile leading to an increased Treg cell polarization in order to counterbalance the accelerated immune activation. Elevated double negative T cell counts in *CTLA4* mutation carriers should prompt investigators to evaluate proposed ALPS diagnostic criteria. (26)

We present first insights into targeted therapeutic strategies: Out of thirteen affected mutation carriers treated with CTLA-4-Fc, eleven responded favorably, especially enteropathy improved. Further clinical studies are necessary to determine the effectiveness and safety of CTLA-4-Fc treatment for individual clinical manifestations. Out of twelve affected mutation carriers undergoing alloHSCT, nine are alive and well (15); although long-term survival still has to be determined, alloHSCT should be considered as a treatment option in carefully selected affected mutation carriers.

In individuals presenting with immunodeficiency, autoimmunity, and lymphoproliferation with impaired Treg development or function, besides CTLA-4 insufficiency, also mutations in *FoxP3, LRBA, IL2RA*, *FAS-L, FAS, PI3K, NFKB1 and 2, STAT3,* and *STAT5b* should be considered as a differential diagnosis.(6), (25, 27-34) Mutations in *FOXP3* lead to a loss of Treg cells and cause IPEX which is an X-linked condition and characterized by enteropathy, immune dysregulation, and polyendocrinopathy, but has an earlier onset, and complete penetrance.(27, 34) Immunological findings in IPEX-syndrome include normal lymphocyte counts and immunoglobulin levels in contrast to CTLA-4 insufficiency. In LRBA deficiency lysosomal CTLA-4 degradation is accelerated and CTLA-4 trafficking to the cell surface is disturbed; hence the inhibitory function of Treg cells is impaired. (25) Biallelic *LRBA* mutations most often lead to complete absence of the LRBA protein; affected mutation carriers present with a phenotype very similar to CTLA-4 insufficiency, characterized by various autoimmune features, lymphoproliferation with dysregulated Treg function, and a defect in production cell homeostasis,(28, 31, 32, 35-44) albeit with an earlier onset, complete penetrance and an autosomal

recessive inheritance. In addition, germline gain-of-function mutations in *STAT3* lead to a broad range of autoimmune disorders such as autoimmune cytopenias and multiorgan autoimmunity (lung, gastrointestinal, hepatic, and endocrine), in combination with an increased susceptibility to infections and a short stature. Further, *STAT3* gain-of-function mutations lead to secondary defects in STAT5 and STAT1 phosphorylation and impair the Treg compartment.(29, 33)

As our results were collected retrospectively, several limiting factors should be considered: affected mutation carriers were treated and evaluated by different physicians and medical departments worldwide. This can lead to an incomplete picture of the clinical phenotype. Also, data was collected at one time point, which often makes it difficult to reconstruct whether symptoms or the immunological phenotype are due to immunosuppressive treatment or the natural course of this immunodeficiency.

In LRBA deficiency, sIL2R, a biomarker for T cell-mediated inflammation, decreases on abatacept treatment.(25) In our cohort sIL2R was only sporadically measured; in one affected mutation carrier sIL2R levels dropped while being on abatacept treatment. Systematical measurement of sIL2R should be considered in all mutation carriers to see whether it indicates disease activity. Affected and unaffected mutation carriers both show impaired *in vitro* CTLA-4 function, indicating the presence of additional factors influencing the clinical phenotype and penetrance such as environmental, genetic, or epigenetic differences. Ethnicity and origin of the mutation carriers could influence age of onset, penetrance, and severity of disease-related symptoms. We cannot assess this, as the world-wide distribution in our study is not equal and the diverse countries of origin varied in diagnostic procedures and standards. In general, there could either be one single modifier, or multiple interacting factors influencing the clinical phenotype. The latter could explain the highly variable expressivity of the phenotype. Another hypothesis suggests an internal threshold within the immune system of CTLA-4-insufficient individuals. Once it is exhausted, immune dysregulation cannot be contained by the organism and individuals develop symptoms; this could explain why healthy mutation carriers may develop life-threatening symptoms late in life (e.g. patient B.II.3 developed

hemophagocytic lymphohistiocytosis and Hodgkin lymphoma at the age of 50 years). These cases teach us to carefully monitor all first-degree relatives for CTLA-4-associated disease activity, while the search for modifying factors in CTLA-4 insufficiency continues.

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Declaration of interest

The authors declare no competing financial or personal interests.

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Figure legends

Figure 1. Pedigrees of families with CTLA-4 insufficiency

Pedigrees of all families with more than one *CTLA4* mutation carrier. Squares, male subjects; circles, female subjects; black filled symbols, mutation carriers classified as affected; gray filled symbols, mutation carriers classified as unaffected; slashed symbols, deceased subjects; *, sequencing of *CTLA4* was performed; §, genotype inferred from clinical symptoms.

Figure 2. Age of onset and age of death in CTLA-4 insufficient individuals

A. Kaplan Meier curve of age of onset of *CTLA4* mutation carriers (n=85).

B. Age of death in affected (n=86) versus unaffected mutation carriers (n=39).

Figure 3. Heterozygous germline mutations within the *CTLA4* gene are distributed throughout exon 1-3.

Figure 3 shows the distribution of the heterozygous germline mutations throughout the *CTLA4* gene. Eight mutations are located in exon 1, 31 are located in exon 2, and six are located in exon 3. §, mutation was functionally tested by transendocytosis assay.

Figure 4. Main clinical findings in CTLA-4 insufficiency

Percentage distribution of clinical manifestations within affected mutation carriers. Clinical data was available for 71 to 90 affected mutation carriers.

Figure 5. Exemplary findings upon CT and MRI in CTLA-4-insufficient individuals

Panel A: splenomegaly (17.5 cm in diameter) and lymphadenopathy in A.III.3. Panel B: large pneumatocele following necrotizing pneumonia in PP.II.1. Panel C: CT scan of ZZ.II.1 showing peripheral bronchiectasis with inflammatory nodules in all lobes of the lung. Panel D: bronchiectasis with peribronchial ground glass nodules in keeping with bronchiolitis in XX.II.1. Panel E: multiple inflammatory nodules in O.II.1. Panel F: signal change in the right temporal lobe and cerebellum consistent with inflammation in KK.II.1. Panel G: enhancement in the thoracic cord in keeping with

849 inflammation in KK.II.1. Panel H: signal change and swelling in the cerebellum in keeping with
850 inflammation in P.II.2.
851
852 **Figure 6. Lymphocytic infiltrations and loss of EBV control define the spectrum of**
853 **inflammatory and neoplastic lesions**
854 Panel A and B: lung samples of PP.II.1 and KK.II.1 with follicular bronchitis/ bronchiolitis,
855 respectively. Lymphoid follicles are marked by asterisks. In Panel A, the follicle contains a germinal
856 center. Panel C: EBV-coded small RNAs (EBER) positive nuclei (dark blue staining) of an early
857 invasive gastric adenocarcinoma of B.II.4. Panel D: autoimmune gastritis with severely atrophic
858 mucosa of the stomach, antral metaplasia and numerous intraepithelial CD8+ T cells (brown staining)
859 of B.II.4. Panel E: nodular T cell lymphocytosis (brown staining) in the bone marrow of Z.II.2. Panel
860 F: perivascular lymphocytes in the brain tissue of KK.II.1 (arteriolar wall highlighted by arrowhead,
861 lumen marked by asterisk). Panel G and H: Hodgkin lymphoma in a lymph node excision sample of
862 MM.II.1. Reed-Sternberg cell is highlighted by an arrowhead (G) or CD30 immunohistochemistry
863 (red staining in H). Nuclei of Hodgkin cells and Reed-Sternberg cells were positive for EBER (dark
864 blue staining, inlet H).
865

Table 1. Baseline description of CTLA-4-insufficient individuals

Subject No.	Case No.	Age of on-set	Age at Evaluation/ Death Δ	Sex	Country of origin	CTLA4-/+ cDNA position; Predicted Amino Acid change	Type of mutation	Reference
1	A.I.2	#	87	F	Germany ¶	c.105C>A; p.C35*; §	Nonsense	Schubert *et al.* (2)
2	A.II.2	#	60	M	Germany ¶	c.105C>A; p.C35*; §	Nonsense	Schubert *et al.* (2)
3	A.II.3	#	59	F	Germany ¶	c.105C>A; p.C35*; §	Nonsense	Schubert *et al.* (2)
4	A.II.5	41	56	M	Germany ¶	c.105C>A; p.C35*; §	Nonsense	Schubert *et al.* (2)
5	A.II.8	12	34 Δ	M	Germany ¶	Φ		Schubert *et al.* (2)
6	A.II.9	17	37 Δ	F	Germany ¶	c.105C>A; p.C35*; §	Nonsense	Schubert *et al.* (2)
7	A.II.10	#	49	M	Germany ¶	c.105C>A; p.C35*; §	Nonsense	Schubert *et al.* (2)
8	A.III.1	10	28	F	Germany ¶	c.105C>A; p.C35*; §	Nonsense	Schubert *et al.* (2)
9	A.III.3	15	20	M	Germany ¶	c.105C>A; p.C35*; §	Nonsense	Schubert *et al.* (2)
10	A.III.5	#	23	F	Germany ¶	c.105C>A; p.C35*; §	Nonsense	Schubert *et al.* (2)
11	A.III.6	#	20	F	Germany ¶	c.105C>A; p.C35*; §	Nonsense	Schubert *et al.* (2)
12	B.I.1	#	66 Δ	M	Germany ¶	Φ		Schubert *et al.* (2)
13	B.II.1	#	57	F	Germany ¶	c.109+1G>T; §	Splice-site	Schubert *et al.* (2)
14	B.II.2	15	23 Δ	M	Germany ¶	Φ		Schubert *et al.* (2)
15	B.II.3	50	51	M	Germany ¶	c.109+1G>T; §	Splice-site	Schubert *et al.* (2)
16	B.II.4	34	43	F	Germany ¶	c.109+1G>T; §	Splice-site	Schubert *et al.* (2)
17	B.III.2	10	16 Δ	F	Germany ¶	Φ		Schubert *et al.* (2)
18	B.III.3	#	17	F	Germany ¶	c.109+1G>T; §	Splice-site	Unpublished
19	C.II.3	7	20 Δ	F	Greece ¶	c.208C>T; p.R70W; §	Missense	Schubert *et al.* (2)
20	C.II.4	#	13	F	Greece ¶	c.208C>T; p.R70W; §	Missense	Schubert *et al.* (2)
21	D.I.2	#	43	F	India/UK †	c.371A>C; p.T124P; §	Missense	Schubert *et al.* (2)
22	D.II.1	10	22	F	India/UK †	c.371A>C; p.T124P; §	Missense	Schubert *et al.* (2)
23	E.II.3	10	22	F	Georgia ¶	c.223C>T; p.R75W; §	Missense	Schubert *et al.* (2)
24	F.II.1	8	23 Δ	M	Germany ¶	c.2T>C; p.?; §	Missense	Schubert *et al.* (2)
25	G.II.1	#	53	F	USA¶	c.179; A<G; p.Y60C	Missense	Zeissig *et al.* (3)
26	G.III.1	12	24 Δ	F	USA¶	c.179; A<G; p.Y60C	Missense	Zeissig *et al.* (3)
27	G.III.2	1.83	22	M	USA¶	c.179; A<G; p.Y60C	Missense	Zeissig *et al.* (3)
28	H.I.2	22	52	F	Germany ¶	c.407C>T; p.P136L	Missense	Unpublished
29	H.II.1	10	21 Δ	M	Germany ¶	Φ		Unpublished
30	H.II.2	7	26	M	Germany ¶	c.407C>T; p.P136L	Missense	Unpublished
31	J.I.2	#	50	F	Germany ¶	c.373G>A; p.G125R	Missense	Unpublished
32	J.II.1	11	22	M	Germany ¶	c.373G>A; p.G125R	Missense	Unpublished
33	K.II.1	26	53 Δ	F	Germany ¶	c.308G>C; p.C103S	Missense	Unpublished
34	L.I.2	20	40 Δ	F	UK ¶	c.437G>T; p.G146V	Missense	Slatter, *et al.* (15)
35	L.II.1	5	20	F	UK ¶	c.437G>T; p.G146V	Missense	Slatter, *et al.* (15)
36	L.II.2	14	16	M	UK ¶	c.437G>T; p.G146V	Missense	Slatter, *et al.* (15)
37	M.II.3	10	35 Δ	M	Japan †	c.76_77insT; p.F28Sfs*40	Frameshift	Hayakawa*et al.*(16)
38	N.I.2	#	71	F	Japan †	c.529_530insA; p.Y177*	Nonsense	Unpublished
39	N.II.1	#	47	F	Japan †	c.529_530insA; p.Y177*	Nonsense	Unpublished
40	N.II.3	#	42	M	Japan †	c.529_530insA; p.Y177*	Nonsense	Unpublished
41	N.III.2	10	10	M	Japan †	c.529_530insA; p.Y177*	Nonsense	Unpublished
42	O.II.1	8	13	M	Spain ¶	c.342_342delC; p.T115Lfs*5	Frameshift	Unpublished
43	P.II.2	2	13	M	Germany ¶	c.534C>G; p.S178R	Missense	Unpublished
44	Q.II.1	10	15 Δ	M	UK ¶	c.529T>G; p.Y177D	Missense	Slatter, *et al.* (15)
45	R.II.5	24	44	F	Italy ¶	c.410C>T; p.P137L	Missense	Unpublished
46	R.III.1	#	18	F	Italy ¶	c.410C>T; p.P137L	Missense	Unpublished
47	S.II.1	2	22	M	UK ¶	c.410C>G; p.P137R	Missense	Slatter, *et al.* (15)
48	T.II.1	1.5	21	M	UK ¶	c.518G>A; p.G173E	Missense	Slatter, *et al.* (15)
49	U.I.1	#	40	M	Japan †	c.494G>A; p.W165*	Nonsense	Unpublished
50	U.II.1	3.75	9	M	Japan †	c.494G>A; p.W165*	Nonsense	Unpublished
51	U.II.2	#	8	M	Japan †	c.494G>A; p.W165*	Nonsense	Unpublished
52	U.II.3	#	6	F	Japan †	c.494G>A; p.W165*	Nonsense	Unpublished
53	V.II.1	9	14	F	Japan †	c.436G>A; p.G146R	Missense	Unpublished
54	W.I.1	19	43	M	Japan †	c.34C>T; p.Q12*	Nonsense	Unpublished
55	W.II.1	#	16	M	Japan †	c.34C>T; p.Q12*	Nonsense	Unpublished
56	W.II.2	9	14	F	Japan †	c.34C>T; p.Q12*	Nonsense	Unpublished
57	W.II.3	4	6	F	Japan †	c.34C>T; p.Q12*	Nonsense	Unpublished
58	X.I.2	#	55	F	USA ‡	c.223C>T; p.R75W; §	Missense	Kucuk *et al.* (18)
59	X.II.1	6	15	F	USA ‡	c.223C>T; p.R75W; §	Missense	Kucuk *et al.* (18)
60	Y.I.1	uk	49	M	Germany ¶	c.226C>T; p.Q76*	Nonsense	Unpublished
61	Y.II.1	10	20	M	Germany ¶	c.226C>T; p.Q76*	Nonsense	Unpublished
62	Z.I.2	#	81	F	Norway ¶	c.94_101delinsTTCTCTTCATCA; p.P32Ffs*29	Frameshift	Unpublished

63	Z.II.1	#	50	F	Norway ¶	c.94_101delinsTTCTCTTCATCA; p.P32Ffs*29	Frameshift	Unpublished
64	Z.II.2	43	49	M	Norway ¶	c.94_101delinsTTCTCTTCATCA; p.P32Ffs*29	Frameshift	Unpublished
65	Z.II.3	#	uk	F	Norway ¶	c.94_101delinsTTCTCTTCATCA; p.P32Ffs*29	Frameshift	Unpublished
66	Z.II.6	#	uk	M	Norway ¶	c.94_101delinsTTCTCTTCATCA; p.P32Ffs*29	Frameshift	Unpublished
67	Z.III.1	16	21	F	Norway ¶	c.94_101delinsTTCTCTTCATCA; p.P32Ffs*29	Frameshift	Unpublished
68	AA.III.3	#	46	M	Japan †	c.155G>V; p.G52V	Missense	Unpublished
69	AA.IV.1	18	18	M	Japan †	c.155G>V; p.G52V	Missense	Unpublished
70	BB.I.2	uk	45	F	Japan †	c.119T>C; p.V40A	Missense	Unpublished
71	BB.II.1	#	20	F	Japan †	c.119T>C; p.V40A	Missense	Unpublished
72	BB.II.2	10	17	F	Japan †	c.119T>C; p.V40A	Missense	Unpublished
73	CC.II.1	10	43	F	Japan †	c.25_26insACAAGGCTCAGCTG; p.N14Tfs*5	Frameshift	Unpublished
74	DD.I.2	#	37	F	Japan †	c.232_232delG; p.D78Tfs*4	Frameshift	Unpublished
75	DD.II.1	13	15	M	Japan †	c.232_232delG; p.D78Tfs*4	Frameshift	Unpublished
76	EE.II.1	11	18	M	TheNetherlands¶	c.436G>T; p.G146*	Nonsense	Unpublished
77	FF.II.1	6	22 Δ	M	USA	c.208C>T; p.R70W; §	Missense	Unpublished
78	GG.I.1	#	47	M	Germany ¶	c.347T>C; p.I116T; §	Missense	Unpublished
79	GG.II.1	9	20	F	Germany ¶	c.347T>C; p.I116T; §	Missense	Unpublished
80	GG.II.2	#	18	M	Germany ¶	c.347T>C; p.I116T; §	Missense	Unpublished
81	GG.II.3	#	14	F	Germany ¶	c.347T>C; p.I116T; §	Missense	Unpublished
82	HH.II.1	2	28	F	USA ‡	c.254G>A; p.C85Y	Missense	Unpublished
83	JJ.II.1	11	28	M	Germany ¶	c.223C>T; p.R75W; §	Missense	Unpublished
84	KK.I.1	#	58	M	Czech Republic ¶	c.402_415del; p.M123Ifs*15	Frameshift	Unpublished
85	KK.II.1	21	36	F	Czech Republic ¶	c.402_415del; p.M123Ifs*15	Frameshift	Unpublished
86	LL.II.1	1	14 Δ	F	Czech Republic ¶	c.407C>T; p.P136L	Missense	Unpublished
87	MM.II.1	14	38	M	Germany¶	c.530_543del; p.F179Cfs*29	Framshift	Unpublished
88	NN.I.1	12	61	M	Uruguay¶	c.280G>T; p.E94*	Nonsense	Unpublished
89	NN.II.1	#	uk	F	Uruguay¶	c.280G>T; p.E94*	Nonsense	Unpublished
90	NN.II.6	23	29	F	Uruguay¶	c.280G>T; p.E94*	Nonsense	Unpublished
91	NN.II.8	13	20	F	Uruguay¶	c.280G>T; p.E94*	Nonsense	Unpublished
92	NN.II.9	18	23	M	Uruguay¶	c.280G>T; p.E94*	Nonsense	Unpublished
93	NN.II.10	#	17	F	Uruguay¶	c.280G>T; p.E94*	Nonsense	Unpublished
94	NN.II.11	6	21	M	Uruguay¶	c.280G>T; p.E94*	Nonsense	Unpublished
95	OO.II.1	18	24	M	Germany¶	c.224G>A; p.R75Q, §	Missense	Unpublished
96	PP.II.1	8	40	M	Canada¶	c.406C>T; p.P136S	Missense	Unpublished
97	QQ.II.1	13	31	M	Germany¶	c.410C>T; p.P137L	Missense	Unpublished
98	RR.II.1	14	16	F	USA¶	c.356T>G; p.L119R	Missense	Unpublished
99	SS.II.1	15	27	F	USA¶	c.436G>A; p.G146R	Missense	Unpublished
100	TT.I.1	5	50	M	Czech Republic¶	c.178T>A; p.Y60N	Missense	Unpublished
101	TT.II.2	21	26	M	Czech Republic¶	c.178T>A; p.Y60N	Missense	Unpublished
102	TT.II.3	11	24	F	Czech Republic¶	c.178T>A; p.Y60N	Missense	Unpublished
103	TT.II.4	4	10	M	Czech Republic¶	c.178T>A; p.Y60N	Missense	Unpublished
104	TT.II.5	1	6	M	Czech Republic¶	c.178T>A; p.Y60N	Missense	Unpublished
105	UU.II.1	#	86 Δ	F	Spain¶	Φ		Unpublished
106	UU.II.2	40	73	F	Spain¶	Φ		Unpublished
107	UU.III.2	6	65	F	Spain¶	c.223C>T; p.R75W; §	Missense	Hou *et al.* (17)
108	UU.III.3	59	60 Δ	F	Spain¶	Φ		Unpublished
109	UU.III.4	57	68	M	Spain¶	c.223C>T; p.R75W; §	Missense	Hou *et al.* (17)
110	UU.III.6	#	62	F	Spain¶	c.223C>T; p.R75W; §	Missense	Hou *et al.* (17)
111	UU.III.7	14	63	F	Spain¶	c.223C>T; p.R75W; §	Missense	Hou *et al.* (17)
112	UU.III.9	uk	55	M	Spain¶	c.223C>T; p.R75W; §	Missense	Hou *et al.* (17)
113	UU.III.10	#	53	F	Spain¶	c.223C>T; p.R75W; §	Missense	Hou *et al.* (17)
114	UU.IV.1	#	46	M	Spain¶	c.223C>T; p.R75W; §	Missense	Hou *et al.* (17)
115	UU.IV.2	31	42	M	Spain¶	c.223C>T; p.R75W; §	Missense	Hou *et al.* (17)
116	UU.IV.3	#	40	F	Spain¶	c.223C>T; p.R75W; §	Missense	Hou *et al.* (17)
117	UU.IV.4	#	33	M	Spain¶	c.223C>T; p.R75W; §	Missense	Hou *et al.* (17)
118	UU.IV.9	#	40	M	Spain¶	c.223C>T; p.R75W; §	Missense	Hou *et al.* (17)
119	UU.IV.10	uk	uk	M	Spain¶	c.223C>T; p.R75W; §	Missense	Hou *et al.* (17)
120	UU.IV.12	0.25	34	F	Spain¶	c.223C>T; p.R75W; §	Missense	Hou *et al.* (17)
121	UU.V.1	0.25	10	M	Spain¶	c.223C>T; p.R75W; §	Missense	Hou *et al.* (17)
122	UU.V.2	0.83	3	M	Spain¶	c.223C>T; p.R75W; §	Missense	Hou *et al.* (17)
123	VV.I.1	#	uk	M	Saudi Arabia¶	c.359_359delG; p.A121fs*23	Frameshift	Unpublished
124	VV.II.1	7	13	M	Saudi Arabia¶	c.359_359delG; p.A121fs*23	Frameshift	Unpublished

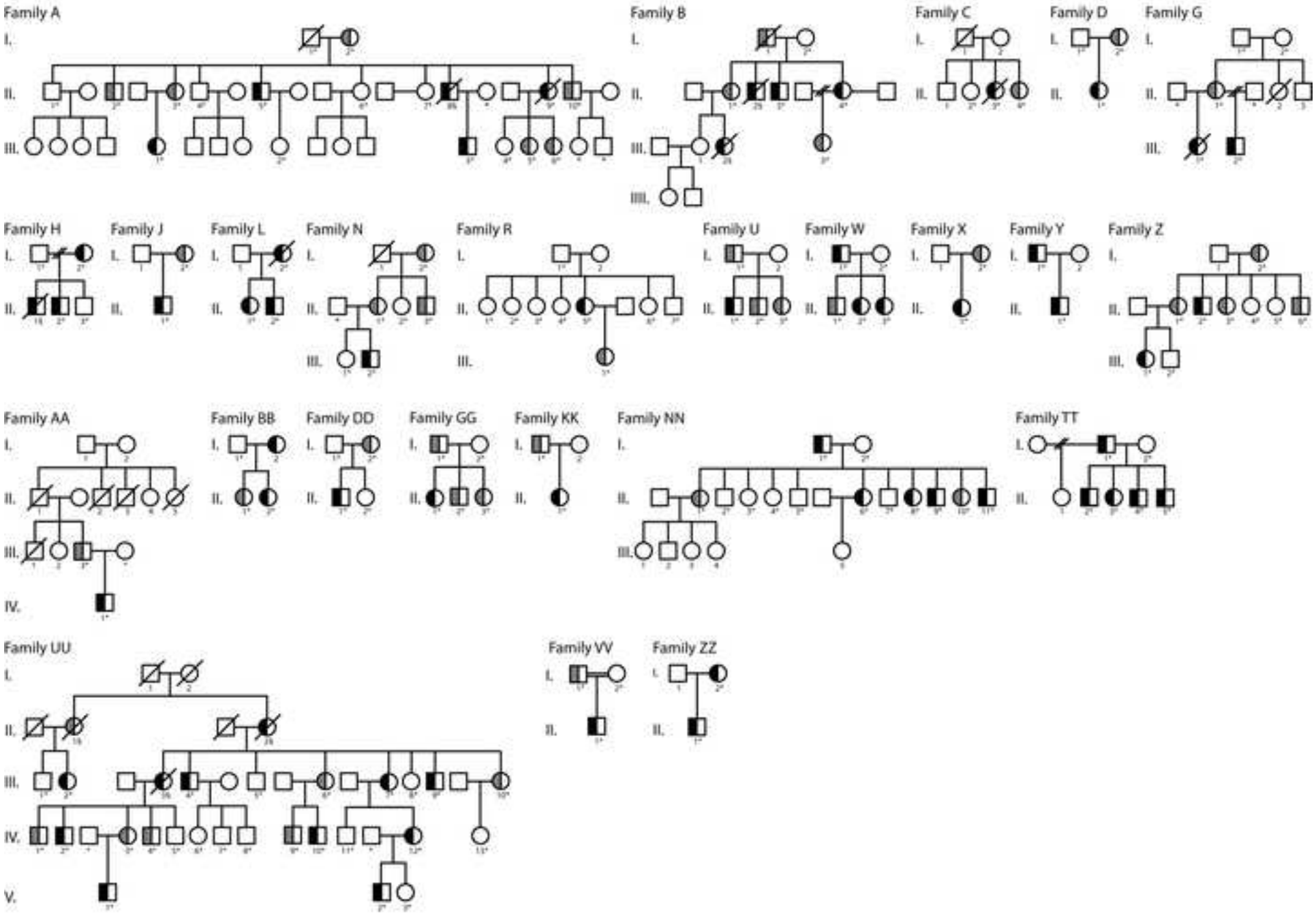
125	WW.II.1	8	12	M	UK¶	c.410C>G; p.P137R	Missense	Unpublished
126	XX.II.1	12	40	M	Belgium¶	c.407C>T; p.P136L	Missense	Unpublished
127	YY.II.1	3	14	F	Germany¶	c.326G>A; p.G109E; §	Missense	Unpublished
128	ZZ.I.2	uk	39	F	Germany¶	c.151C>T; p.R51*	Nonsense	Unpublished
129	ZZ.II.1	16	19	M	Germany¶	c.151C>T; p.R51*	Nonsense	Unpublished
130	AAA.II.1	23	46	M	Switzerland¶	c.257C>T; p.A86V; §	Missense	Navarini *et al.* (19)
131	BBB.II.1	1	17	F	USA¶	c.56_57insCTGG; p.T19Tfs*42	Frameshift	Unpublished
132	CCC.II.1	14	14	M	USA¶	c.406C>G;p.P136A	Missense	Unpublished
133	DDD.II.1	38	38	F	USA¶	c.173G>T; p.C58F	Missense	Unpublished
[Chr2_1	P1	5	37	F	Canada¶	2q33.2-2q33.3	Deletion	Unpuplished] Ω
[Chr2_2	P2	14	20	M	Australia¶	2q33.2-2q33.3	Deletion	Unpublished] Ω
Total no: 133	**54 different families**		**Penetrance** 90 affected mutation carriers 67.6%	**66 Female 67 Male**		**45 different mutations 28 novel mutations**		**82 unpublished mutation carriers**

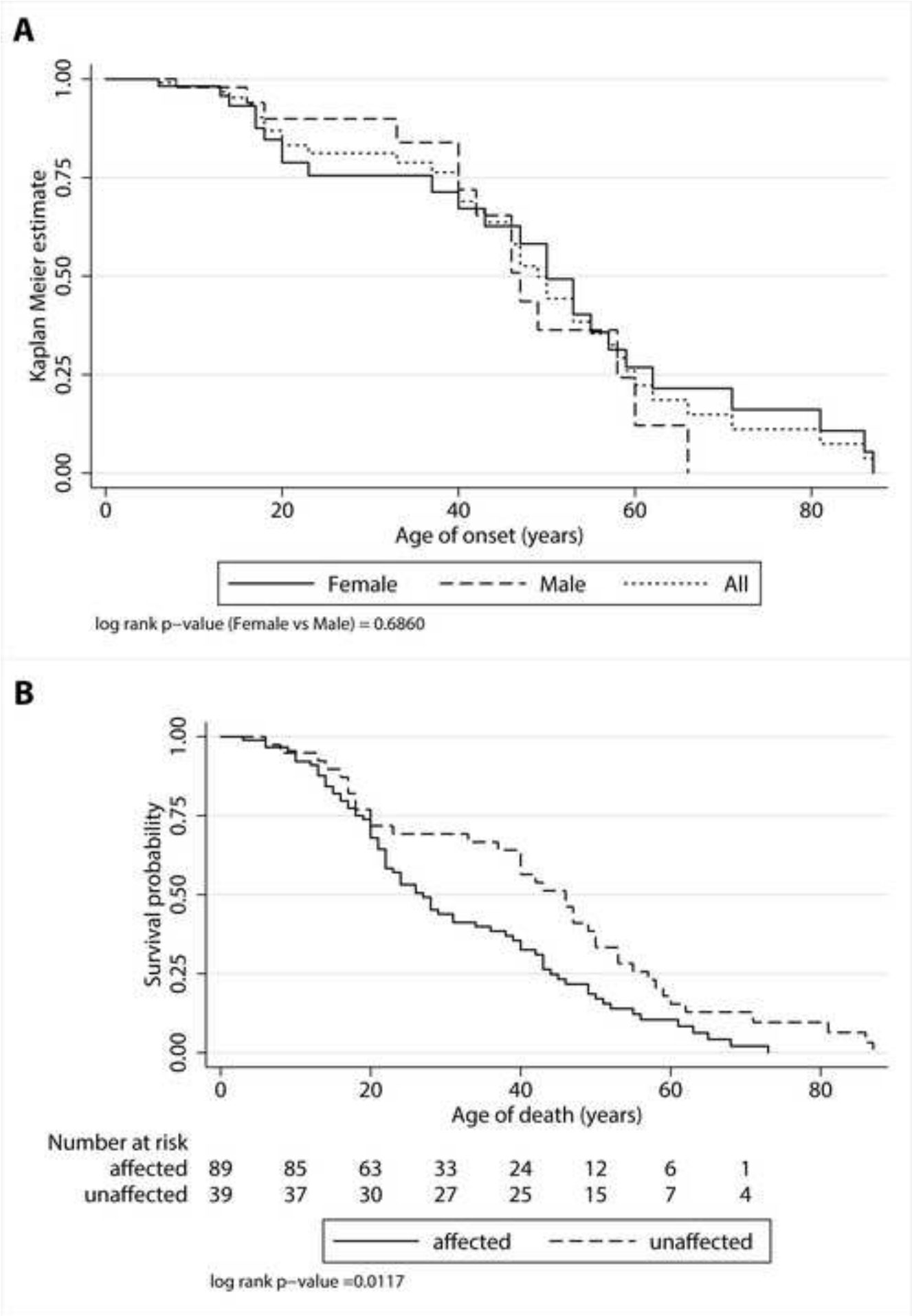
= unaffected Mutation carrier; Φ = died prior to being genotyped; Δ = deceased due to disease associated manifestations or complications; age at death is shown; ¶= Caucasian; †= Asian; ‡ = African-American; § = disease causing effect of the mutation is functionally proven by transendocytosis assay (Figure S2); Ω = P1 and P2 with Chromosome 2 contiguous gene deletion involving *CTLA4* are not included within all calculations of the clinical spectrum. UK = United Kingdom, uk = unknown. F = female, M =male, USA = United States of America

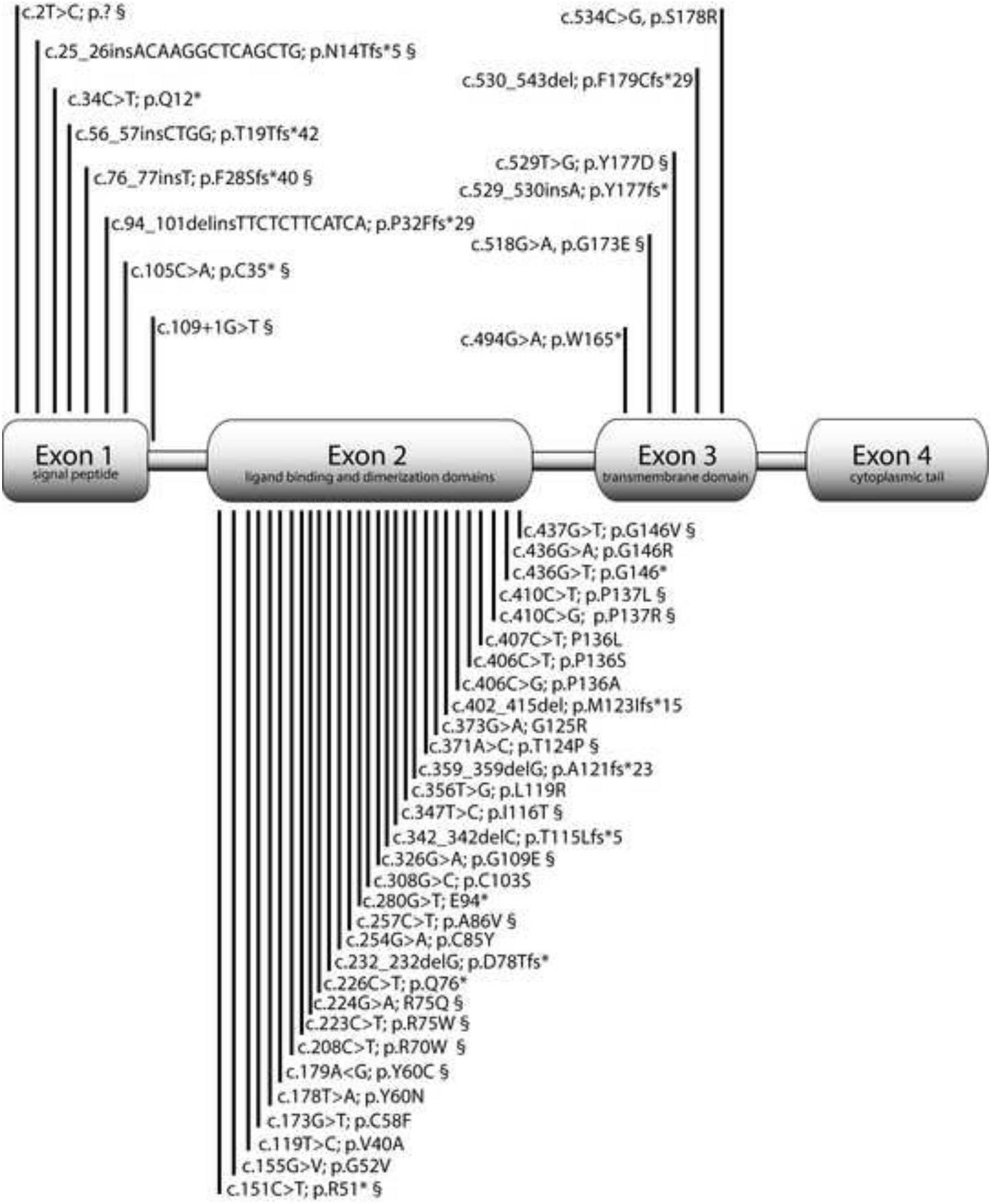
Table 2. Mutations identified in multiple families.

Exon	AA position	Mutations	Families
2	60	p.Y60C (c.179A>G); p.Y60N (c.178T>A)	Family G; Family TT
2	70	p.R70W (c.208C>T)	Family C, Family FF
2	75	p.R75W (c.223C>T); p.R75Q (c.224G>A)	Family E, Family X, Family JJ, Family UU; Family OO
2	136	p.P136L (c.407C>T); p.P136A (c.406C>G); p.P136S (c.406C>T)	Family H, Family LL, Family XX; Family CCC; Family PP
2	137	p.P137L (c.410C>T); p.P137R (c.410C>G)	Family R, Family QQ; Family S, Family WW
2	146	p.G146* (c.436G>T); p.G146R (c.436G>A); p.G146V (c.437G>T)	Family EE; Family V, Family SS; Family L
2	177	p.Y177* (c.529_530insA); p.Y177D (c.529T>G)	Family N; Family Q

At seven loci mutations were identified in multiple families.







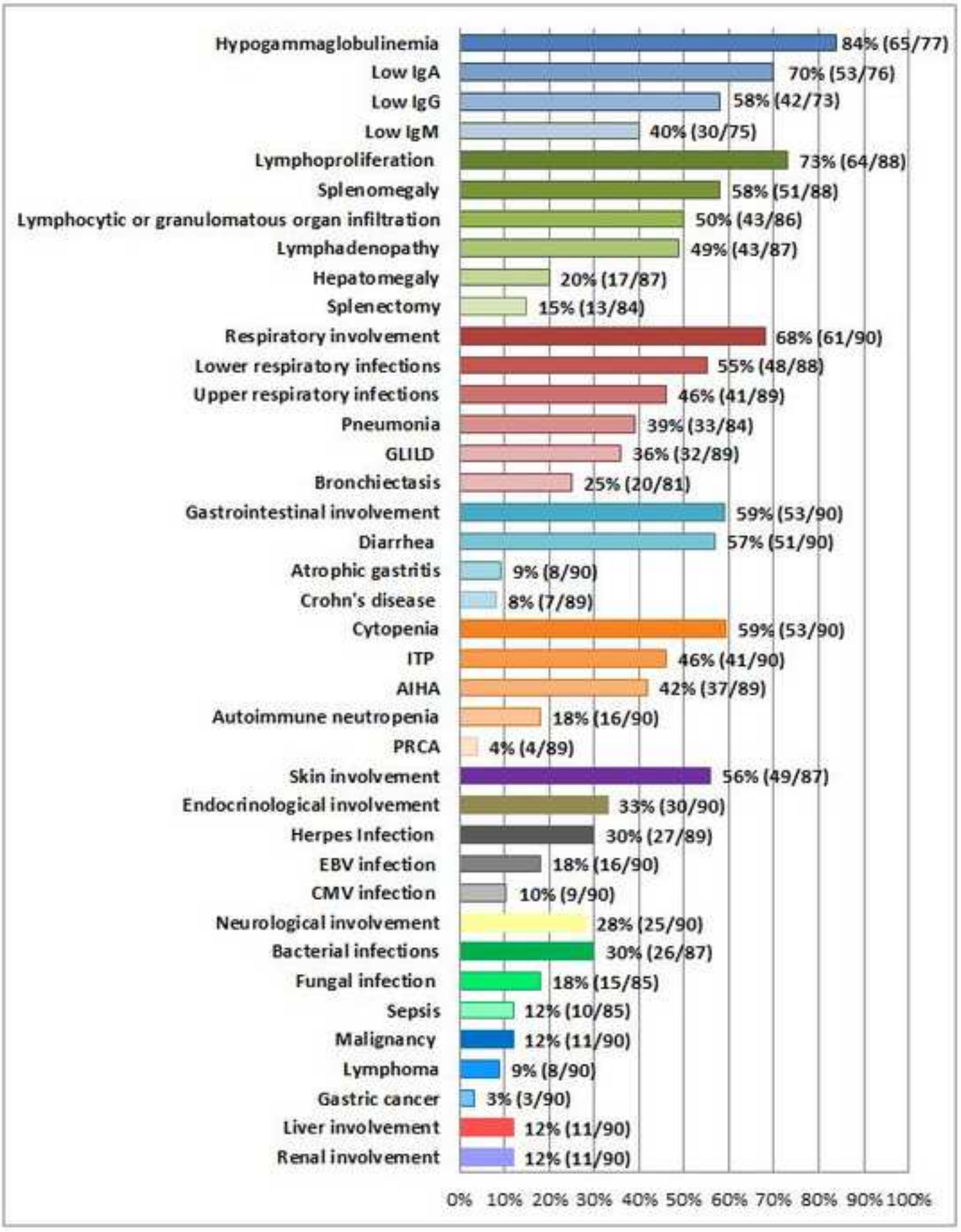
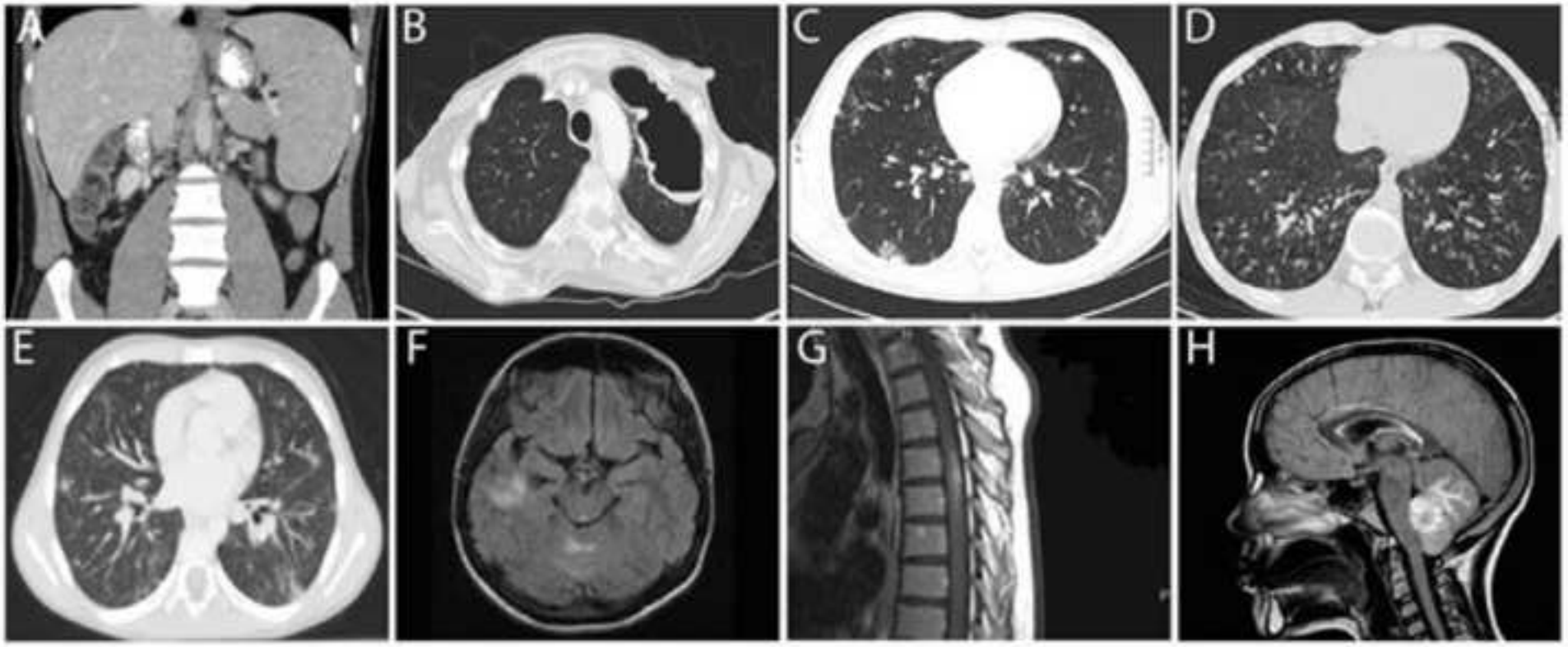
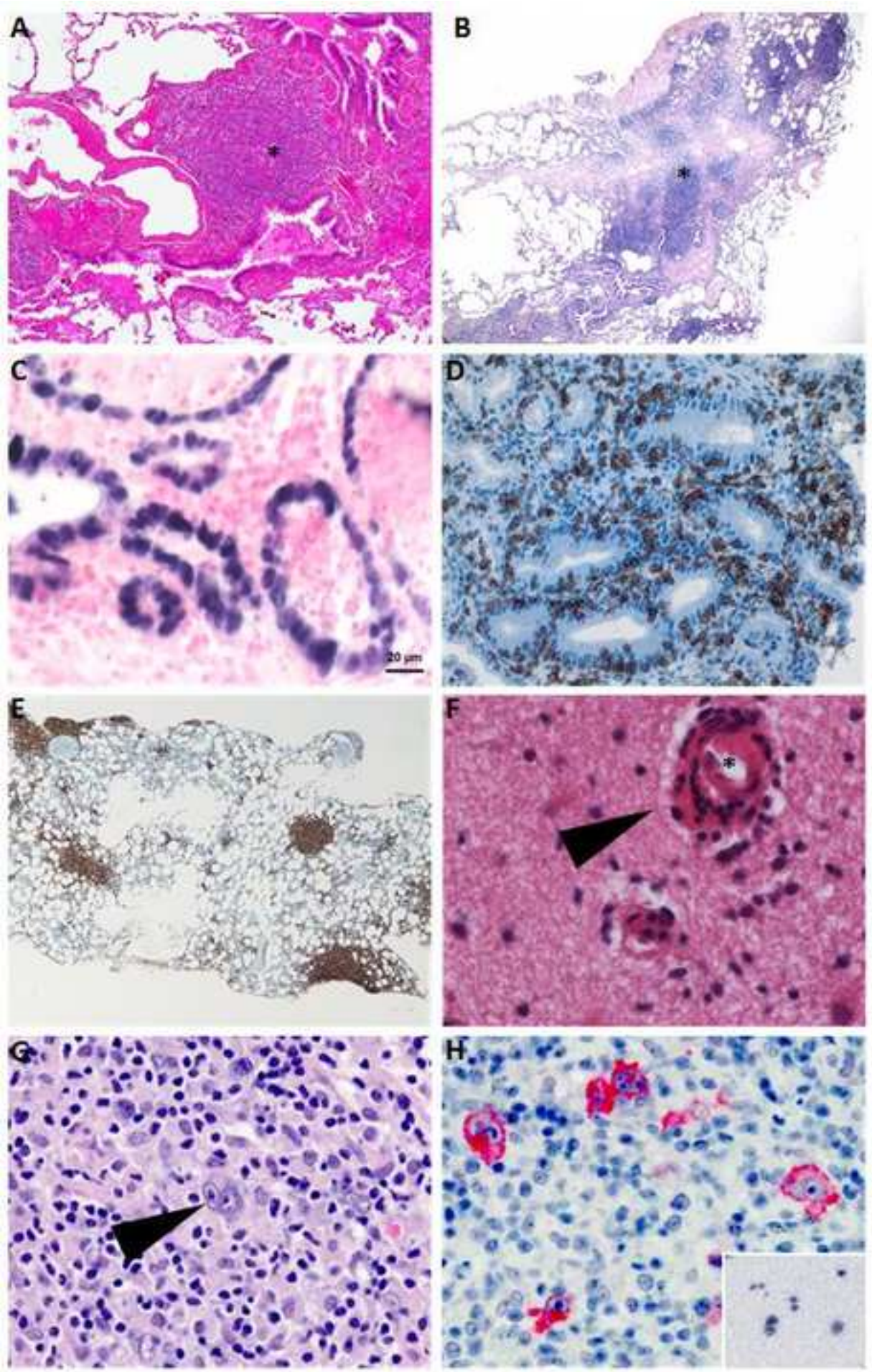


Figure No.5

Click here to download Figure No. Fig 5. Exemplary findings upon CT and MRI in CTLA-4-insufficient individuals.jpg





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Methods

We report on 133 individuals, including 90 affected and 43 unaffected mutation carriers (relatives who did not seek medical attention) from diverse ethnic backgrounds (Table 1). Twenty-six out of the 43 unaffected carriers were classified as completely unaffected as they were reported to be completely free of CTLA-4 associated symptoms. One-hundred-twenty-five individuals were genetically proven heterozygous *CTLA4* mutation carriers; eight were relatives who had that deceased prior to being genotyped without availability of genomic DNA. They were either diagnosed with CTLA-4 insufficiency following their typical clinical presentation, or they were mandatory mutation carriers following Mendelian inheritance. Fifty-one mutation carriers have previously been published (1-9) and 99 individuals are here described for the first time.
All samples were donated following informed written consent under local ethics board–approved protocols 239/99_BG, 251/13_KW, and 282/11_SE version 140023. Written consent was obtained of all included individuals or their legal guardians. This research was performed following approved protocols of the Albert-Ludwigs-Universität Freiburg, Germany.
Samples and clinical information were collected between October 2014 and July 2016. For all individuals, treating physicians completed a detailed questionnaire including genetics, clinical history, laboratory values, treatment, and outcome. Immunoglobulin levels under replacement therapy and B cell values after rituximab treatment were excluded. For some affected mutation carriers, treating physician could not provide us with the exact immunoglobulin serum levels but only with the information whether IgG, IgM and IgA were reduced. We included available serum levels in Figure S2 whereas Figure 4 includes all affected mutation carriers with hypogammaglobulinemia.
Mutation carriers were classified as affected mutation carried if they showed clinically apparent symptoms related to CTLA-4 deficiency requiring medical care or treatment. Lymphocyte phenotyping, CTLA-4 staining, and transendocytosis assay were performed in selected individuals as previously described.(4, 10) Statistical analysis was performed using GraphPad Prism version 6, and *p* values were calculated by two-tailed unpaired Student's *t*-test for the means with a 95% confidence interval, and Fisher's exact test for probing the gender bias, assuming the null hypothesis that male and female mutation carriers are equally affected. CVID was diagnosed on the basis of the revised European society of immune deficiencies (ESID) registry. (11)

Case vignettes

Case vignette – Respiratory involvement
PP.II.1 presented aged 17 with cough and dyspnea and was treated with multiple antibiotics. X-ray revealed confluent opacities and lung biopsy showed a mixed cellular and follicular bronchiolitis (Figure 6 Panel A) and EBV+ lymphocytic T cell infiltrations, consistent with bronchiolitis obliterans organized pneumonia (BOOP). He was therefore treated with prednisone; whenever trying to wean him off prednisone other symptoms (e.g. arthritis, uveitis) appeared and he became steroid dependent. He had multiple pneumonias, including one episode of necrotizing pneumonia with cavitation at age 38 (see Figure 5 Panel B).

Case vignette – Gastrointestinal involvement
HH.II.1 first presented at age two with diarrhea. Throughout the next years she developed malabsorption and nutritional deficiencies necessitating total parenteral nutrition (TPN) for prolonged periods of time. Biopsies revealed villous blunting in the small bowel, apoptotic bodies in the colonic mucosa, and CD3+CD8+ infiltrations in the small and large bowel. In her twenties, enteropathy worsened with eight stools per day requiring potassium and magnesium replacement in addition to TPN. Treatment including steroids, calcineurin inhibitors, mycophenolate, methotrexate, TNF-alpha inhibitors, rituximab, and ustekinumab had no lasting effect. Following genetic diagnosis at age 29, abatacept was started. On abatacept she is doing well, stool frequency decreased from eight to four stools per day, she reported to have the first formed stools since early childhood, and is off TPN and intravenous electrolyte replacement.

Case vignette – Cytopenia
GG.II.1 presented aged nine with petechial bleeding and platelets 2/nl. ITP was diagnosed and treated with high-dose steroids. Within the next two years she had relapsing episodes of ITP and AIHA. Treatment with steroids and immunoglobulins was only intermittently effective. At the age of eleven years, the platelet count was 10/nl and she developed cerebral bleeding leading to seizures and transient hemiparesis. Over the next years, treatment including plasmapheresis, rituximab, methotrexate, cyclosporin, sirolimus and abatacept had no long-lasting effect. Aged 15, she developed autoimmune neutropenia. Following a severe autoimmune hemolytic episode at age 18 (Hb 21g/l) and cerebral vasculitis resulting in paraparesis, she had a splenectomy and ultimately underwent successful alloHSCT at age 20.

Case vignette – Neurological involvement
KK.II.1 presented aged 30 with headache and Bell's palsy. MRI imaging revealed multiple supra- and infratentorial lesions of unknown etiology (Figure 5 Panel F) and steroid treatment was started after excluding borreliosis in the cerebrospinal fluid. Clinical response was incomplete and ten days prior to biopsy, steroid treatment was discontinued leading to a worsening of the CNS lesions and development of lower extremity paraparesis. The biopsy revealed non-malignant perivascular infiltration of lymphocytes (Figure 6 Panel F), immunohistochemically CD3+CD8+ T cells. Following the brain biopsy, steroid treatment was restarted and led to partial improvement of the paraparesis. Two weeks later, magnetic resonance imaging revealed spinal cord inflammation (Figure 5 Panel G) and she developed palsy of the musculus sphincter ani. Currently, aged 36, she is on 8 mg of methylprednisolone per day. Repeat MRI shows partial regression of the lesions, and she can walk without support, while fecal incontinence remains.

Case vignette – Treatment
B.II.4 had CVID, wasting diarrhea and developed EBV-associated gastric cancer aged 41, which was resected. Abatacept was started, and after three months stool frequency normalized and she regained 18 kg of weight. Immunological findings included a decrease in the MFI of activation markers CD4+HLA-DR+ and CD8+HLA-DR+ and CD21low B cells while total B cells normalized (Figure S1). After one year, abatacept was discontinued due to elevated EBV copies up to 12,000 copies/ml, and switched to Rituximab under which EBV was cleared in blood, but stool became loose, stool frequency rose, and *Helicobacter pylori* associated gastritis occurred.

Additional treatment options
Seventy percent of affected mutation carriers received corticosteroids (60/86), including 46 affected mutation carriers requiring long-term treatment and 41 undergoing pulse treatment. Forty-eight affected mutation carriers had at least one course of immunosuppressive steroid-sparing agents for autoimmune or inflammatory conditions. B cells are potent APCs (12), which, once activated, express the co-stimulatory B7-molecules (CD80 and CD86), binding and stimulating CD28 and not being sufficiently counteracted by CTLA-4 in case of *CTLA4* mutations. Anti-CD-20 treatment with rituximab efficiently depletes these potent immune-stimulatory cells and is frequently used in the treatment of refractory cytopenias(13). In our cohort, rituximab (n=25) was used more often than abatacept/belatacept (n=13) and sirolimus (n=13), with thirteen affected mutation carriers responding with good or partial effect. These included improved pancytopenia (D.II.1), resolution of AIHA with concomitant permanent loss of B cells (LL.II.1), and possible contribution to improvement of enteropathy (WW.II.1).
Thirty-five percent of affected mutation carriers (27/78) were under antibiotic prophylaxis. In one affected mutation carrier treatment with vedolizumab (blocking $\alpha_4\beta_7$ integrin(14)) improved colitis, and in the same individual PRCA responded well to cyclosporine A (AAA.II.1).(6)

Chromosome 2 contiguous gene deletion involving *CTLA4*
In two unrelated individuals from Canada (P1) and Australia (P2) a large heterozygous 2q33.2-2q33.3 deletion was detected, implicating the known immune relevant genes *CTLA4*, *ICOS*, and *CD28*. Whereas the mutation in P1 has been proven to have occurred *de novo*, the same deletion was detected in the clinically healthy mother of P2. In both *ICOS* was sequenced to exclude a mutation on the second allele causing ICOS deficiency(15). The phenotype of both individuals is characterized by immune dysregulation, similar to our cohort of affected *CTLA4* mutation carriers, even though an influence of the heterozygous loss of other genes located at this locus has to be considered.
The age of onset in these two individuals was five years (P1) and 14 years (P2) respectively, and age at evaluation was 37 years (P1) and 20 years (P2). Clinical features common for both individuals included hypogammaglobulinemia, lymphoproliferation, recurrent respiratory tract infections, GLILD, and a diagnosis of CVID made at the age of 12 years (P1) and 14 years (P2). However, primary clinical complications were autoimmune enteropathy in P1 and AIHA in P2.
P1 first presented with arthritis at the age of five years. She developed diarrhea at the age of 9, respiratory tract infections at the age of 12, and was further diagnosed with growth retardation. She had recurrent *Varicella zoster*- and *Herpes simplex virus*, and esophageal *Candida* infections. Her immunoglobulin levels revealed low IgG (1.73 g/l) and low IgA (0.11 g/l) at the age of 25 years. At 32 years she had recurrent fevers and at 36 years she presented with seizures. Infiltrations into the brain were described at that time but were not further investigated. Lymphocytic infiltrations into gastrointestinal tract, lung, and kidney were confirmed by biopsy. This individual received rituximab for one year without clinical improvement and was further treated with abatacept, corticosteroids, immunoglobulin substitution, and prophylactic antibiotics. At age 37, anticoagulation

was stopped following a traumatic leg hematoma. A few months later she developed acute severe dyspnea, chest pain, hypoxemia, and suddenly died. Autopsy was not performed.
P2 had recurrent respiratory tract infections, developed AIHA at the age of 14 years, splenomegaly at the age of 16, and GLILD at the age of 17: biopsy revealed B and T cell infiltrations. At that time, he had low IgG (1.43 g/l), low IgM (0.24 g/l), and low IgA (<0.05 g/l), after being treated with one course of rituximab at age 14 for his AIHA. At age 19, blurred vision of the right eye and bilateral optic disc swelling led to magnetic resonance imaging and revealed a swollen right ocular medial rectus muscle, responding to methylprednisolone/sirolimus treatment.

Possible influences of disease manifestation

As the penetrance of CTLA-4 insufficiency is incomplete, we searched for modifying factors predicting overt disease.
We saw an association between male gender and the occurrence of clinical symptoms, as 76% (51/67) of male, but only 59% (39/66) of female mutation carriers were classified as affected. This difference was highly significant (p=0.04). However, there was no gender preponderance within the 15 deceased individuals.
We hypothesized that a second germline or somatic hit or a reversion of the mutation, the exposure to a specific viral infection, or the HLA locus may influence the clinical penetrance, but no correlation was found.
Second, we hypothesized that affected individuals manifested following a second somatic genetic hit in *CTLA4* or that unaffected mutation carriers stayed healthy due to a somatic reversion of the heterozygous *CTLA4* germline mutation. To this end we sequenced isolated DNA from CD4+ T cells from five affected and eight unaffected mutation carriers, by next generation sequencing (Illumina MiSeq) to detect small clones of cells with potential biallelic mutations, but always only identified the one predicted germline mutation in *CTLA4*. Likewise, no somatic reversion was detected.
We studied whether the affected individuals had been exposed to a different set of viral infections than the currently healthy individuals. This analysis was complicated by the fact that some affected individuals either were hypogammaglobulinemic or on immunoglobulin replacement therapy, affecting their serological response. However, we found that four tested unaffected individuals were EBV-positive, while four of seven tested affected individuals were EBV-negative. Regarding CMV, two out of four tested unaffected mutation carriers were CMV-positive, and two were negative. Out of six tested affected mutation carrier, only two have had a CMV infection. One affected mutation carrier (B.II.3) was tested for CMV both before first symptoms manifested and after first manifestation of CTLA-4 deficiency and results were negative at both times. Concerning parvovirus B19, four tested unaffected mutation carriers, as well as five affected mutation carriers were positive. Due to the variation of the age of onset, even within one family, a viral infection triggering the symptoms is not unlikely, but EBV, CMV, and parvovirus B19 are unlikely to be the underlying cause.
We asked the question whether there is an HLA restriction within the affected or unaffected cohort, as CVID and IgA deficiency have previously shown strong HLA-associations.(16) However, when genotyping two of our largest families (A and B) for HLA-class I (HLA-A, -B, -C) and HLA-class II (HLA-DRB1*, -DQB1*) the individual HLA-types did not segregate with the disease phenotype. This data does not support the hypothesis of HLA as modifier in disease pathogenesis in families A and B.
Last, we analyzed whole exome sequencing data from 13 affected and twelve unaffected mutation carriers to screen for genetic loci within the coding sequences of the human genome. However, we were unable to identify SNPs or Indels occurring exclusively in affected but not in unaffected mutation carriers or *vice versa*.

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Figure legends

Figure S1. CTLA-4 expression and function in heterozygous mutation carriers

Panel A: Mean fluorescence intensity of total CTLA-4 in CD4+CD45RO+FoxP3+ Tregs is significantly decreased in mutation carriers compared to wildtype controls (**** = $p < 0.0001$), and in affected compared to unaffected mutation carriers (* = $p < 0.05$). Panel B: Impaired CTLA-4-mediated transendocytosis of CD80-GFP was observed in CD4+CD45RO+FoxP3+ Tregs, after co-culture of mutation carriers' CD4+ T cells with CHO cells presenting CD80-GFP. §, This mutation is already published, further information can be found in Table 1.

Figure S2. Immunological phenotype of CTLA-4 insufficiency

Percentages and absolute numbers of lymphocytes and various B and T cell subsets in the peripheral blood of affected and unaffected mutation carriers. IgA-, IgM-, and IgG serum levels in the peripheral blood of affected and unaffected mutation carriers [g/l]. For immunoglobulin levels only exact values were included in this figure (further information see methods). B cell values and immunoglobulin levels of affected mutation carriers treated with RTX or IVIG were excluded. Gray background indicates normal range.

Figure S3. Immunological changes during abatacept treatment in affected mutation carriers B.II.4

Panel A: Percentage of CD45RA+ cells within the group of CD4+ T cells during a time span of 400 days post first abatacept infusion. Panel B: Percentage of CD45RO+ cells within the group of CD4+ T cells during a time span of 400 days post first abatacept infusion. Panel C: Percentage of CD4+HLA-DR+ cells within the lymphocytes. Panel D: Mean fluorescence intensity of CD4+HLA-DR+ cells (black) and CD8+HLA-DR+ cells (gray). Day 1, day 142, day 290, and day 330 after starting abatacept treatment.

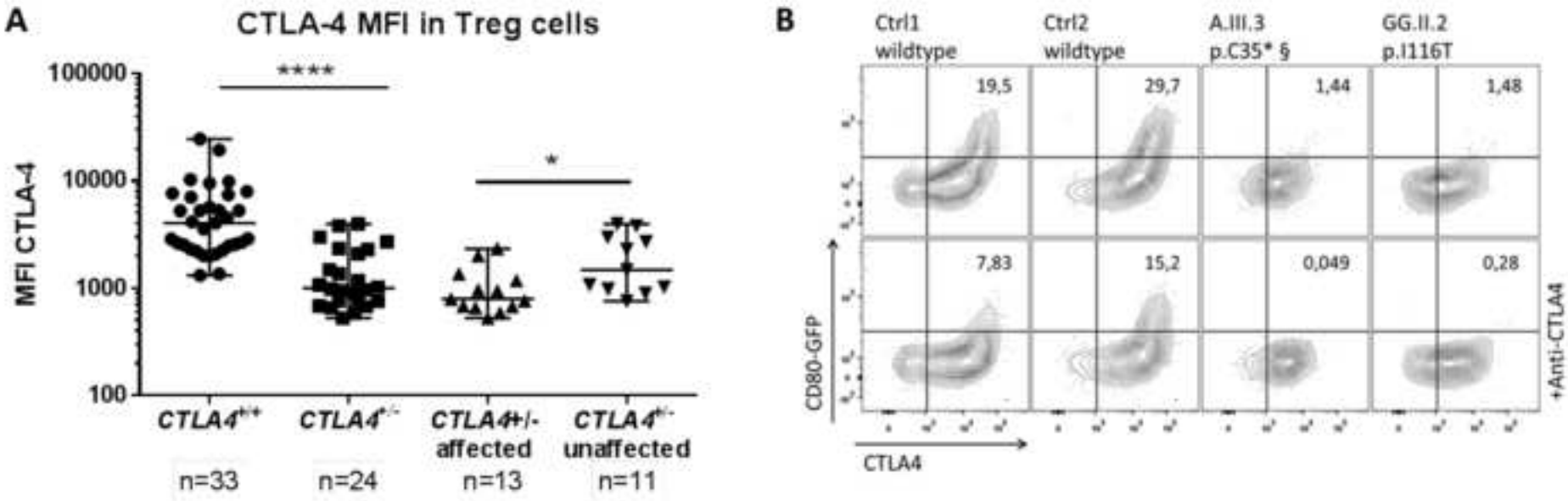

Figure No.S2

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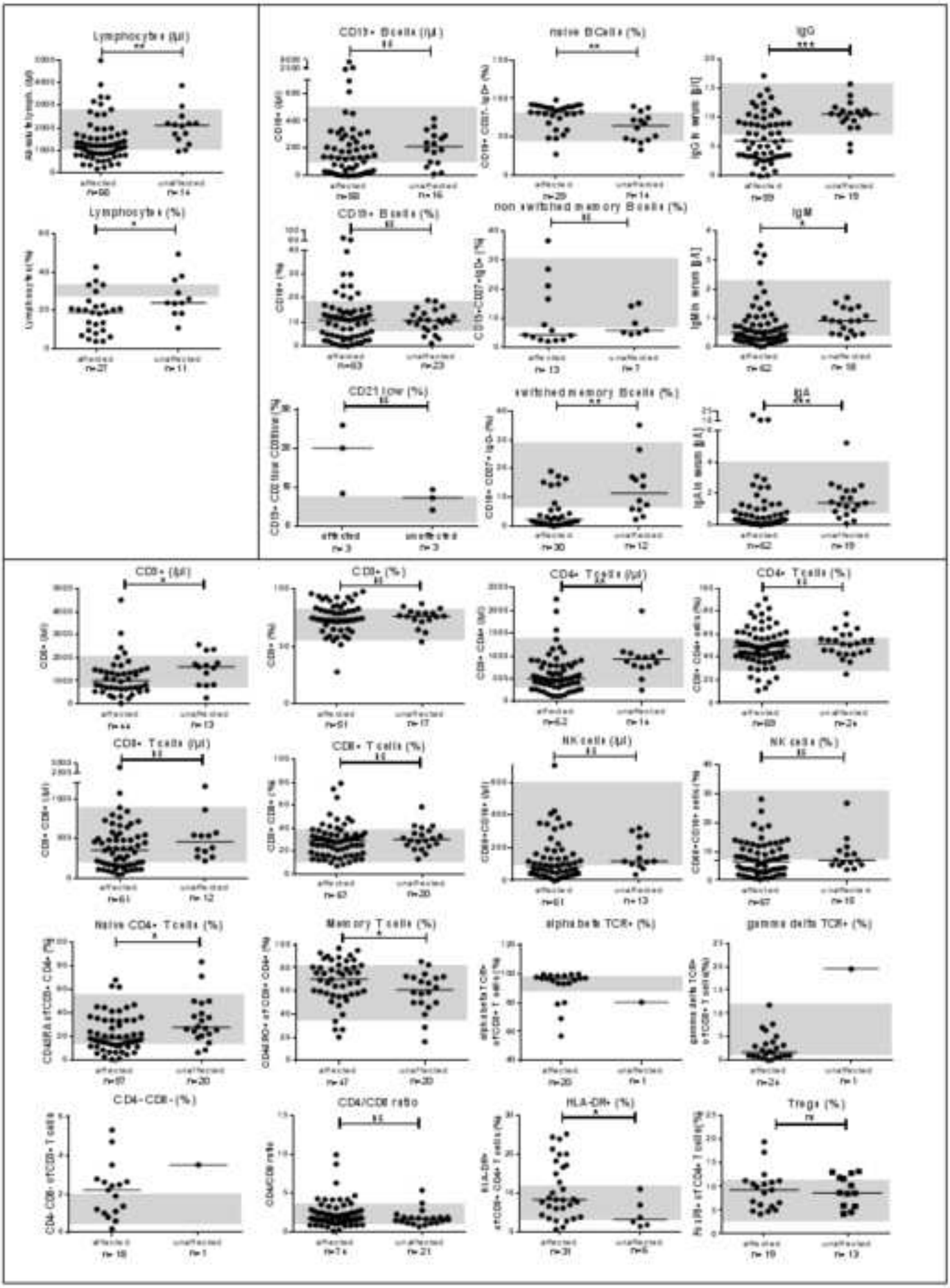

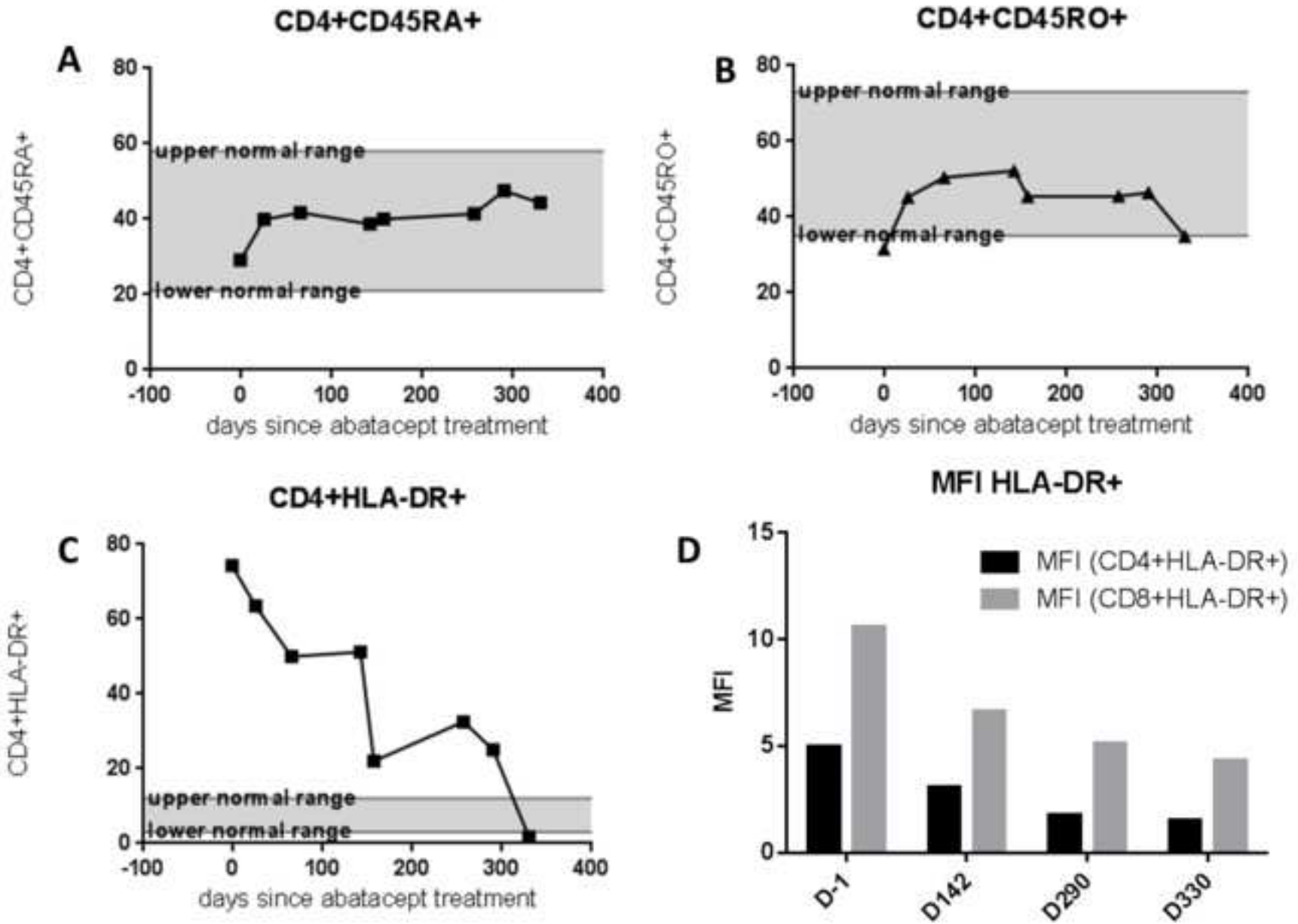

Table S1 . Clinical spectrum of CTLA4 mutation carriers

Clinical phenotype and treatment options in CTLA4 mutation carriers	1	2	3	4	5	6	7	8	9
patient ID	**A.I.2**	**A.II.2**	**A.II.3**	**A.II.5**	**A.II.8**	**A.II.9**	**A.II.10**	**A.III.1**	**A.III.3**
Date of birth	1/13/1928	9/7/1955	11/21/1956	7/2/1959	7/11/1963	1/10/1965	4/14/1966	11/9/1987	6/20/1995
Sex	f	m	f	m	m	f	m	f	m
Age of onset [years]	na	na	na	41	12	17	na	10	15
Age at evaluation [years]	87	60	59	56	34	37	49	28	20
Age of death [years]	na	na	na	na	34	37	na	na	na
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Country of Origin	Germany	Germany	Germany	Germany	Germany	Germany	Germany	Germany	Germany
Consanguinity	no	no	no	no	no	no	no	no	no
Classification	completely unaffected	completely unaffected	unaffected	affected	affected	affected	unaffected	affected	affected
First manifestations/symptoms									
Respiratory involvement	0	0	0	1	0	1	0	0	0
Gastrointestinal involvement	0	0	0	0	0	0	0	0	1
Cytopenia	0	0	0	0	0	0	0	0	0
Neurological involvement	0	0	0	0	1	0	0	1	0
Fever, night sweats	0	0	0	0	0	0	0	0	0
Growth retardation	0	0	0	0	0	0	0	0	0
Arthritis	0	0	0	0	0	0	0	0	0
Atopic dermatitis	0	0	0	0	0	0	0	0	0
Type 1 diabetes	0	0	0	0	0	0	0	0	0
Thyroid disease	0	0	0	0	0	0	0	0	0
Others	0	0	0	0	0	0	0	0	0
Details	0	0	0	0	0	0	0	0	0
Primary Diagnosis									
CVID	0	0	0	1	0	0	0	0	0
ALPS-like phenotype	0	0	0	0	0	0	0	0	0
IPEX-like phenotype	0	0	0	0	0	0	0	0	0
Lymphoproliferation	0	0	0	0	0	0	0	0	0
Respiratory involvement	0	0	0	0	1	1	0	0	0
Gastrointestinal involvement	0	0	0	0	0	0	0	0	0
Cytopenia	0	0	0	0	0	0	0	0	1
Evans syndrome	0	0	0	0	0	0	0	0	0
ITP	0	0	0	0	0	0	0	0	1
AIHA	0	0	0	0	0	0	0	0	0
PRCA	0	0	0	0	0	0	0	0	0
Neurological involvement	0	0	0	0	0	0	0	1	0
Malignancy	0	0	0	0	0	0	0	0	0
Endocrinological involvement	0	0	0	0	0	0	0	0	0
Warts	0	0	0	0	0	0	0	0	0
Psoriatic arthritis	0	0	0	0	0	0	0	0	0
Others	0	0	0	0	0	0	0	0	0
Hypogammaglobulinemia	1	uk	0	1	1	1	uk	1	1
Low IgG	0	uk	0	0	1	1	uk	1	0
Low IgM	0	uk	0	1	0	0	uk	1	1
Low IgA	1	uk	0	1	1	1	uk	1	1
Lymphoproliferation	0	0	0	1	1	1	0	1	1
Splenomegaly	0	0	0	1	0	1	0	0	1
Splenectomy	0	0	0	0	0	0	0	0	0
Hepatomegaly	0	0	0	0	1	0	0	0	0
Lymphadenopathy	0	0	0	0	0	0	0	0	1
Lymphocytic or granulomatous organ infiltration	**uk**	**uk**	**uk**	**0**	**uk**	**1**	**uk**	**1**	**1**
Bone marrow	uk	uk	uk	0	uk	1	uk	0	0
Brain	uk	uk	uk	0	uk	0	uk	1	0
Lung	uk	uk	uk	0	uk	1	uk	0	1
Spleen	uk	uk	uk	0	uk	0	uk	0	0
Kidney	uk	uk	uk	0	uk	0	uk	0	0
Liver	uk	uk	uk	0	uk	0	uk	0	0
Retroperitoneum	uk	uk	uk	0	uk	0	uk	0	0
Gut	uk	uk	uk	0	uk	0	uk	0	0
Retrobulbar space	uk	uk	uk	0	uk	0	uk	0	0
Biopsied	**uk**	**uk**	**uk**	**na**	**uk**	**uk**	**uk**	**uk**	**1**
B cell infitration	uk	uk	uk	na	uk	uk	uk	uk	0
T cell infiltration	uk	uk	uk	na	uk	uk	uk	uk	1
CD4 T cells	uk	uk	uk	na	uk	uk	uk	uk	1
CD8 T cells	uk	uk	uk	na	uk	uk	uk	uk	0
Respiratory tract involvement	0	0	0	1	1	1	0	0	1
Upper respiratory infections	0	0	0	1	1	1	0	0	0
Lower respiratory infections	0	0	0	1	1	1	0	0	0
Pneumonia	0	0	0	0	1	1	0	0	0
GLILD	0	0	0	0	1	1	0	0	1
Bronchiectasis	0	0	0	0	1	1	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

Others	0	0	0	0	0	Recurrent pneumo-thoraces	0	0	0
Clinically reactivated/apparent Infections	0	0	0	1	1	1	0	0	0
Sepsis	0	0	0	0	uk	0	0	0	0
Herpes Infection	0	0	0	0	0	0	0	0	0
clinical EBV infection	0	0	0	0	0	0	0	0	0
clinical CMV infection	0	0	0	0	0	0	0	0	0
HHV6 viremia	0	0	0	0	0	0	0	0	0
HHV7 viremia	0	0	0	0	0	0	0	0	0
Severe varizella zoster infection	0	0	0	0	0	0	0	0	0
Recurrent herpes simplex infection	0	0	0	uk	uk	uk	0	uk	uk
Mycoplasma or TBC	0	0	0	0	1	1	0	0	0
Bacterial infections	0	0	0	1	1	1	0	0	0
Staphylococcus aureus	0	0	0	0	1	0	0	0	0
Hemophilus influenzae	0	0	0	0	1	1	0	0	0
Escherichia coli	0	0	0	0	0	0	0	0	0
Salmonella	0	0	0	1	0	0	0	0	0
Streprococcus pneumoniae	0	0	0	uk	1	1	0	0	0
Helicobacter pylori	0	0	0	uk	0	0	0	0	0
Others	0	0	0	uk	0	0	0	0	0
Viral infections (other than Herpes infection)	0	0	0	uk	uk	uk	0	uk	uk
Fungal infection	0	0	0	0	1	1	0	0	0
Aspergillus	0	0	0	0	0	0	0	0	0
Candida	0	0	0	0	1	1	0	0	0
Others	0	0	0	0	0	0	0	0	0
Gastrointestinal involvement	0	0	1	1	1	1	1	1	1
Diarrhea/ Enteropathy	0	0	1	1	1	1	1	1	1
Crohn's disease	0	0	0	0	0	0	0	0	0
Coeliac disease	0	0	0	0	0	0	0	0	0
Atrophic gastritis	0	0	0	0	0	0	0	0	0
Pancreatitis	0	0	0	0	0	0	0	0	0
Others	0	0	0	0	0	0	0	0	0
Cytopenia	0	0	0	0	0	0	0	1	1
Autoimmune cytopenia	0	0	0	0	0	0	0	1	1
ITP	0	0	0	0	0	0	0	1	1
AIHA	0	0	0	0	0	0	0	0	0
Autoimmune neutropenia	0	0	0	0	0	0	0	0	0
PRCA	0	0	0	0	0	0	0	0	0
Arterial thrombosis	0	0	0	0	0	0	0	0	0
Deep vein thrombosis	0	0	0	0	0	0	0	0	0
Pernicious anemia	0	0	0	0	0	0	0	0	0
Others	0	0	0	0	0	0	0	0	0
Neurological involvement	0	0	0	0	1	0	0	1	0
Malignancy	1	0	0	0	0	0	0	0	0
Lymphoma	0	0	0	0	0	0	0	0	0
Gastric cancer/colon cancer	1	0	0	0	0	0	0	0	0
EBV-associated malignancy	0	0	0	0	0	0	0	0	0
Dermatological involvement	0	0	1	0	1	0	0	1	0
Psoriasis	0	0	1	0	0	0	0	1	0
Atopic dermatitis	0	0	0	0	0	0	0	0	0
Eczema	0	0	0	0	0	0	0	0	0
Alopecia	0	0	0	0	0	0	0	0	0
Vitiligio	0	0	0	0	0	0	0	0	0
Trachyonychia	0	0	0	0	0	0	0	0	0
Warts	0	0	0	0	1	0	0	0	0
Angiofibroma	0	0	0	0	0	0	0	0	0
Others	0	0	0	0	0	0	0	0	0
Endocrinological involvement	0	0	0	0	0	0	0	0	1
Thyroiditis/Hypothyroidism	0	0	0	1	0	0	0	0	1
Autoimmune Thyroiditis/Hypothyroidism	na	na	na	1	na	na	na	na	1
Type 1 diabetes	0	0	0	0	0	0	0	0	0
Hypogonadism	0	0	0	0	0	0	0	0	0
Hypopituitarism	0	0	0	0	0	0	0	0	0
Hypophysitis	0	0	0	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

Others	0	0	0	uk	uk	uk	0	uk	uk
Other clinical manifestations	0	0	0	1	0	0	0	1	0
Growth retardation	0	0	0	0	0	0	0	0	0
Kidney involvement	0	0	0	0	0	0	0	0	0
Liver involvement	0	0	0	0	0	0	0	0	0
Arthritis	0	0	0	1	0	0	0	1	0
Others	0	0	0	uk	uk	uk	0	uk	uk
Specific Antibody responses; Serology/Virology									
Tetanus §	uk	uk	0	0	uk	0	uk	0	1
Diphtheria §	uk	uk	0	0	uk	0	uk	0	1
Pneumococcal vaccination §	uk	uk	uk	1	uk	uk	uk	1	1
Antibodies									
Coombs	uk	uk	uk	uk	uk	uk	uk	uk	uk
ANA	uk	uk	uk	0	uk	uk	uk	uk	0
ANCA	uk	uk	uk	0	uk	uk	uk	uk	0
Autoantibodyscreening	uk	uk	uk	uk	uk	uk	uk	uk	uk
Antiphospholipid antibodies	uk	uk	uk	uk	uk	uk	uk	uk	uk
LISS	uk	uk	uk	uk	uk	uk	uk	uk	uk
Anti_GAD antibodies	uk	uk	uk	uk	uk	uk	uk	uk	uk
C3d	uk	uk	uk	0	uk	uk	uk	uk	1
CH50	uk	uk	uk	0	uk	uk	uk	uk	1
Anti-microsomal antibodies	uk	uk	uk	uk	uk	uk	uk	uk	uk

Table S1. Clinical spectrum of CTLA4 mutation carriers

0, not present; 1, present; uk, unknown; na, not applicable; f, female; m, male; Immunoglobulins: #, no values available before IVIG or Rituximab; normal values: IgG, 7-

Table S1 . Clinical spectrum of CTLA4 mutation carriers

10	11	12	13	14	15	16	17	18	19	20
A.III.5	**A.III.6**	**B.I.1**	**B.II.1**	**B.II.2**	**B.II.3**	**B.II.4**	**B.III.2**	**B.III.3**	**C.II.3**	**C.II.4**
10/1/1992	8/9/1995	5/31/1934	9/12/1958	6/18/1962	9/21/1964	1971	1984	8/6/1998	1993	2002
f	f	m	f	m	m	f	f	f	f	f
na	na	na	na	15	50	34	10	na	7	na
23	20	66	57	23	51	43	16	17	20	13
na	na	66	na	23	na	na	16	na	20	na
Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Germany	Germany	Germany	Germany	Germany	Germany	Germany	Germany	Germany	Greece	Greece
no	no	no	no	no	no	no	no	no	no	no
completely unaffected	completely unaffected	completely unaffected	unaffected	affected	affected	affected	affected	completely unaffected	affected	unaffected
0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	1	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0
0	0	0	1	1	0	1	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	1	uk	uk	uk	1	1	1	1	1	0
0	0	uk	uk	uk	1	0	0	0	1	0
0	0	uk	uk	uk	1	0	0	0	0	0
1	1	uk	uk	uk	1	1	1	1	1	0
0	0	0	0	uk	1	1	1	0	1	0
0	0	0	0	uk	1	0	1	0	1	0
0	0	0	0	1	0	0	0	0	1	0
0	0	0	0	uk	0	0	uk	0	1	0
0	0	0	0	uk	1	0	uk	0	1	0
uk	**uk**	**uk**	**0**	**uk**	**1**	**1**	**0**	**0**	**0**	**0**
uk	uk	uk	0	uk	0	1	0	0	0	0
uk	uk	uk	0	uk	0	0	0	0	0	0
uk	uk	uk	0	uk	0	1	0	0	0	0
uk	uk	uk	0	uk	0	0	0	0	0	0
uk	uk	uk	0	uk	0	1	0	0	0	0
uk	uk	uk	0	uk	0	0	0	0	0	0
uk	uk	uk	0	uk	0	0	0	0	0	0
uk	uk	uk	0	uk	1	1	0	0	0	0
uk	uk	uk	0	uk	0	0	0	0	0	0
uk	**uk**	**uk**	**na**	**uk**	**1**	**1**	**na**	**na**	**na**	**na**
uk	uk	uk	na	uk	0	0	na	na	na	na
uk	uk	uk	na	uk	1	1	na	na	na	na
uk	uk	uk	na	uk	1	1	na	na	na	na
uk	uk	uk	na	uk	1	0	na	na	na	na
0	0	1	0	0	1	1	1	0	1	0
0	0	0	0	0	0	1	1	0	1	0
0	0	0	0	0	0	1	1	0	1	0
0	0	1	0	0	0	0	1	0	1	0
0	0	0	0	uk	0	0	1	0	1	0
0	0	0	0	uk	0	0	1	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	0	0	0	0	Lesions in CT (asymp-tomatic)	Asthma	0	0	0	0
0	0	0	0	0	1	1	0	0	1	0
0	0	0	0	uk	1	1	0	0	1	0
0	0	0	0	0	1	1	0	0	0	0
0	0	0	0	0	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	uk	uk	0	uk	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	uk	0	0	0	0	1	0
0	0	0	0	uk	0	0	0	0	0	0
0	0	0	0	uk	0	0	0	0	0	0
0	0	0	0	uk	0	0	0	0	1	0
0	0	0	0	uk	0	0	0	0	0	0
0	0	0	0	uk	0	0	0	0	0	0
0	0	0	0	uk	0	0	0	0	0	0
0	0	0	0	uk	0	0	0	0	0	0
0	0	0	0	uk	uk	uk	uk	0	H1N1	0
0	0	0	0	uk	1	0	0	0	0	0
0	0	0	0	uk	1	0	0	0	0	0
0	0	0	0	uk	0	0	0	0	0	0
0	0	0	0	uk	0	0	0	0	uk	0
0	0	0	1	1	1	1	0	0	0	1
0	0	0	1	1	0	1	0	0	0	1
0	0	0	0	uk	0	0	0	0	0	0
0	0	0	0	uk	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	uk	0	0	0	0	0	0
0	0	0	0	0	Imaging proof (asymp-tomatic)	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	1	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	1	1	0	0	0	0
0	0	0	0	1	1	1	0	0	0	0
0	0	0	0	1	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	Lichen simplex chronicus	uk	0	uk	0
0	0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
na	na	na	na	na	na	na	na	na	na	na
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	0	0	0	uk	uk	0	uk	0	uk	0
0	0	0	0	0	1	1	0	0	1	1
0	0	0	0	0	0	0	0	0	1	1
0	0	0	0	0	0	1	0	0	1	0
0	0	0	0	0	1	0	0	0	1	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	uk	Hemangioma	uk	uk	0	uk	0
1	1	uk	uk	uk	uk	1	uk	uk	uk	1
1	0	uk	uk	uk	uk	uk	uk	uk	uk	1
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	1	0
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	1
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	0	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	0	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk

16g/l; IgM, 0.4-2.3g/l; IgA, 0.7-4g/l; § Vaccination response: 0, vaccination response (>0.1IU/ml); 1, no vaccination response (<0.1IU/ml); UK, United Kingdom

Table S1 . Clinical spectrum of CTLA4 mutation carriers

21	22	23	24	25	26	27	28	29	30	31
D.I.2	**D.II.1**	**E.II.3**	**F.II.1**	**G.II.1**	**G.III.1**	**G.III.2**	**H.I.2**	**H.II.1**	**H.II.2**	**J.I.2**
uk	1993	12/14/1993	1988	27.05.1962	06.02.1985	Jan 7, 1993	10/16/1963	7/16/1988	10/7/1989	7/5/1966
f	f	f	m	f	f	m	f	m	m	f
na	10	10	8	na	12	1.83	22	10	7	na
43	22	22	23	53	24	22.00	52	21	26	50
na	na	na	23	na	24	na	na	21	na	na
asian	asian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
India	India/UK	Georgia	Germany	USA	USA	USA	Germany	Germany	Germany	Germany
no	no	no	no	no	no	no	no	no	no	no
unaffected	affected	affected	affected	completely unaffected	affected	affected	affected	affected	affected	completely unaffected
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	1	1	0	0	0
0	1	0	1	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	1	0
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	1	0	1	1	1	0	0	0
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
Skin involvement	0	0	0	0	0	0	0	0	0	0
uk	1	1	1	uk	1	0	uk	1	1	uk
uk	1	1	1	uk	0	0	uk	1	0	uk
uk	1	0	1	uk	0	0	uk	1	1	uk
uk	1	1	1	uk	1	0	uk	1	1	uk
0	1	1	1	0	1	1	0	1	1	0
0	1	1	1	0	1	1	0	1	1	0
0	0	0	1	0	uk	0	0	1	0	0
0	0	1	0	0	1	1	0	1	1	0
0	1	1	0	0	0	0	0	1	1	0
0	**1**	**1**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**0**
0	0	0	0	0	1	1	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	1	0	0	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
na	**1**	**1**	**na**	**na**	**1**	**1**	**na**	**na**	**na**	**na**
na	0	0	na	na	1	1	na	na	na	na
na	1	1	na	na	1	1	na	na	na	na
na	0	1	na	na	uk	uk	na	na	na	na
na	0	1	na	na	uk	uk	na	na	na	na
0	1	1	1	1	1	1	0	0	1	0
0	0	1	1	0	1	1	0	0	0	0
0	0	1	1	0	0	uk	0	0	1	0
0	0	1	1	0	1	1	0	0	1	0
0	1	1	0	0	1	0	0	0	1	0
0	1	1		0	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	Asthma	0	0	Pulmonary nodules (asymptomatic)	0		0	0	0	0
1	1	0	1	0	1	1	0	1	1	0
0	0	0	1	0	uk	0	0	1	1	0
0	1	0	0	0	1	1	0	1	1	0
0	0	0	0	0	0	0	0	1	1	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0
0	0	0	uk	0	1	0	0	uk	uk	0
0	0	0	0	0	uk	0	0	1	0	0
1	1	uk	uk	0	1	1	0	0	0	0
1	0	uk	uk	0	0	1	0	0	0	0
0	1	uk	uk	0	0	0	0	0	0	0
0	0	uk	uk	0	0	0	0	0	0	0
0	0	uk	uk	0	0	1	0	0	0	0
uk	1	uk	uk	0	uk	0	0	0	0	0
uk	0	uk	uk	0	uk	uk	0	0	0	0
0	Pneumocystis jirovecii, Pseudo-monas aeruginosa	uk	uk	0	uk	0	0	0	0	0
0	NPV	0	uk	0	uk	1	0	uk	uk	0
0	1	0	0	0	1	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	1	0	0	0	0	0
0	uk	uk	uk	0	uk	uk	0	uk	uk	0
0	1	1	1	0	1	1	1	1	1	0
0	1	1	1	0	1	1	1	1	1	0
0	0	0	1	0	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	1	1	0	1	1	0	1	1	0
0	1	1	1	0	1	1	0	1	1	0
0	0	1	1	0	0	1	0	1	1	0
0	1	1	1	0	0	1	0	1	1	0
0	0	0	0	0	1	1	0	1	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	uk	0	0	0	1	0
0	0	0	0	0	uk	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	1	1	1	0
0	0	0	0	0	0	1	0	1	0	0
0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0
1	0	1	0	0	0	1	1	1	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0
1	0	0	0	0	0	1	0	1	0	0
0	0	0	0	0	uk	0	0	1	1	0
0	0	0	0	0	uk	0	1	0	0	0
0	0	0	0	0	uk	0	0	0	0	0
0	0	0	0	0	uk	0	0	0	0	0
0	0	0	0	0	uk	0	0	0	0	0
Lichenified, acute, and chronic inflammation	uk	erythema nodosum	uk	0	uk	0	0	uk	uk	0
0	1	1	0	0	1	1	1	0	1	0
0	1	1	0	0	1	0	1	0	0	0
na	0	1	na	na	1	na	1	na	na	na
0	0	0	0	0	1	1	1	0	1	0
0	0	0	0	0	uk	uk	0	0	1	0
0	0	0	0	0	uk	uk	0	0	1	0
0	0	0	0	0	uk	uk	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	uk	uk	uk	0	uk	uk	0	uk	uk	0
0	1	0	0	0	1	1	0	1	1	0
0	0	0	0	0	1	1	0	1	1	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	1	0	0
0	0	1	0	0	1	1	0	0	0	0
0	uk	0	uk	0	uk	uk	0	uk	uk	0
uk	0	1	uk	0	0	0	uk	uk	uk	uk
uk	1	1	uk	0	0	0	uk	uk	uk	uk
1	0	1	uk	0	0	uk	uk	uk	uk	uk
uk	uk	0	uk	uk	0	1	uk	uk	1	uk
uk	0	uk	uk	uk	0	uk	uk	uk	0	uk
uk	uk	uk	uk	uk	0	uk	uk	0	1	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	0	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk

; USA, United States of America; ITP, immune thrombocytopenia; AIHA, autoimmune hemolytic anemia; PRCA, pure red blood cell aplasia; C3d: 0 if ≤ 9 mg/L,

Table S1 . Clinical spectrum of CTLA4 mutation carriers

32	33	34	35	36	37	38	39	40	41	42
J.II.1	**K.II.1**	**L.I.2**	**L.II.1**	**L.II.2**	**M.II.3**	**N.I.2**	**N.II.1**	**N.II.3**	**N.III.2**	**O.II.1**
21.12.1993	09.11.1962	13.01.1972	20.02.1995	21.09.1998	21.02.1979	28.11.1944	11.02.1969	02.09.1973	23.02.2003	1/28/2002
m	f	f	f	m	m	f	f	m	m	m
11	26	20	5	14	10	na	na	na	10	8
22	53	40	20	16	35	71	47	42	10	13
na	53	40	na	na	35	na	na	na	na	na
Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	asian	asian	asian	asian	asian	Caucasian
Germany	Germany	UK	UK	UK	Japan	Japan	Japan	Japan	Japan	Spain
no	no	no	no	no	no	no	no	no	no	no
affected	affected	affected	affected	affected	affected	completely unaffected	completely unaffected	completely unaffected	affected	affected
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
1	1	0	1	0	0	0	0	0	1	1
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	1	0	0	0	0	0	0
1	0	1	0	0	0	0	0	0	0	1
0	0	1	0	0	0	0	0	0	0	1
1	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0
0	1	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
uk	1	1	1	1	1	uk	uk	uk	1	1
#	1	1	0	1	1	uk	uk	uk	1	1
#	1	0	0	1	1	uk	uk	uk	1	0
#	1	0	1	0	1	uk	uk	uk	1	1
1	1	1	1	1	0	0	0	0	1	1
0	1	1	1	1	0	0	0	0	1	1
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	1	1	1	0	0	0	0	1	1
1	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**
0	1	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	1	0
1	0	0	0	0	0	0	0	0	1	1
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
uk	**1**	**1**	**na**	**na**	**na**	**na**	**na**	**na**	**1**	**0**
uk	1	1	na	na	na	na	na	na	0	uk
uk	1	0	na	na	na	na	na	na	1	uk
uk	1	0	na	na	na	na	na	na	0	uk
uk	0	0	na	na	na	na	na	na	1	uk
1	1	1	1	1	1	0	0	0	1	1
0	0	0	1	0	1	0	0	0	1	0
1	1	1	1	1	1	0	0	0	1	0
uk	1	uk	1	uk	1	0	0	0	1	0
1	0	0	0	0	0	0	0	0	1	1
0		uk	1	uk	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	0	0	0	0	0	0	0	0	0	0
1	1	1	0	0	1	0	0	0	1	1
0	0	1	0	0	1	0	0	0	0	0
0	1	0	0	0	1	0	0	0	1	1
0	1	0	0	0	0	0	0	0	1	0
0	1	0	0	0	1	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
uk	uk	uk	0	uk	uk	0	0	0	uk	0
1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	1	uk	uk	uk	0	0
0	0	0	0	0	0	uk	uk	uk	0	0
0	0	0	0	0	1	uk	uk	uk	0	0
0	0	0	0	0	0	uk	uk	uk	0	0
0	0	0	0	0	uk	uk	uk	uk	uk	uk
0	0	0	0	0	uk	uk	uk	uk	uk	uk
0	0	0	0	0	uk	uk	uk	uk	uk	uk
uk	uk	uk	0	uk	uk	0	0	0	uk	0
0	0	uk	0	0	1	0	0	0	0	0
0	0	uk	0	0	0	0	0	0	0	0
0	0	uk	0	0	1	0	0	0	0	0
uk	0	uk	0	uk	uk	0	0	0	uk	uk
0	1	1	1	1	1	0	0	0	1	0
0	1	1	1	1	1	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	Pancreatic insufficiency	0
1	1	1	1	0	0	0	0	0	1	1
1	1	1	1	0	0	0	0	0	1	1
1	1	1	1	0	0	0	0	0	1	1
1	1	1	1	0	0	0	0	0	0	1
1	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	1	0	0	0	0	0	0	0	1	0
0	1	0	0	1	1	0	0	0	0	0
0	1	0	0	1	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	1	1	1	1	1				0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	1	1	1	1	uk	uk	uk	0	0
0	0	0	0	0	0	uk	uk	uk	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	1	1	0	0	uk	uk	uk	0	0
0	0	0	0	0	0	0	0	0	0	0
uk	0	uk	uk	uk	uk	0	0	0	uk	uk
0	1	1	0	0	1	0	0	0	0	0
0	1	1	0	0	0	0	0	0	0	0
na	0	1	na	na	na	na	na	na	na	na
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

uk	uk	uk	uk	uk	uk	0	0	0	uk	uk
1	0	0	1	0	1	0	0	0	1	0
1	0	0	1	0	1	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0
uk	0	uk	uk	uk	uk	0	0	0	uk	uk
uk	uk	1	1	0	uk	uk	uk	uk	uk	1
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	1
uk	uk	1	1	uk	0	uk	uk	uk	0	0
uk	uk	1	1	0	0	uk	uk	uk	uk	uk
0	uk	0	0	0	0	uk	uk	uk	0	uk
0	uk	0	0	0	0	uk	uk	uk	0	uk
uk	uk	uk	uk	0	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	0	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	0	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk		uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	0	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	1	uk	uk	1	uk	uk	uk	uk	uk
, 1 if > 9 mg/L; CH50: 0 if ≥ 20%, 1 if < 20%.										

Table S1 . Clinical spectrum of CTLA4 mutation carriers

43	44	45	46	47	48	49	50	51	52	53
P.II.2	**Q.II.1**	**R.II.5**	**R.III.1**	**S.II.1**	**T.II.1**	**U.I.1**	**U.II.1**	**U.II.2**	**U.II.3**	**V.II.1**
2/3/2002	4/29/1993	3/21/1971	9/24/1997	10/10/1988	8/11/1994	uk	8/23/2006	uk	uk	2002
m	m	f	f	m	m	m	m	m	f	f
2	10	24	na	2	1.5	na	3.75	na	na	9
13	15	44	18	22	21	40	9	8	6	14
na	15	na	na	na	na	na	na	na	na	na
Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	asian	asian	asian	asian	asian
Germany	UK	Italy	Italy	UK	UK	Japan	Japan	Japan	Japan	Japan
no	no	no	no	no	no	no	no	no	no	no
affected	affected	affected	unaffected	affected	affected	unaffected	affected	completely unaffected	completely unaffected	affected
1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	1	0	0	0	0	1	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	1	0	0	0
1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	1
0	0	0	0	0	1	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	1	1	1	1	uk	1	uk	uk	uk
#	0	1	0	0	0	uk	1	uk	uk	uk
#	0	1	1	0	0	uk	0	uk	uk	uk
1	0	1	1	1	1	uk	1	uk	uk	uk
1	1	1	0	1	1	0	0	0	0	1
1	1	1	0	1	1	0	0	0	0	0
0	1	0	0	1	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	1	1	0	1	1	0	0	0	0	1
1	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
0	0	0	0	0	0	0	0	0	0	0
1	1	0	0	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	**1**	**1**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**0**
1	1	1	na	na	na	na	na	na	na	uk
1	1	1	na	na	na	na	na	na	na	uk
0	0	0	na	na	na	na	na	na	na	uk
1	0	0	na	na	na	na	na	na	na	uk
1	1	1	1	1	0	0	0	0	0	0
1	0	1	1	0	0	0	0	0	0	0
1	1	1	1	1	0	0	0	0	0	0
1	uk	1	1	0	0	0	0	0	0	0
1	1	1	0	1	0	0	0	0	0	0
0	0	uk	0	1	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	uk	uk	0	0	0	0	0	0	0	0
1	0	1	0	1	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	1	0	0	1	0	0	0
0	0	0	0	1	0	0	1	0	0	0
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	uk	0	0	0	0	uk	0	0	uk
0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	1	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0
0	0	uk	0	0	0	0	0	0	uk	0
0	0	uk	0	0	0	0	0	0	uk	0
0	0	uk	0	Pseudo-monas aeruginosa	0	0	0	0	0	0
uk	0	0		HPV, Adenovirus	0	0	uk	0	0	uk
1	0	0		1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	1	0	0	0	0	0	0
0	0	uk		0	0	0	0	0	0	0
0	0	1	0	1	0	0	0	0	0	0
0	0	1	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	1	1	0	1	1	0	1	0	0	1
1	1	1	0	1	1	0	1	0	0	1
1	1	1	0	1	1	0	1	0	0	1
1	0	1	0	1	1	0	0	0	0	1
0	1	0	0	0	1	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	1	0	1	1	1	1	0	0	0	0
0	0	0	1	0	1	0	0	0	0	0
1	0	0	0	0	0	1	0	0	0	0
1	1	0	1	1	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
oral aphthae	uk	uk	0	uk	uk	0	uk	0	0	uk
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
na	na	na	na	na	na	na	na	na	na	na
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

uk	uk	uk	0	uk	uk	0	uk	0	0	uk
0	0	0	0	1	1	0	0	0	1	1
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
uk	uk	uk	0	uk	uk	0	uk	0	Congenital sensorineural hearing loss	uk
uk	1	uk	1	1	1	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	1	uk	uk	1	uk	uk	uk	uk	uk	uk
1	1	0	uk	1	1	uk	1	uk	uk	1
1	0	0	1	uk	1	uk	0	uk	uk	uk
0	uk	uk	uk	uk	0	uk	0	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	1	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk

Table S1 . Clinical spectrum of CTLA4 mutation carriers

54	55	56	57	58	59	60	61	62	63	64
W.I.1	**W.II.1**	**W.II.2**	**W.II.3**	**X.I.2**	**X.II.1**	**Y.I.1**	**Y.II.1**	**Z.I.2**	**Z.II.1**	**Z.II.2**
21.12.1972	29.07.1999	30.01.2002	18.08.2009	23.10.1959	1/11/1999	11/20/1966	7/14/1995	1934	1965	1966
m	m	f	f	f	f	m	m	f	f	m
19	na	9	4	na	6	uk	10	na	na	43
43	16	14	6	55	15.00	49	20	81	50	49
na	na	na	na	na	na	na	na	na	na	na
asian	asian	asian	asian	african american	africain americain	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Japan	Japan	Japan	Japan	USA	USA	Germany	Germany	Norway	Norway	Norway
no	no	no	no	no	no	no	no	no	no	no
affected	unaffected	affected	affected	completely unaffected	affected	affected	affected	unaffected	completely unaffected	affected
0	0	1	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	1	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0	0
0	0	0	Alopecia, Eczema	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0
1	0	0	1	0	1	0	1	0	0	1
1	0	0	0	0	uk	0	1	0	0	0
0	0	0	0	0	uk	0	0	0	0	0
0	0	0	1	0	uk	0	0	0	0	0
0	0	0	0	0	uk	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	Eczema	0	0	0	0	0	0	0	0	0
0	0	1	1	0	0	uk	1	uk	0	1
0	0	1	0	0	0	uk	0	uk	0	0
0	0	1	0	0	0	uk	0	uk	uk	0
0	0	1	1	0	0	uk	1	uk	0	1
1	1	1	1	0	1	uk	1	0	0	0
0	0	1	0	0	0	uk	1	0	0	0
0	0	0	0	0	0	uk	0	0	0	0
0	0	1	0	0	0	uk	1	0	0	0
1	1	1	1	0	1	uk	1	0	0	0
0	**0**	**1**	**0**	**0**	**0**	**uk**	**0**	**uk**	**uk**	**0**
0	0	0	0	0	0	uk	0	uk	uk	0
0	0	0	0	0	0	uk	0	uk	uk	0
0	0	1	0	0	0	uk	0	uk	uk	0
0	0	0	0	0	0	uk	0	uk	uk	0
0	0	0	0	0	0	uk	0	uk	uk	0
0	0	0	0	0	0	uk	0	uk	uk	0
0	0	1	0	0	0	uk	0	uk	uk	0
0	0	0	0	0	0	uk	0	uk	uk	0
0	0	0	0	0	0	uk	0	uk	uk	0
na	**na**	**0**	**na**	**na**	**na**	**uk**	**na**	**uk**	**uk**	**na**
na	na	uk	na	na	na	uk	na	uk	uk	na
na	na	uk	na	na	na	uk	na	uk	uk	na
na	na	uk	na	na	na	uk	na	uk	uk	na
na	na	uk	na	na	na	uk	na	uk	uk	na
0	0	1	0	0	1	0	0	0	0	0
0	0	1	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	1	0	1	0	0	1
0	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	1	0	0	0	0	0
1	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	uk	0	0	uk
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	uk
0	0	0	0	0	0	0	0	0	0	uk
0	0	0	0	0	0	0	0	0	0	uk
0	0	0	0	0	0	0	0	0	0	uk
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	uk
0	0	1	0	0	0	0	0	1	0	1
0	0	1	0	0	0	0	0	1	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	1	1	0	1	0	1	1	0	1
1	0	1	1	0	1	0	1	0	0	1
1	0	0	0	0	1	0	1	0	0	0
1	0	1	1	0	0	0	1	0	0	0
0	0	0	0	0	1	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	1	1	0	1	0	0	1	0	1
0	0	0	0	0	0	0	0	1	0	1
0	1	1	1	0	1	0	0	0	0	0
0	1	0	1	0	1	0	0	0	0	0
0	0	1	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	uk	0	uk	0	0	uk
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
na	na	na	na	na	na	na	na	na	na	na
0	0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	0	0	0	0	uk	0	uk	0	0	uk
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	uk	0	uk	0	0	uk
uk	uk	uk	uk	1	1	uk	1	uk	uk	uk
uk	uk	uk	uk	1	1	uk	1	uk	uk	uk
uk	uk	uk	uk	1	1	uk	1	uk	uk	uk
uk	uk	1	1	0	1	uk	1	uk	uk	uk
uk	uk	0	0	0	1	uk	0	uk	uk	0
uk	uk	uk	uk	uk	0	uk	0	uk	uk	0
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	0	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	1	uk	uk	uk	uk	
uk	uk	uk	uk	1	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	0	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	0	uk	uk	uk	uk	uk	uk

Table S1 . Clinical spectrum of CTLA4 mutation carriers

65	66	67	68	69	70	71	72	73	74	75
Z.II.3	**Z.II.6**	**Z.III.1**	**AA.III.3**	**AA.IV.1**	**BB.I.2**	**BB.II.1**	**BB.II.2**	**CC.II.1**	**DD.I.2**	**DD.II.1**
1969	1971	1994	uk	10/11/1996	uk	uk	1998	1972	uk	1999
f	m	f	m	m	f	f	f	f	f	m
na	na	16	na	18	uk	na	10	10	na	13
uk	uk	21	46	18	45	20	17	43	37	15
na	na	na	na	na	na	na	na	na	na	na
Caucasian	Caucasian	Caucasian	asian	asian	asian	asian	asian	asian	asian	asian
Norway	Norway	Norway	Japan	Japan	Japan	Japan	Japan	Japan	Japan	Japan
no	no	no	no	no	no	no	no	no	no	no
completely unaffected	completely unaffected	affected	unaffected	affected	affected	completely unaffected	affected	affected	completely unaffected	affected
0	0	0	0	1	0	0	0	1	0	0
0	0	0	0	0	0	0	1	0	0	0
0	0	1	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	Asthma	0	0	0	0	0	0
0	0	0	0	1	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0
0	0	1	0	0	0	0	0	0	0	1
0	0	1	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	uk	1	uk	1	uk	uk	1	1	uk	0
0	uk	1	uk	1	uk	uk	0	1	uk	0
0	uk	1	uk	0	uk	uk	0	1	uk	0
0	uk	1	uk	1	uk	uk	1	1	uk	0
0	uk	1	0	1	0	0	1	1	0	0
0	uk	1	0	1	0	0	1	1	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	1	0	0	0	1	0	0
uk	**uk**	**1**	**uk**	**1**	**uk**	**uk**	**0**	**0**	**uk**	**0**
uk	uk	1	uk	0	uk	uk	0	0	uk	0
uk	uk	1	uk	0	uk	uk	0	0	uk	0
uk	uk	0	uk	1	uk	uk	0	0	uk	0
uk	uk	0	uk	0	uk	uk	0	0	uk	0
uk	uk	1	uk	0	uk	uk	0	0	uk	0
uk	uk	0	uk	0	uk	uk	0	0	uk	0
uk	uk	0	uk	0	uk	uk	0	0	uk	0
uk	uk	0	uk	0	uk	uk	0	0	uk	0
uk	uk	0	uk	0	uk	uk	0	0	uk	0
uk	**uk**	**1**	**uk**	**0**	**uk**	**uk**	**na**	**na**	**uk**	**na**
uk	uk	0	uk	uk	uk	uk	na	na	uk	na
uk	uk	1	uk	uk	uk	uk	na	na	uk	na
uk	uk	1	uk	uk	uk	uk	na	na	uk	na
uk	uk	0	uk	uk	uk	uk	na	na	uk	na
0	uk	1	0	0	0	0	0	1	0	1
0	uk	1	0	0	0	0	0	1	0	0
0	uk	1	0	0	0	0	0	1	0	1
0	uk	uk	0	0	0	0	0	0	0	1
0	uk	1	0	0	0	0	0	0	0	0
0	uk	uk	0	0	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	uk	0	0	0	0	0	0	Asthma	0	0
0	uk	1	0	0	0	0	0	0	0	0
0	uk	uk	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	uk	0	uk	0	0	uk	uk	0	uk
0	uk	0	0	0	0	0	0	0	0	0
0	uk	1	0	0	0	0	0	0	0	0
0	uk	1	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	1	0	0	0	0	0	0	0	0
0	uk	uk	uk	0	uk	uk	0	0	uk	0
0	uk	uk	uk	0	uk	uk	0	0	uk	0
0	uk	uk	0	0	0	0	0	0	0	0
0	uk	uk	0	uk	0	0	uk	uk	0	uk
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	uk	0	0	0	0	0	0	0	0
0	uk	1	0	0	0	0	1	0	0	0
0	uk	1	0	0	0	0	1	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	1	0	0	0	0	1	0	0	1
0	uk	1	0	0	0	0	1	0	0	1
0	uk	1	0	0	0	0	1	0	0	1
0	uk	0	0	uk	0	0	1	0	0	1
0	uk	0	0	0	0	0	0	0	0	0
0	uk	1	0	uk	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	1
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	1	1	1	0	0	0	1	0	0
0	uk	1	0	0	0	0	0	0	0	0
0	uk	0	1	1	0	0	0	1	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	1	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	uk	0	uk	0	0	uk	uk	0	uk
0	uk	0	0	0	1	1	0	0	0	0
0	uk	0	0	0	1	0	0	0	0	0
na	na	na	na	na	1	na	na	na	na	na
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	uk	uk	0	uk	0	Hyperthyroidism	uk	uk	0	uk
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	0	0	0	0	0	0	0	0	0
0	uk	uk	0	uk	0	0	uk	uk	0	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	0	uk	0	uk	uk	1	uk	uk	1
uk	uk	0	uk	0	uk	uk	0	uk	uk	uk
uk	uk	0	uk	0	uk	uk	0	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk		uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk

Table S1 . Clinical spectrum of CTLA4 mutation carriers

76	77	78	79	80	81	82	83	84	85	86
EE.II.1	**FF.II.1**	**GG.I.1**	**GG.II.1**	**GG.II.2**	**GG.II.3**	**HH.II.1**	**JJ.II.1**	**KK.I.1**	**KK.II.1**	**LL.II.1**
8/24/1996	8/20/1992	8/9/1968	2/2/1995	1/15/1997	2/28/2001	11/9/1987	5/20/1987	1958	12/12/1979	9/11/2001
m	m	m	f	m	f	f	m	m	f	f
11	6	na	9	na	na	2	11	na	21	1
18	22	47	20	18	14	28	28	58	36	14
na	22	na	na	na	na	na	na	na	na	14
Caucasian	uk	Caucasian	Caucasian	Caucasian	Caucasian	Africain americain	Caucasian	Caucasian	Caucasian	Caucasian
Netherlands	USA	Germany	Germany	Germany	Germany	USA	Germany	Czech Republic	Czech Republic	Czech Republic
no	no	no	no	no	no	no	no	no	no	no
affected	affected	unaffected	affected	unaffected	completely unaffected	affected	affected	unaffected	affected	affected
1	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	1	0	1	0	0	1	1	0	0	0
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	1	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	1	0	0	0	0
0	0	0	1	0	0	0	0	0	0	1
0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	Pernicious anemia	0	0
1	1	uk	uk	uk	uk	1	1	0	1	1
0	0	uk	#	uk	uk	#	1	0	1	1
1	1	uk	#	uk	uk	#	1	0	1	1
0	1	uk	#	uk	uk	#	1	0	1	1
1	1	0	1	0	0	0	1	0	1	1
1	0	0	1	0	0	0	1	0	1	1
0	0	0	1	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	1
1	1	0	0	0	0	0	1	0	1	1
1	**0**	**uk**	**0**	**uk**	**uk**	**0**	**1**	**0**	**1**	**1**
0	0	uk	0	uk	uk	0	0	0	0	0
0	0	uk	0	uk	uk	0	0	0	1	0
0	0	uk	0	uk	uk	0	1	0	1	1
0	0	uk	0	uk	uk	0	0	0	0	0
0	0	uk	0	uk	uk	0	1	0	0	0
0	0	uk	0	uk	uk	0	0	0	0	0
0	0	uk	0	uk	uk	0	0	0	0	0
1	0	uk	0	uk	uk	0	0	0	0	0
0	0	uk	0	uk	uk	0	0	0	0	0
1	**na**	**uk**	**na**	**uk**	**uk**	**na**	**1**	**na**	**1**	**0**
uk	na	uk	na	uk	uk	na	uk	na	1	uk
uk	na	uk	na	uk	uk	na	uk	na	1	uk
uk	na	uk	na	uk	uk	na	uk	na	0	uk
uk	na	uk	na	uk	uk	na	uk	na	0	uk
1	0	0	0	0	0	1	1	0	1	1
1	0	0	0	0	0	1	1	0	1	1
0	0	0	0	0	0	1	1	0	0	1
0	0	0	0	0	0	uk	1	0	0	1
0	0	0	0	0	0	0	1	0	1	1
0	0	0	0	0	0	uk	0	0	uk	1

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	1	0	1	1
0	0	0	0	0	0	0	1	0	0	0
1	0	0	0	0	0	0	1	0	0	1
0	0	0	0	0	0	0	1	0	0	1
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	1
1	uk	0	uk	0	0	uk	uk	0	uk	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	1	0	1	1
1	0	0	0	0	0	0	uk	0	1	0
0	0	0	0	0	0	0	uk	0	1	0
0	0	0	0	0	0	0	uk	0	1	1
0	0	0	0	0	0	0	uk	0	0	0
0	uk	uk	uk	uk	uk	uk	1	uk	0	0
0	uk	uk	uk	uk	uk	uk	0	uk	0	0
0	uk	0	uk	0	0	uk	0	0	0	0
0	uk	0	uk	0	0	uk	uk	0	uk	Rotavirus
0	0	0	0	0	0	0	uk	0	1	0
0	0	0	0	0	0	0	uk	0	0	0
0	0	0	0	0	0	0	uk	0	1	0
0	uk	0	uk	0	0	uk	uk	0	uk	0
1	0	0	0	0	0	1	1	0	1	1
1	0	0	0	0	0	1	1	0	1	1
0	0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	1	0	1	0	0	0	1	1	0	1
1	1	0	1	0	0	0	1	0	0	1
0	1	0	1	0	0	0	1	0	0	0
0	0	0	1	0	0	0	1	0	0	1
1	0	0	1	0	0	0	1	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	1	0	0	0	0	0	1	1
0	1	0	0	0	0	0	1	0	0	0
0	1	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0
1	0	0	0	0	0	1	1	0	0	1
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	1	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	1
uk	uk	0	uk	0	0	uk	uk	0	0	uk
1	0	1	1	1	0	1	1	0	0	0
0	0	1	0	1	0	0	0	0	0	0
na	na	uk	na	uk	na	na	na	na	na	na
1	0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

uk	uk	0	uk	0	0	uk	uk	0	uk	uk
0	0	0	0	0	0	1	1	0	1	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	1	1
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	1	0	0	0	0
uk	uk	0	uk	0	0	uk	uk	0	uk	0
1	1	uk	uk	uk	uk	uk	uk	uk	1	uk
1	1	uk	uk	uk	uk	uk	uk	uk	1	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	1	uk
0	0	uk	1	uk	uk	0	1	uk	0	1
1	0	uk	uk	uk	uk	0	uk	uk	0	0
1	0	uk	uk	uk	uk	0	uk	uk	0	1
uk	uk	uk	1	uk	uk	uk	1	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	0	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	1	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	0	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	0	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk

Table S1 . Clinical spectrum of CTLA4 mutation carriers

87	88	89	90	91	92	93	94	95	96	97
MM.II.1	**NN.I.1**	**NN.II.1**	**NN.II.6**	**NN.II.8**	**NN.II.9**	**NN.II.10**	**NN.II.11**	**OO.II.1**	**PP.II.1**	**QQ.II.1**
4/27/1978	01.05.1951	uk	16.10.1983	24.09.1987	08.05.1889	04.02.1999	29.06.1995	6/25/1992	3/18/1975	3/13/1985
m	m	f	f	f	m	f	m	m	m	m
14	12	na	23	13	18	na	6	18	8	13
38	61	uk	29	20	23.00	17	21	24.00	40.00	31
na	na	na	na	na	na	na	na	na	na	na
Caucasian	Caucasian	Caucasian	caucasien	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Germany	Uruguay	Uruguay	Uruguay	Uruguay	Uruguay	Uruguay	Uruguay	Germany	Canada	Germany
no	no	no	no	no	no	no	no	no	no	no
affected	affected	completely unaffected	affected	affected	affected	completely unaffected	affected	affected	affected	affected
0	1	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	1
1	0	0	0	0	1	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0	0
0	0	0	Primary biliary cirrhosis	0	0	0	0	0	0	0
1	1	0	0	0	0	0	0	1	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	Herpes labialis	Primary biliary cirrhosis	Rheumatoid arthritis	0	0	0	0	0	0
1	1	uk	1	1	uk	0		1	1	1
0	1	uk	1	1	#	0	uk	1	1	1
1	0	uk	0	1	#	0	uk	1	0	1
1	1	uk	0	1	#	0	uk	1	0	0
1	0	0	1	1	1	0	0	1	1	1
1	0	0	1	0	1	0	0	1	1	1
0	uk	uk	uk	uk	1	uk	uk	0	1	0
0	0	0	1	0	0	0	0	0	0	0
1	0	0	1	0	1	0	0	0	0	0
1	**0**	**0**	**1**	**1**	**1**	**0**	**0**	**1**	**1**	**1**
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	1	0	0	1	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	1	1	0	0	0	1	1
0	0	0	0	0	0	0	0	0	0	0
1	**na**	**na**	**1**	**1**	**1**	**na**	**na**	**0**	**1**	**1**
uk	na	na	uk	uk	1	na	na	na	0	0
uk	na	na	1	uk	1	na	na	na	1	1
uk	na	na	uk	uk	1	na	na	na	uk	0
uk	na	na	uk	uk	1	na	na	na	uk	1
1	1	1	1	1	1	0	1	1	1	1
1	1	0	1	1	0	0	1	1	1	0
1	1	0	1	1	1	0	0	1	1	1
1	0	1	1	1	1	0	0	1	1	1
0	0	0	1	0	1	0	0	1	0	0
0	0	0	1	1	1	0	0	1	1	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	0	0	0	0	0	0	0	0	0	0
1	0	1	1	0	1	0	0	1	1	1
0	0	0	0	0	0	0	0	0	0	0
1	0	1	1	0	1	0	0	0	1	1
1	0	0	1	0	0	0	0	0	1	1
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	uk	0	0	0	0	0	0
0	0	0	0	uk	0	0	0	0	0	0
1	0	0	0	uk	0	0	0	0	1	1
0	0	1	1	uk	1	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	1	1	0
uk	0	0	0	0	0	0	0	1	1	0
uk	0	0	1	0	0	0	0	1	0	0
uk	0	0	0	0	0	0	0	0	0	0
uk	0	0	0	0	0	0	0	0	0	0
uk	0	uk	uk	0	0	uk	uk	0	0	0
uk	0	uk	uk	0	0	uk	uk	0	0	0
uk	0	0	uk	0	0	0	0	0	0	0
uk	0	0	uk	uk	0	0	0	0	Measles	0
uk	0	0	0	0	1	0	0	0	1	1
uk	0	0	0	0	0	0	0	0	0	0
uk	0	0	0	0	1	0	0	0	1	1
uk	0	0	0	0	0	0	0	0	uk	0
1	0	0	1	1	1	0	0	0	1	1
1	0	0	1	1	1	0	0	0	1	1
1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	Pancreatic Insufficiency
1	0	0	0	0	1	0	0	1	1	0
1	0	0	0	0	1	0	0	1	1	0
1	0	0	0	0	1	0	0	1	1	0
1	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	uk	uk	uk	uk	0	uk	uk	0	0	0
0	uk	uk	uk	uk	0	uk	uk	0	0	0
0	0	uk	0	0	0	uk	uk	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
1	uk	0	uk	uk	0	0	1	0	1	1
0	0	0	0	0	0	0	1	0	0	0
uk	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	uk	0	uk	uk	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	1
0	uk	0	uk	uk	0	0	0	0	0	0
Urtikaria	0	0	uk	uk	uk	0	0	0	uk	uk
0	0	0	0	0	0	0	1	0	1	1
0	0	0	0	0	0	0	1	0	1	1
na	na	na	na	na	na	na	uk	na	0	0
0	0	0	0	0	0	0	1	0	1	1
0	0	uk	0	0	0	uk	0	0	0	0
0	0	uk	0	0	0	uk	0	0	0	0
0	0	uk	0	0	0	uk	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	0	uk	uk	uk	uk	uk	0	0	uk	uk
0	0	0	1	1	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0
0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	1	0
uk	0	0	uk	uk	uk	0	0	0	uk	uk
uk	uk	uk	0	1	1	uk	uk	0	1	uk
uk	uk	uk	uk	uk	uk	uk	uk	0	1	uk
0	uk	uk	uk	uk	uk	uk	uk	0	1	uk
uk	uk	uk	0	0	0	uk	uk	uk	uk	0
uk	uk	uk	0	0	0	uk	uk	uk	0	0
uk	uk	uk	0	0	0	uk	uk	uk	0	0
uk	uk	uk	uk	uk	uk	uk	uk	uk	0	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	0
uk	uk	uk	uk	uk	uk	uk	uk	uk	0	0
uk	uk	uk	uk	uk	uk	uk	uk	uk	1	uk
1	uk	uk	uk	uk	uk	uk	uk	uk	uk	0
1	uk	uk	uk	uk	uk	uk	uk	uk	uk	0
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk

Table S1 . Clinical spectrum of CTLA4 mutation carriers

98	99	100	101	102	103	104	105	106	107	108
RR.II.1	**SS.II.1**	**TT.I.1**	**TT.II.2**	**TT.II.3**	**TT.II.4**	**TT.II.5**	**UU.II.1**	**UU.II.2**	**UU.III.2**	**UU.III.3**
12/14/1998	8/27/1988	08.02.1966	21.06.1989	02.08.1992	05.10.2006	02.02.2010	uk	uk	27.08.1949	uk
f	f	m	m	f	m	m	f	f	f	f
14	15	5	21	11	4	1	na	40	6	59
16	27	50	26	24	10	6	86	73	65	60
na	na	na	na	na	na	na	86	na	na	60
Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	caucasien	Caucasian	Caucasian	Caucasian
USA	USA	Czech Republic	Czech Republic	Czech Republic	Czech Republic	Czech Republic	Spain	Spain	Spain	Spain
no	No	no	no	no	no	no	no	no	no	no
affected	affected	affected	affected	affected	affected	affected	completely unaffected	affected	affected	affected
1	1	0	0	0	0	0	0	1	0	0
0	1	0	0	0	0	0	0	0	1	0
0	0	0	1	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0
0	0	0	0	1	0	0	0	0	0	1
0	0	1	0	0	0	0	0	0	0	0
0	0	Alopecia	0	Grave's disease	0	0	0	0	0	0
0	1	0	0	0	0	0	0	1	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	1	1	1	1	1	0	0	0	0
0	0	0	0	0	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	1	1	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	1	0	1	0	1	0	uk	uk	1	uk
0	1	0	0	0	0	0	uk	uk	1	uk
0	1	0	0	0	0	0	uk	uk	0	uk
1	1	0	1	0	1	0	uk	uk	0	uk
1	1	0	0	0	0	0	0	0	1	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	1	0
1	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**
0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
1	1	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	**1**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**0**	**na**
uk	1	na	na	na	na	na	na	na	uk	na
uk	0	na	na	na	na	na	na	na	uk	na
uk	0	na	na	na	na	na	na	na	uk	na
uk	0	na	na	na	na	na	na	na	uk	na
1	1	0	0	0	0	0	0	1	1	1
0	1	0	0	0	0	0	0	0	1	uk
1	1	0	0	0	0	0	0	1	1	uk
1	1	0	0	0	0	0	0	0	1	0
1	1	0	0	0	0	0	0	0	1	0
1	1	0	0	0	0	0	0	1	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	1	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	uk
0	0	0	0	0	0	0	0	0	0	uk
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	uk	0	0	0	0	0	0	0	0
0	0	uk	0	0	0	0	0	0	0	0
0	0	uk	0	0	0	0	0	0	0	0
0	0	uk	0	0	0	0	0	0	0	0
0	0	uk	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	HCV	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	1	0	0	0	0	0	0	1	0
0	1	1	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	1	1	1	1	1	0	0	0	0
0	0	1	1	1	1	1	0	0	0	0
0	0	0	0	0	1	1	0	0	0	0
0	0	1	1	1	1	1	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	Agranulocytosis	0	0	0	0	0
0	1	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	uk
1	0	1	0	1	1	0	0	0	1	0
0	0	0	0	0	0	0	0	0	1	0
1	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	1	0	0	0	1	0
0	0	1	0	1	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	1	1	1	0	0	1	1
0	0	1	0	0	0	0	0	0	1	1
na	na	1	na	na	na	na	na	na	0	0
0	0	0	0	0	1	1	0	0	0	0
uk	0	0	0	0	na	na	0	0	0	0
uk	0	0	0	0	0	0	0	0	0	0
uk	0	0	0	0	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

uk	0	0	0	Grave's disease	0	0	0	0	0	0
1	1	0	0	0	0	0	1	1	0	0
1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	1	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	uk	uk	uk	1	1	uk	uk	uk	uk
uk	0	uk	uk	uk	0	1	uk	uk	uk	uk
1	uk	uk	uk	uk	1	1	uk	uk	uk	uk
0	0	1	uk	0	1	1	uk	uk	uk	uk
0	0	0	0	0	0	0	uk	uk	uk	uk
uk	uk	0	0	0	0	0	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	0	0	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	1	1	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk

Table S1 . Clinical spectrum of CTLA4 mutation carriers

109	110	111	112	113	115	114	116	117	118	119
UU.III.4	**UU.III.6**	**UU.III.7**	**UU.III.9**	**UU.III.10**	**UU.IV.1**	**UU.IV.2**	**UU.IV.3**	**UU.IV.4**	**UU.IV.9**	**UU.IV.10**
20.08.1947	22.08.1950	03.11.1952	07.11.1960	28.07.1963	03.07.1969	11.02.1973	14.09.1974	18.10.1982	16.06.1974	6/12/1977
m	f	f	m	f	m	m	f	m	m	m
57	na	14	uk	na	na	31	na	na	na	uk
68	62	63	55	53	46	42	40	33	40	uk
na	na	na	na	na	na	na	na	na	na	na
Caucasian	Caucasian	Caucasian	caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	causain	Caucasian
Spain	Spain	Spain	Spain	Spain	Spain	Spain	Spain	Spain	Spain	Spain
no	no	no	no	no	no	no	no	no	no	no
affected	unaffected	affected	affected	unaffected	completely unaffected	affected	unaffected	completely unaffected	unaffected	affected
0	0	0	0	0	0	0	0	0	0	1
1	0	1	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	HepB, Polio infection	0	0	Psoriasis	0	0	0	Asthma
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1
1	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	1	0	0	0	0
0	Psoriasis	0	Chronic liver disease	0	0	0	Unexpected weight loss	0	Coeliac disease	Asthma
0	1	1	1	0	0	0	0	0	1	0
0	1	1	1	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	1	0
0	0	0	1	0	0	0	0	0	0	0
0	0	1	1	0	0	0	0	0	0	0
0	0	1	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	1	1	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0
0	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
na	**na**	**0**	**na**	**na**	**na**	**na**	**na**	**na**	**na**	**na**
na	na	uk	na	na	na	na	na	na	na	na
na	na	uk	na	na	na	na	na	na	na	na
na	na	uk	na	na	na	na	na	na	na	na
na	na	uk	na	na	na	na	na	na	na	na
0	0	1	0	1	0	0	0	0	0	1
0	0	1	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	1
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	0	0	0	Spontaneous pneumothorax	0	0	0	0	0	Asthma
1	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	uk	0	0	uk	uk	0	uk	uk	uk	0
1	uk	0	0	uk	uk	0	uk	uk	uk	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	Polio, HBV	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	0	0	1	0	1	0
1	0	1	1	1	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0
0	0	1	0	1	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
H.pylori, mesenteric panniculitis	gastric adenoma	Gastric polyps (tubular adenoma)	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	1	0	1	0	1	0	0	0	0
0	1	0	0	1	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	Chronic urticaria	0	Erythema anulare	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
0	na	na	na	na	na	na	na	na	na	na
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	0	0	0	0	0	0	0	0	0	0
1	0	0	1	0	0	1	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0
Wolf-Parkinson-White Syndrome	0	0	0	0	0	0	Arthralgia	0	0	0
uk	uk	uk	uk	1	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	1	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	1	uk	uk	uk	uk	uk	uk
uk	uk	0	uk	uk	uk	uk	uk	uk	uk	uk
uk	0	0	0	uk	uk	0	uk	uk	uk	uk
uk	0	0	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	0	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk

Table S1 . Clinical spectrum of CTLA4 mutation carriers

120	121	122	123	124	125	126	127	128	129	130
UU.IV.12	**UU.V.1**	**UU.V.2**	**VV.I.1**	**VV.II.1**	**WW.II.1**	**XX.II.1**	**YY.II.1**	**ZZ.I.2**	**ZZ.II.1**	**AAA.II.1**
08.06.1979	15.11.2004	17.08.2011	uk	11/15/2001	04.09.2003	12.03.1974	16.03.1999	1977	30.05.1997	01.02.1968
f	m	m	m	m	m	m	f	f	m	m
0.25	0.25	0.83	na	7	8	12	3	uk	16	23
34	10	3	uk	13	12	40	14	39	19	46
na	na	na	na	na	na	na	na	na	na	na
Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Spain	Spain	Spain	Saudi Arabia	Saudi Arabia	UK	Belgium	Germany	Germany	Germany	Switzerland
no	no	no	no	yes	no	no	no	no	no	no
affected	affected	affected	completely unaffected	affected	affected	affected	affected	affected	affected	affected
0	0	0	0	1	0	0	0	0	0	0
1	0	1	0	0	0	1	0	0	0	0
0	0	0	0	0	1	0	0	0	1	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	1	1
Salmonella sepsis	West syndrome	0	0	0	0	0	0	0	Spleno-megaly, wound healing disorder	Addison's disease
1	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	1	1	1	1	0	0	1
0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	West syndrome	0	0	0	0	0	0	0	0	0
1	1	1	uk	1	#	1	0	uk	1	1
1	1	1	uk	#	#	1	0	uk	1	#
0	0	0	uk	1	#	0	0	uk	0	0
0	1	0	uk	1	#	0	0	uk	1	1
0	0	0	0	1	1	1	1	0	1	1
0	0	0	0	1	1	1	1	0	1	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	1	0	1	1	0	1	0
0	0	0	0	1	1	0	1	0	1	0
0	**0**	**0**	**0**	**1**	**0**	**1**	**1**	**0**	**1**	**1**
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	1	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	1	0	0	0
0	0	0	0	1	0	1	1	0	0	1
0	0	0	0	0	0	0	0	0	0	0
na	**na**	**na**	**na**	**1**	**na**	**1**	**uk**	**na**	**0**	**uk**
na	na	na	na	uk	na	0	uk	na	na	uk
na	na	na	na	uk	na	1	uk	na	na	uk
na	na	na	na	uk	na	uk	uk	na	na	uk
na	na	na	na	uk	na	uk	uk	na	na	uk
0	0	1	0	1	1	1	1	0	1	0
0	0	1	0	1	0	1	1	0	0	0
0	0	1	0	1	1	1	1	0	0	0
0	0	0	0	1	0	1	0	0	0	0
0	0	0	0	1	0	1	0	0	1	0
0	0	0	0	1	0	1	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	1	0	1	1	0	0	0
1	0	0	0	0	uk	0	0	0	0	0
0	0	0	0	1	0	1	0	0	0	uk
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	1	0	0	0	0
0	0	0	0	0	uk	uk	uk	0	0	uk
0	0	0	0	0	uk	uk	uk	0	0	uk
0	0	0	0	0	uk	0	0	0	0	0
0	0	0	0	0	uk	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0	0	0	0
uk	0	0	uk	0	0	0	0	0	0	0
0	0	0	uk	0	0	0	0	0	0	0
Streptococcus	0	0	0	0	0	0	0	0	0	0
HPV	0	0	0	0	uk	HPV	HPV	0	0	0
0	0	0	0	0	uk	0	0	0	0	0
0	0	0	0	0	uk	0	0	0	0	0
0	0	0	0	0	uk	0	0	0	0	0
0	0	0	0	0	uk	0	0	0	0	0
1	0	1	0	1	1	1	1	0	0	1
1	0	1	0	1	1	1	1	0	0	1
0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	1	1	0	0	1	1
0	0	0	0	1	1	1	0	0	1	1
0	0	0	0	1	1	0	0	0	1	0
0	0	0	0	0	1	1	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	uk	0	0	0	0	0
0	0	0	0	0	uk	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	uk	0	0	0	0	0
0	1	0	0	0	1	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	1	1	0	0	0	1	1	0	1	0
1	1	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	1	1	0	1	0
0	0	1	0	0	0	0	1	0	1	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	1	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
Chronic urticaria	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	1	0	1
0	0	0	0	0	0	0	0	0	0	0
na	na	na	na	na	na	na	na	na	na	na
0	0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0

Table S1 . Clinical spectrum of CTLA4 mutation carriers

Hyperparathyroidism	0	0	0	0	0	0	0	0	0	Addison's disease
1	0	1	0	1	0	0	1	0	0	1
0	0	1	0	0	0	0	1	0	0	0
1	0	0	0	0	0	0	0	0	0	1
0	0	0	0	1	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
uk	uk	uk	uk	uk	1	uk	1	uk	1	uk
uk	uk	uk	uk	uk	uk	uk	1	uk	1	uk
uk	uk	1	uk	uk	1	uk	1	uk	1	uk
uk	uk	uk	uk	0	1	uk	uk	uk	1	uk
0	uk	0	uk	0	0	0	0	uk	0	uk
0	uk	0	uk	uk	0	0	0	uk	0	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	0	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	uk	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	0	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	0	uk	uk	uk	uk
uk	uk	uk	uk	uk	uk	0	uk	uk	uk	uk
uk	uk	uk	uk	0	uk	0	0	uk	uk	uk

Table S1 . Clinical spectrum of CTLA4 mutation carriers

131	132	133	Chr2_1	Chr2_2	**Clinical phenotype and treatment options in CTLA4 mutation carriers**	
BBB.II.1	**CCC.II.1**	**DDD.II.1**	**P1**	**P2**	**patient ID**	
2/22/1999	06.09.1992	09.02.1971	5/22/1978	4/2/1996	**Date of birth**	
f	m	f	f	m	**Sex**	
1	14	38	5	14	**Age of onset [years]**	
17	24	45	37	20	**Age at evaluation [years]**	
na	na	na	na	na	**Age of death [years]**	
Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	**Ethnicity**	
USA	USA	USA	Canada	Australia	**Country of Origin**	
no	no	no	no	no	**Consanguinity**	
affected	affected	affected	affected	affected	**Classification**	
					First manifestations/symptoms	
1	1	1	0	1	**Respiratory involvement**	
1	0	0	0	0	**Gastrointestinal involvement**	
1	1	1	0	0	**Cytopenia**	
0	0	0	0	0	**Neurological involvement**	
0	0	0	0	0	**Fever, night sweats**	
1	0	0	0	0	**Growth retardation**	
0	0	0	1	0	**Arthritis**	
0	0	0	0	0	**Atopic dermatitis**	
0	0	0	0	0	**Type 1 diabetes**	
0	0	0	0	0	**Thyroid disease**	
0	0	0	0	0	**Others**	
0	0	0	0	0	**Details**	
					Primary Diagnosis	
1	1	1	1	1	**CVID**	
0	0	0	0	0	**ALPS-like phenotype**	
0	0	0	0	0	**IPEX-like phenotype**	
1	1	0	0	0	**Lymphoproliferation**	
1	1	0	0	0	**Respiratory involvement**	
1	0	0	1	0	**Gastrointestinal involvement**	
1	1	1	0	1	**Cytopenia**	
1	1	0	0	0	Evans syndrome	13
1	1	0	0	0	ITP	
1	1	0	0	1	AIHA	
0	0	0	0	0	PRCA	
0	0	0	0	0	**Neurological involvement**	
0	0	0	0	0	**Malignancy**	
0	0	0	0	0	**Endocrinological involvement**	
0	0	0	0	0	**Warts**	
0	0	0	0	0	**Psoriatic arthritis**	
0	0	0	0	0	**Others**	
1	1	1	1	1	**Hypogammaglobulinemia**	
1	1	1	1	1	**Low IgG**	
1	0	1	0	1	**Low IgM**	
1	1	1	1	1	**Low IgA**	
1	1	0	1	1	**Lymphoproliferation**	
1	1	0	0	1	**Splenomegaly**	
0	0	0	uk	0	**Splenectomy**	
0	0	0	0	0	**Hepatomegaly**	
1	1	0	0	0	**Lymphadenopathy**	
1	**1**	**0**	1	1	**Lymphocytic or granulomatous organ infiltration**	
0	0	0	0	0	Bone marrow	
0	1	0	uk	0	Brain	
1	1	0	1	1	Lung	
uk	0	0	0	0	Spleen	
0	1	0	1	0	Kidney	
0	0	0	uk	0	Liver	
0	1	0	0	0	Retroperitoneum	
1	0	0	1	0	Gut	
0	0	0	0	1	Retrobulbar space	
1	**1**	**0**	1	1	**Biopsied**	
1	1	0	uk	1	B cell infitration	
1	1	0	uk	1	T cell infiltration	
1	1	0	uk	uk	CD4 T cells	
1	1	0	uk	uk	CD8 T cells	
1	1	1	1	1	**Respiratory tract involvement**	
1	1	1	1	1	**Upper respiratory infections**	
1	1	1	1	1	**Lower respiratory infections**	
1	0	1	uk	0	**Pneumonia**	
1	1	0	1	1	**GLILD**	
1	1	0	uk	0	**Bronchiectasis**	

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	follicular bronchiolitis	0	0	0	**Others**	
1	0	0	1	0	**Clinically reactivated/apparent Infections**	
0	0	0	0	0	**Sepsis**	
0	0	0	0	0	**Herpes Infection**	
0	0	0	0	0	clinical EBV infection	
0	0	0	0	0	clinical CMV infection	
0	0	0	0	0	HHV6 viremia	
0	0	0	0	0	HHV7 viremia	
0	0	0	1	0	Severe varizella zoster infection	
0	0	0	1	0	Recurrent herpes simplex infection	
0	0	0	0	0	**Mycoplasma or TBC**	
1	1	1	0	0	**Bacterial infections**	
0	1	0	0	0	Staphylococcus aureus	
0	0	0	0	0	Hemophilus influenzae	
0	0	0	0	0	Escherichia coli	
0	0	1	0	0	Salmonella	
0	0	0	0	0	Streprococcus pneumoniae	
0	0	0	0	0	Helicobacter pylori	
0	0	0	0	0	Others	
0	1	0	0	0	**Viral infections** (other than Herpes infection)	
0	0	0	1	0	**Fungal infection**	
0	0	0	0	0	Aspergillus	
0	0	0	1	0	Candida	
0	0	0	0	0	Others	
1	0	0	1	0	**Gastrointestinal involvement**	
1	0	0	1	0	**Diarrhea/ Enteropathy**	
0	0	0	0	0	**Crohn's disease**	
0	0	0	0	0	**Coeliac disease**	
0	0	0	0	0	**Atrophic gastritis**	
0	0	0	0	0	**Pancreatitis**	
0	otitis media, sinusitis	0	0	0	**Others**	
1	1	0	0	1	**Cytopenia**	
1	1	0	0	1	**Autoimmune cytopenia**	
1	1	0	0	0	ITP	
1	1	0	0	1	AIHA	
0	0	0	0	0	Autoimmune neutropenia	
0	0	0	0	0	PRCA	
0	0	0	0	0	**Arterial thrombosis**	
0	0	0	0	0	**Deep vein thrombosis**	
0	0	0	0	0	**Pernicious anemia**	
0	0	0	0	0	**Others**	
0	1	0	1	0	**Neurological involvement**	
0	0	0	0	0	**Malignancy**	
0	0	0	0	0	**Lymphoma**	
0	0	0	0	0	**Gastric cancer/colon cancer**	
0	0	0	0	0	**EBV-associated malignancy**	
1	1	0	1	0	**Dermatological involvement**	
0	1	0	0	0	**Psoriasis**	
0	0	0	0	0	**Atopic dermatitis**	
0	1	0	0	0	**Eczema**	
0	0	0	0	0	**Alopecia**	
0	0	0	0	0	**Vitiligio**	
0	0	0	0	0	**Trachyonychia**	
1	0	0	0	0	**Warts**	
0	0	0	0	0	**Angiofibroma**	
erythema nodosum	0	0	Poro-keratosis, perforating folliculitis, recurrent molluscum contagiosum	0	**Others**	
0	0	0	1	0	**Endocrinological involvement**	
0	0	0	0	0	**Thyroiditis/Hypothyroidism**	
0	0	0	0	0	**Autoimmune Thyroiditis/Hypothyroidism**	
0	0	0	0	0	**Type 1 diabetes**	
0	0	0	1	0	**Hypogonadism**	
0	0	0	0	0	**Hypopituitarism**	
0	0	0	0	0	**Hypophysitis**	

Table S1 . Clinical spectrum of CTLA4 mutation carriers

0	0	0	0	0	**Others**	
1	0	0	1	0	**Other clinical manifestations**	
1	0	0	1	0	**Growth retardation**	
0	0	0	1	0	**Kidney involvement**	
0	0	0	uk	0	**Liver involvement**	
0	0	0	1		**Arthritis**	
0	0	0	Recurrent fevers	0	**Others**	
					Specific Antibody responses; Serology/Virology	
0	uk	uk	0	uk	**Tetanus §**	
0	uk	uk	0	uk	**Diphtheria §**	
0	uk	uk	0	uk	**Pneumococcal vaccination §**	
					Antibodies	
uk	1	0	uk	uk	**Coombs**	
0	0	0	uk	uk	**ANA**	
0	0	0	uk	uk	**ANCA**	
0	0	0	uk	uk	**Autoantibodyscreening**	
uk	uk	0	uk	uk	**Antiphospholipid antibodies**	
uk	uk	0	uk	uk	**LISS**	
uk	uk	0	uk	uk	**Anti_GAD antibodies**	
uk	uk	0	uk	uk	**C3d**	
uk	uk	0	uk	uk	**CH50**	
uk	uk	0	uk	uk	**Anti-microsomal antibodies**	

Table S2. HSCT

ID Gender	Age (year) of HSCT	Reason for transplantation	Conditioning regimen	Serotherapy	GvHD prophylaxis	HLA/ Donor source	Chimerism	GvHD/ Complications	Outcome
L.II.1 **Female**	10 (2005)	life-threatening, multisystem and immune-suppressant drug resistant nature of her gastrointestinal and haematological disease	Flu, Mel	Alem	CsA	BM 9/10 MUD	100%	No GvHD, No complications	Alive and well 10.5 years Complete remission
L.II.2 **Male**	17 (2015)	serious clinical complications of disease - Hodgkin disease and difficult to control autoinflammatory gut disease, mother died of disease, sister cured of disease by MUD BMT	Treo, Flu, TT	Alem	MMF, CsA	BM 10/10 MUD	100%	Chronic GVHD from first week post BMT treated with methyl-prednisolone and belatacept. Required 3-4 months on TPN. CMV colitis.	12 month follow-up: chronic CMV and gut GvHD, finally improving after 11 months. Currently fully orally fed, on oral budesonide 3mg/d and IV infliximab; 4 stools a day. Not requiring any treatment for CMV.
Q.II.1 **Male**	15 (2008)	Thrombocytopenia and widespread lymphoid hyperplasia despite Rituximab	Flu, Mel	Alem	CsA, MMF	PBSC 10/10 MUD	100%	Acute GvHD Grade IV of the gut	Died 4 months after HSCT due to a GvHD disease
S.II.1 **Male**	20 (2008)	Cytopenias, bronchiectasis, enteropathy (TPN dependent); mother had died of GI lymphoma	Flu, Mel	Alem,	CsA, MMF	PBSC 10/10 MUD	100%	Acute Grade II skin; resolved	Died 2.5 years later due to DKA, Before: complete remission
T.II.1 **Male**	16 (2010)	Arthritis, cytopenias, lymph-adenopathy: father had died post autologous HSCT for non-Hodgkin lymphoma.	Treo, Flu	Alem	CsA, MMF	PBSC 10/10 MUD	T 90% B 95% CD15+ 96%	No GvHD CMV re-activation (resolved) 1 episode of AIHA 6 months post HSCT	Alive and well, 6.1 years, Complete remission
Y.II.1 **Male**	16 (2011)	Therapy-refractory and partially life-threatening autoimmune cytopenias and lympho-proliferative disorder of unknown dignity	Treo, Flu	Alem	CsA, MMF	PBSC 9/10 MUD	100%	No GvHD No complications	Alive and well 5.6 years, complete remission
B.II.3 **Male**	51 (2015)	Life-threatening HLH, Hodgkin lymphoma	Bu, Flu, TT	ATG	CsA, MMF	PBSC 10/10 MUD	100%	Acute GvHD Grade II of the skin. Treated with Steroids. HSV-mucositis Grade II, resolved	100 day follow-up: alive and well

P.II.2 Male	13 (2015)	Life-threatening autoimmune complications including recurrent, treatment resistant cytopenias and AI-encephalitis	Treo, Flu, TT	ATG	CsA, MTX	BM 10/10 MUD	100%	Acute GvHD Grade II of the skin. Treated with Steroids.	10 months follow-up: alive and well
GG.II.1 Female	20 (2015)	Life-threatening autoimmune cytopenias, hemolysis, vasculitis with paraplegia; therapy resistant, low life quality	Treo, Flu, TT	ATG	CsA, MTX	BM 9/10 MUD	96%	Bacterial sepsis, Adeno-virus-, CMV- and EBV-re-activation requiring virus specific T cell transfer, resolved	10 months follow-up: alive and well
LL.II.1 Female	14 (2015)	Uncontrollable CMV infection, immune deficiency, lack of B cells, impossibility of immuno-suppressive therapy for lung disease	Treo, Flu, TT	ATG	CsA, MTX	PBSC 9/10 MUD	100%	GvHD Grade IV of the GIT (bleeding, hyperbili-rubinemia), Candida dubli-niensis, CMV, BKV infection, multiple organ failure, cytopenia	Died 4 months after HSCT due to multiple complications (see complications)
W.II.2 Female	14 (2016)	Recurrent infections	Flu, Mel TBI	??	Tacrolimus, PT-Cy	BM 7/8 MMUD	100%	Acute GvHD: skin stage 2, Grade I	Alive and well 7 months after; improvement of diarrhea and alopecia
VV.II.1 Male	14 (2016)	Drug resistant nature of respiratory and gastrointestinal disease	Bu, Flu	Alem	CsA, MMF	BM 10/10 MUD	>97% in all lineages except T cells (60%)	CMV reactivation despite foscarnet (42 mg/kg) and CMV Ig. Treated with increased foscarnet (54 mg/kg). ARDS in the setting of CMV re-activation, successful methyl-prednisolone treatment (2 mg/kg) led to resolution	Primary graft failure, remains lymphopenic and requires steroid treatment (1mg/kg) for pulmonary disease at day >100 post transplantation.

Alem = Alemtuzumab total dose 1.0 mg/kg; Flu = Fludarabine total dose G.II.1, L.II.1, S.II.1, T.II.1, Y.II.1, LL.II.1: 150 mg/m2 B.II.3 60 mg/m2, P.II.2: 160 mg/m2; Treo = Treosulfan total dose 42g /m2; Mel = Melphalan total dose 140 mg/m2; TT = Thiopeta 5 mg/kg, LL.II.1: 10 mg/kg, P.II.2: 8 mg/kg; Bu = Busulfan totale dosis 6.4 mg/kg; PBSC = peripheral blood stem cells; BM = bone marrow; PBSC= peripheral blood stem cell; MUD= matched unrelated donor; MMUD= mismatched unrelated donor; GvHD=Graft versus host disease; CsA = Cyclosporin; Pred = Prednisolon; MMF= Mycophenolatmofetil; ATG= Antithymocyte globulin P.II.1: 3x15 mg/kg; DKA = diabetic ketoacidosis.

Table S3. Frequency of genetic changes in the *CTLA4* gene (Ensembl)

Exon	Variant ID	Chr: bp	Alleles	Global MAF	Highest population MAF (highest MAF in any data base)	Conseq. Type	AA	AA cord	Transcript
Exon 1 (+)	rs767352102	2:203867946	G/T	T=0.00002/2 (ExAC); T=0.00003/1 (TOPMED)	< 0.01	missense variant	A/S	2	ENST00000302823.7
Exon 1 (+)	rs896306346	2:203867958	T/A	A=0.00003/1 (TOPMED)	< 0.01	missense variant	F/I	6	ENST00000302823.7
Exon 1 (+)	rs201778935	2:203867964	C/T	T=0.00002/3 (ExAC); T=0.00008/1 (GO-ESP)	< 0.01	missense variant	R/W	8	ENST00000302823.7
Exon 1 (§)	rs138279736	2:203867965	G/A/T	A=0.0006/3 (1000 Genomes); A=0.0002/3 (GO-ESP); A=0.00003/1 (TOPMED)	0.02	missense variant	R/Q	8	ENST00000302823.7
Exon 1 (§)	rs138279736	2:203867965	G/A/T	T=0.00005/6 (ExAC)	0.02	missense variant	R/L	8	ENST00000302823.7
Exon 1 (+)	rs146541851	2:203867970	A/G	G=0.000008/1 (ExAC); G=0.00008/1 (GO-ESP); G=0.0001/3 (TOPMED)	< 0.01	missense variant	K/E	10	ENST00000302823.7
Exon 1 (§)	rs755080468	2:203867979	C/T	T=0.000008/1 (ExAC)	< 0.01	synonymous variant	L	13	ENST00000302823.7
Exon 1 (§)	rs376591332	2:203867984	C/T	T=0.000008/1 (ExAC); T=0.00008/1 (GO-ESP)	< 0.01	synonymous variant	N	14	ENST00000302823.7
Exon 1 (+)	rs748599835	2:203867988	G/A	A=0.000008/1 (ExAC)	< 0.01	missense variant	A/T	16	ENST00000302823.7
Exon 1 (+)	rs772433747	2:203867989	C/A	A=0.00003/4 (ExAC)	< 0.01	missense variant	A/D	16	ENST00000302823.7
Exon 1 (§)	rs231775	2:203867991	A/G/T	G=0.4112/49892	0.48	missense	T/A	17	ENST00000302823.7

				(ExAC); G=0.4273/2140 (1000 Genomes); G=0.3694/4805 (GO-ESP); G=0.3890/11326 (TOPMED)		variant			
Exon 1 (§)	rs231775	2:203867991	A/G/T		0.48	missense variant	T/S	17	ENST00000302823.7
Exon 1 (+)	rs769368847	2:203868001	G/T	T=0.000008/1 (ExAC)	< 0.01	missense variant	W/L	20	ENST00000302823.7
Exon 1 (+)	rs886041906	2:203868002	G/A	NA		stop gained	W/*	20	ENST00000302823.7
Exon 1 (+)	rs1041117695	2:203868004	C/G	NA	< 0.01	missense variant	P/R	21	ENST00000302823.7
Exon 1 (+)	rs606231418	2:203868017	G/-	NA		frameshift variant	L/X	25	ENST00000302823.7
Exon 1 (+)	rs16840275	2:203868017	G/C	C=0.0023/275 (ExAC); C=0.0064/32 (1000 Genomes); C=0.0094/122 (GO-ESP); C=0.0106/308 (TOPMED)		synonymous variant	L	25	ENST00000302823.7
Exon 1 (+)	rs748802696	2:203868019	T/C	C=0.000008/1 (ExAC)	< 0.01	missense variant	F/S	26	ENST00000302823.7
Exon 1 (§)	rs145950656	2:203868029	C/T	T=0.00002/2 (ExAC); T=0.0002/2 (GO-ESP)	< 0.01	synonymous variant	L	29	ENST00000302823.7
Exon 1 (+)	rs774434261	2:203868033	A/G	G=0.000008/1 (ExAC)	< 0.01	missense variant	I/V	31	ENST00000302823.7
Exon 1 (+)	rs369567630	2:203868036	C/T	T=0.00004/5 (ExAC); T=0.00008/1 (GO-ESP)	< 0.01	missense variant	P/S	32	ENST00000302823.7
Exon 1 (§)	rs767441580	2:203868038	T/C	C=0.00002/2 (ExAC)	< 0.01	synonymous variant	P	32	ENST00000302823.7
Exon 1 (*)	rs606231420	2:203868047	C/A	NA		stop gained	C/*	35	ENST00000302823.7
Exon 1 (*)	rs606231421	2:203868052	G/T	NA		splice donor variant			ENST00000302823.7
Exon 2 (+)	rs200180357	2:203870579	A/**G**/T	G=0.00002/2 (ExAC); G=0.0002/1 (1000 Genomes)	< 0.01	splice region variant~intron variant			ENST00000302823.7

Exon 2 (+)	rs200180357	2:203870579	A/G/**T**		< 0.01	splice region variant~intron variant			ENST00000302823.7
Exon 2 (+)	rs767100685	2:203870582	C/G	G=0.00002/2 (ExAC)	< 0.01	splice region variant~intron variant			ENST00000302823.7
Exon 2 (+)	rs773279316	2:203870583	T/C	C=0.000008/1 (ExAC)	< 0.01	splice region variant~intron variant			ENST00000302823.7
Exon 2 (§)	rs760446668	2:203870593	C/T	T=0.00003/3 (ExAC); T=0.00003/1 (TOPMED)	< 0.01	synonymous variant	H	39	ENST00000302823.7
Exon 2 (+)	rs767995794	2:203870602	GCCTGCTGTGGTACTG/-	-=0.000008/1 (ExAC)	< 0.01	frameshift variant	QPAVVL/X	42	ENST00000302823.7
Exon 2 (+)	rs766143912	2:203870612	G/A	A=0.000008/1 (ExAC)	< 0.01	missense variant	V/I	46	ENST00000302823.7
Exon 2 (§)	rs146200342	2:203870615	C/T	T=0.00008/1 (GO-ESP)	< 0.01	synonymous variant	L	47	ENST00000302823.7
Exon 2 (§)	rs776726776	2:203870617	G/A	A=0.000008/1 (ExAC)	< 0.01	synonymous variant	L	47	ENST00000302823.7
Exon 2 (*)	rs606231417	2:203870627	C/T	NA		stop gained	R/*	51	ENST00000302823.7
Exon 2 (+)	rs759766975	2:203870628	G/A	A=0.000008/1 (ExAC)	< 0.01	missense variant	R/Q	51	ENST00000302823.7
Exon 2 (§)	rs765325921	2:203870635	C/T	T=0.000008/1 (ExAC)	< 0.01	synonymous variant	I	53	ENST00000302823.7
Exon 2 (§)	rs373393185	2:203870647	G/A	A=0.00008/1 (GO-ESP)	< 0.01	synonymous variant	V	57	ENST00000302823.7
Exon 2 (+)	rs752811424	2:203870655	A/T	T=0.000008/1 (ExAC)	< 0.01	missense variant	Y/F	60	ENST00000302823.7
Exon 2 (+)	rs758465752	2:203870667	G/A	A=0.000008/1 (ExAC)	< 0.01	missense variant	G/D	64	ENST00000302823.7
Exon 2 (§)	rs979522213	2:203870674	C/T	NA		synonymous variant	A	66	ENST00000302823.7
Exon 2 (§)	rs764639840	2:203870677	T/G	G=0.000008/1 (ExAC)	< 0.01	synonymous	T	67	ENST00000302823.7

						variant			
Exon 2 (+)	rs557116456	2:203870681	G/A	A=0.00002/2 (ExAC); A=0.0002/1 (1000 Genomes);A=0.00003/1 (TOPMED)	< 0.01	missense variant	V/I	69	ENST00000302823.7
Exon 2 (+)	rs757566658	2:203870682	T/C	C=0.00002/2 (ExAC)	< 0.01	missense variant	V/A	69	ENST00000302823.7
Exon 2 (*)	rs606231422	2:203870684	C/T	NA		missense variant	R/W	70	ENST00000302823.7
Exon 2 (+)	rs781579729	2:203870691	C/T	T=0.000008/1 (ExAC)	< 0.01	missense variant	T/I	72	ENST00000302823.7
Exon 2 (§)	rs199943943	2:203870692	A/G	G=0.000008/1 (ExAC)	< 0.01	synonymous variant	T	72	ENST00000302823.7
Exon 2 (§)	rs866679318	2:203870695	G/T	NA		synonymous variant	V	73	ENST00000302823.7
Exon 2 (+)	rs754725143	2:203870712	G/T	T=0.000008/1 (ExAC)	< 0.01	missense variant	S/I	79	ENST00000302823.7
Exon 2 (§)	rs778678737	2:203870716	G/A	A=0.000008/1 (ExAC)	< 0.01	synonymous variant	Q	80	ENST00000302823.7
Exon 2 (§)	rs139154557	2:203870722	T/C	C=0.00008/1 (GO-ESP)	< 0.01	synonymous variant	T	82	ENST00000302823.7
Exon 2 (+)	rs775877536	2:203870729	TG/-	-=0.000008/1 (ExAC)	< 0.01	frameshift variant	C/X	85	ENST00000302823.7
Exon 2 (*)	rs376038796	2:203870733	C/T	T=0.00002/3 (ExAC); T=0.0002/1 (1000 Genomes); T=0.00008/1 (GO-ESP); T=0.0001/3 (TOPMED)	< 0.01	missense variant	A/V	86	ENST00000302823.7
Exon 2 (§)	rs771695723	2:203870734	G/A	A=0.00004/5 (ExAC)	< 0.01	synonymous variant	A	86	ENST00000302823.7
Exon 2 (+)	rs370443546	2:203870744	A/G	G=0.000008/1 (ExAC); G=0.00008/1 (GO-ESP); G=0.00003/1	< 0.01	missense variant	M/V	90	ENST00000302823.7

				(TOPMED)					
Exon 2 (+)	rs746900785	2:203870747	A/T	T=0.00002/2 (ExAC)	< 0.01	missense variant	M/L	91	ENST00000302823.7
Exon 2 (+)	rs1065442	2:203870748	T/C	NA	< 0.01	missense variant	M/T	91	ENST00000302823.7
Exon 2 (+)	rs1052219132	2:203870749	G/A	NA	< 0.01	missense variant	M/I	91	ENST00000302823.7
Exon 2 (+)	rs770666846	2:203870757	A/T	T=0.000008/1 (ExAC)	< 0.01	missense variant	E/V	94	ENST00000302823.7
Exon 2 (+)	rs896360225	2:203870780	A/G	NA		missense variant	I/V	102	ENST00000302823.7
Exon 2 (+)	rs776440178	2:203870782	C/G	G=0.000008/1 (ExAC)	< 0.01	missense variant	I/M	102	ENST00000302823.7
Exon 2 (+)	rs759232662	2:203870787	C/T	T=0.00002/2 (ExAC)	< 0.01	missense variant	T/M	104	ENST00000302823.7
Exon 2 (§)	rs770065318	2:203870788	G/A	A=0.00005/6 (ExAC)	< 0.01	synonymous variant	T	104	ENST00000302823.7
Exon 2 (§)	rs949253264	2:203870791	C/T	NA	< 0.01	synonymous variant	G	105	ENST00000302823.7
Exon 2 (+)	rs777843969	2:203870793	C/T	NA		missense variant	T/I	106	ENST00000302823.7
Exon 2 (*)	rs144988077	2:203870802	G/A	A=0.0002/29 (ExAC); A=0.0002/1 (1000 Genomes); A=0.0002/2 (GO-ESP)	< 0.01	missense variant	G/E	109	ENST00000302823.7
Exon 2 (+)	rs763030646	2:203870808	A/T	T=0.000008/1 (ExAC)	< 0.01	missense variant	Q/L	111	ENST00000302823.7
Exon 2 (+)	rs764089901	2:203870828	G/C	C=0.000008/1 (ExAC); C=0.00003/1 (TOPMED)	< 0.01	missense variant	G/R	118	ENST00000302823.7
Exon 2 (+)	rs752037577	2:203870840	A/G	G=0.00002/3 (ExAC)	< 0.01	missense variant	M/V	122	ENST00000302823.7
Exon 2 (+)	rs757773669	2:203870847	C/T	T=0.000008/1 (ExAC)	< 0.01	missense variant	T/M	124	ENST00000302823.7

Exon 2 (§)	rs372929906	2:203870848	G/A	A=0.00007/9 (ExAC); A=0.0002/2 (GO-ESP); A=0.0004/11 (TOPMED)	< 0.01	synonymous variant	T	124	ENST00000302823.7
Exon 2 (§)	rs943460449	2:203870854	C/G	G=0.00003/1 (TOPMED)	< 0.01	synonymous variant	L	126	ENST00000302823.7
Exon 2 (+)	rs750841862	2:203870860	C/G	G=0.00002/2 (ExAC)	< 0.01	missense variant	I/M	128	ENST00000302823.7
Exon 2 (§)	rs147679342	2:203870866	G/A	A=0.00008/1 (GO-ESP)	< 0.01	synonymous variant	K	130	ENST00000302823.7
Exon 2 (§)	rs188862082	2:203870887	G/A	A=0.00006/7 (ExAC); A=0.0002/1 (1000 Genomes)	< 0.01	synonymous variant	P	137	ENST00000302823.7
Exon 2 (§)	rs376126248	2:203870890	A/T	T=0.00002/2 (ExAC); T=0.00008/1 (GO-ESP)	< 0.01	synonymous variant	P	138	ENST00000302823.7
Exon 2 (§)	rs752339954	2:203870897	C/T	T=0.000008/1 (ExAC)	< 0.01	synonymous variant	L	141	ENST00000302823.7
Exon 2 (+)	rs757989570	2:203870903	A/G	G=0.000008/1 (ExAC)	< 0.01	missense variant	I/V	143	ENST00000302823.7
Exon 2 (§)	rs777300741	2:203870911	C/T	T=0.00003/4 (ExAC); T=0.00003/1 (TOPMED)	< 0.01	synonymous variant	N	145	ENST00000302823.7
Exon 2 (§)	rs746944635	2:203870929	A/C	C=0.000009/1 (ExAC)	< 0.01	synonymous variant	V	151	ENST00000302823.7
Exon 3 (+)	rs756706504	2:203871387	C/T	T=0.000008/1 (ExAC)	< 0.01	missense variant	P/L	156	ENST00000302823.7
Exon 3 (§)	rs200657280	2:203871388	G/A	A=0.00009/11 (ExAC); A=0.0002/2 (GO-ESP); A=0.0003/9 (TOPMED)	< 0.01	synonymous variant	P	156	ENST00000302823.7
Exon 3 (+)	rs745734610	2:203871393	C/T	T=0.000008/1 (ExAC)	< 0.01	missense variant	P/L	158	ENST00000302823.7
Exon 3 (+)	rs778733155	2:203871399	C/G	NA		missense variant	S/C	160	ENST00000302823.7
Exon 3 (§)	rs1018212735	2:203871406	C/T	NA		synonymous	F	162	ENST00000302823.7

						variant			
Exon 3 (§)	rs755931042	2:203871409	C/T	T=0.000008/1 (ExAC)	< 0.01	synonymous variant	L	163	ENST00000302823.7
Exon 3 (+)	rs779775271	2:203871416	A/G	G=0.000008/1 (ExAC)	< 0.01	missense variant	I/V	166	ENST00000302823.7
Exon 3 (§)	rs749059847	2:203871421	T/C	C=0.000008/1 (ExAC)	< 0.01	synonymous variant	L	167	ENST00000302823.7
Exon 3 (+)	rs768961499	2:203871432	G/C	C=0.000008/1 (ExAC)	< 0.01	missense variant	S/T	171	ENST00000302823.7
Exon 3 (+)	rs963824682	2:203871435	C/T	T=0.00007/2 (TOPMED)	< 0.01	missense variant	S/L	172	ENST00000302823.7
Exon 3 (§)	rs375949600	2:203871436	G/A	A=0.00007/9 (ExAC); A=0.0002/2 (GO-ESP); A=0.0005/16 (TOPMED)	< 0.01	synonymous variant	S	172	ENST00000302823.7
Exon 3 (§)	rs146571801	2:203871466	A/T	T=0.000008/1 (ExAC); T=0.00008/1 (GO-ESP); T=0.00003/1 (TOPMED)	< 0.01	synonymous variant	T	182	ENST00000302823.7
Exon 3 (+)	rs771987351	2:203871467	G/T	T=0.000008/1 (ExAC)	< 0.01	missense variant	A/S	183	ENST00000302823.7
Exon 3 (§)	rs199659316	2:203871469	T/C	NA		synonymous variant	A	183	ENST00000302823.7
Exon 3 (+)	rs773775010	2:203871473	T/A	A=0.00002/3 (ExAC)	< 0.01	missense variant	S/T	185	ENST00000302823.7
Exon 3 (+)	rs761227535	2:203871477	T/C	C=0.00002/2 (ExAC)	< 0.01	missense variant	L/S	186	ENST00000302823.7
Exon 3 (+)	rs766875859	2:203871483	A/G	G=0.00003/4 (ExAC)	< 0.01	missense variant	K/R	188	ENST00000302823.7
Exon 3 (+)	rs199912925	2:203871485	A/G	G=0.00003/4 (ExAC)	< 0.01	missense variant~splice region variant	M/V	189	ENST00000302823.7
Exon 3 (*)	rs606231419	2:203871492	G/C	NA		splice region variant~intron			ENST00000302823.7

						variant			
Exon 4 (§)	rs1037900731	2:203872713	G/A	A=0.00003/1 (TOPMED)	< 0.01	synonymous variant	K	191	ENST00000302823.7
Exon 4 (+)	rs745310078	2:203872722	C/A	NA		missense variant	S/R	194	ENST00000302823.7
Exon 4 (+)	rs767174634	2:203872733	C/A	A=0.000008/1 (ExAC)	< 0.01	missense variant	T/K	198	ENST00000302823.7
Exon 4 (§)	rs749973402	2:203872737	G/T	T=0.000008/1 (ExAC)	< 0.01	synonymous variant	G	199	ENST00000302823.7
Exon 4 (+)	rs1046533169	2:203872738	G/T	NA		missense variant	V/F	200	ENST00000302823.7
Exon 4 (+)	rs755615887	2:203872739	T/C	C=0.000008/1 (ExAC)	< 0.01	missense variant	V/A	200	ENST00000302823.7
Exon 4 (§)	rs74808460	2:203872755	C/G	G=0.00004/5 (ExAC); G=0.0002/1 (1000 Genomes)	< 0.01	synonymous variant	P	205	ENST00000302823.7
Exon 4 (§)	rs753717111	2:203872761	A/G	G=0.000008/1 (ExAC)		synonymous variant	T	207	ENST00000302823.7
Exon 4 (§)	rs754809262	2:203872764	G/A	A=0.000008/1 (ExAC)	< 0.01	synonymous variant	E	208	ENST00000302823.7
Exon 4 (+)	rs778534474	2:203872766	C/G	G=0.000008/1 (ExAC)	< 0.01	missense variant	P/R	209	ENST00000302823.7
Exon 4 (+)	rs527697475	2:203872771	T/C	C=0.0002/1 (1000 Genomes)	< 0.01	missense variant	C/R	211	ENST00000302823.7
Exon 4 (§)	rs752988825	2:203872776	A/G	G=0.000008/1 (ExAC)	< 0.01	synonymous variant	E	212	ENST00000302823.7
Exon 4 (+)	rs758600786	2:203872786	C/T	T=0.000008/1 (ExAC)	< 0.01	stop gained	Q/*	216	ENST00000302823.7
Exon 4 (§)	rs778114608	2:203872794	T/C	C=0.00002/2 (ExAC)	< 0.01	synonymous variant	Y	218	ENST00000302823.7
Exon 4 (§)	rs747155841	2:203872803	C/T	T=0.000008/1 (ExAC)	< 0.01	synonymous variant	P	221	ENST00000302823.7
Exon 4 (+)	rs367760388	2:203872804	A/G	NA		missense variant	I/V	222	ENST00000302823.7

Exon 4 (§)	rs370517673	2:203872806	C/T	NA		synonymous variant	I	222	ENST00000302823.7

"*" indicates variants which have previously been published as disease causing or are part of our cohort. "§" indicates variants which have no disease causing effect in mutation carriers. "+" indicates variants whose effect on disease manifestation has to be explored. This data was extracted from Ensembl.