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**Revisiting human IL-12Rβ1 deficiency: A survey of 141 patients from 30 countries**
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# Revisiting human IL-12Rβ1 deficiency: a survey of 141 patients from 30 countries

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**Abbreviations**

IL12Rb1: Interleukin 12 receptor beta 1 chain

MSMD: Mendelian susceptibility to mycobacterial disease

BCG: Bacille Calmette-Guerin

EM: Environmental mycobacteria

TB: Tuberculosis

IFNGR: Interferon gamma receptor

STAT1: Signal transducer and activator of transcription 1

IL12B: Interleukin 12 B

NEMO: Nuclear factor-kB essential modulator

**Abstract**

IL-12Rβ1 deficiency is the most common form of Mendelian susceptibility to mycobacterial disease (MSMD). We undertook an international survey of 141 patients from 102 kindreds from 30 countries. Among 102 probands, the first infection occurred at a mean age of 2.4 years. In 77 patients, this infection was caused by bacille Calmette-Guérin (BCG, n = 65), environmental mycobacteria (EM, n = 8) or *M. tuberculosis* (n = 4). Twenty-two of the remaining 24 probands initially presented with non-typhoidal, extra-intestinal salmonellosis. Twenty of the 28 genetically affected siblings displayed clinical signs (71%); however eight remained asymptomatic (29%). Nine of the ten ungenotyped siblings with symptoms died. Recurrent BCG infection was diagnosed in 15 cases, recurrent EM in 3 cases, recurrent salmonellosis in up to 22 patients. Ninety of the 132 symptomatic patients suffered from infections with a single microorganism. Multiple infections were diagnosed in 40 cases, with combined mycobacteriosis and salmonellosis in 36 individuals. BCG disease strongly protected against subsequent EM disease ($p = 8x10^{-5}$). Various other infectious diseases occurred, albeit each rarely, yet candidiasis was reported in up to 33 of the patients (23%). Up to 99 patients (70%) survived, with a mean age at last follow-up visit of 12.7 years +/- 9.8 years (range 0.5 to 46.4 years). IL-12Rβ1 deficiency is characterized by childhood-onset mycobacteriosis and salmonellosis, rare recurrences of mycobacterial disease, and more frequent recurrence of salmonellosis. There is higher clinical penetrance, broader susceptibility to infections and less favorable outcome than previously thought.

## Introduction

MSMD (MIM 209950) is a clinical syndrome, probably first described in 1951(45), that predisposes otherwise apparently healthy individuals to infections caused by weakly virulent mycobacteria, such as BCG and EM (9). Since 1996, MSMD-causing mutations have been identified in six genes [reviewed in (3, 25)]. Five of these genes are autosomal and encode the two chains of the IFN-γ receptor (*IFNGR1* and *IFNGR2*), the signal transducer and activator of transcription factor 1 (*STAT1*), the p40 subunit of IL-12 and IL-23 (*IL12B*), and the β1 chain shared by the IL-12 and IL-23 receptors (*IL12RB1*), whereas the sixth gene is X-linked and encodes nuclear factor-κB essential modulator (*NEMO*) (25). These defects impair IFN-γ-mediated immunity. The allelic heterogeneity is such that mutations in these six genes define up to 13 different genetic traits, with some genes associated with recessive or dominant inheritance, complete or partial defects, and loss of expression or the expression of non functional molecules [reviewed in (3, 25)]. Patients with MSMD are also susceptible to the more virulent species *Mycobacterium tuberculosis*, and IL-12Rβ1 deficiency was the first identified Mendelian genetic etiology of pediatric tuberculosis in children with normal resistance to BCG and EM (4, 6, 8, 48). These defects also predispose patients to *Salmonella* infections (25, 43). A few other infections have been diagnosed, but mostly in smaller numbers of patients, making it difficult to draw firm conclusions about the relationship between these infections and the underlying genetic defects [reviewed in (25)].

The most common genetic etiology of MSMD is autosomal recessive IL-12Rβ1 deficiency, first reported in 1998 (5, 16). NK and T cells from patients with this condition do not respond to IL-12 and produce low levels of IFN-γ. The first large series of patients was reported in 2003 and included 41 patients from 29 unrelated families in 17 countries (24). This survey described five key clinical features of IL-12Rβ1 deficiency, differentiating this deficiency from other genetic etiologies of MSMD, such as IFN-γR1 deficiency (18): 1)

infections typically appeared in childhood, with no adult onset of disease; 2)the recurrence of mycobacterial disease was exceedingly rare, with BCG disease protecting against subsequent EM disease; 3) clinical penetrance was incomplete, with up to 45% of genetically affected siblings remaining asymptomatic; 4) patients displayed broad resistance to infectious agents other than *Mycobacterium* and *Salmonella* and 5) the outcome was favorable in most cases, with a mortality rate of only 15%. Individual case reports and small series have since brought the number of reported patients with this deficiency to 78 (2, 6-8, 11, 13-15, 19, 20, 22, 23, 27, 28, 30, 33-37, 40, 42, 46-49, 54-60, 63-67, 69, 70, 72). However, improvements in the description of this disorder are required. These improvements will require a decrease in ascertainment bias, through description of the clinical phenotype of a larger number of patients with diverse genetic backgrounds exposed to different microbial flora, including, in particular, genetically affected siblings of index cases. We report here the molecular, cellular, and clinical features of a series of 141 patients (including 63 unpublished patients) with IL-12Rβ1 deficiency from 102 kindreds in 30 countries.

## Patients and methods

*Subjects and kindreds*

Patients (and their families) were recruited into this study through a large, worldwide network of collaborations with clinicians and immunologists. These patients presented with a history of unusual infections, such as disseminated and/or recurrent disease caused by weakly virulent mycobacteria and/or *Salmonella*, corresponding to the description of MSMD and other similar conditions. Patients with severe, disseminated forms of tuberculosis were also studied. Our study was conducted in accordance with the Helsinki Declaration, with informed consent obtained from each patient or the patient's family, as requested and approved by the institutional review boards of the various institutions involved, including the Necker Medical School.

*Whole-blood activation*

Whole-blood activation was used as the first-line screening for the possible mutation in the IL-12/IFN-γ axis. Venous blood samples were collected in tubes containing heparin and were transported, at room temperature and by express mail, to our laboratory for analysis. Blood was diluted 1:2 in RPMI 1640 medium (Invitrogen). Aliquots of diluted blood were dispensed into the wells of a 48-well plate and incubated in four sets of conditions: with medium alone, with live BCG (*Mycobacterium bovis* BCG, Pasteur strain, MOI 20:1), with BCG plus IFN-γ (5000 IU/ml, Imukin Boehringer Ingelheim), or with BCG plus IL-12p70 (20 ng/ml, R&D systems), the final volume within each well being 1 ml (22). Supernatants were collected after 48 hours and centrifuged at 1000 *g* for 5 minutes. All supernatants were stored at -20°C until analysis.

*Determination of cytokine levels by ELISA*

IL-12p40, IL-12p70, and IFN-γ levels (48-hour culture supernatants) were determined by ELISA. We used the capture antibodies, detection antibodies and standards supplied in the R&D Systems kits for IL-12p40 and IL-12p70 (Quantikine SP400) and in the Sanquin kit for IFN-γ (M9333), diluted in HPE dilution buffer (M1940, Sanquin). Milk was used for blocking and antibody binding was detected with streptavidin horseradish peroxidase (M2032, Sanquin) and TMB microwell peroxidase substrate (50-76-00, KPL). The reaction was stopped by adding $H_2SO_4$ (1.8 M). Optical density was determined with an MRX microplate reader (Thermolab Systems). Quantitative analysis with a non linear, four-parameter logistic (4PL) calibration model was carried out with in-house software based on the Microsoft Excel application language developed for this purpose (gift from Max Feinberg). Results for each cytokine are expressed in pg/ml/$10^6$ peripheral blood mononuclear cells [PBMC (22)]. Whole-blood activation and subsequent ELISA were repeated only in cases when the blood arrived in poor condition due to long travel. The results of this assay, when performed with optimal conditions, were strictly consistent for the same patient as well as between patients.

*Cell culture*

Epstein-Barr virus-transformed lymphoblastoid cell lines (EBV-B cell lines) were cultured in RPMI 1640 medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Invitrogen). *Saimiri* herpesvirus-transformed T cells were cultured in a 1:2 mixture of RPMI 1640 medium/Panserin 401 medium (Pan Biotech) with 10% FBS, 2 mM L-glutamine (Invitrogen), 10 U/ml IL-2 (Roche), and 100 μg/ml gentamycin. For the production of phytohemagglutinin (PHA)-activated T cells, PBMCs were purified by

centrifugation on a Ficoll-Hypaque gradient (GE Healthcare), resuspended in RPMI medium supplemented with 10% FBS and activated by incubation with 1/700 PHA (Becton Dickinson) for 72 to 96 hours. PHA-T-cell blasts were then stimulated by incubation for 48 hours with IL-2 (50 IU/ml, Proleukin, Chiron) and cultured at a density of 2 x $10^5$ cells/ml in Panserin 401 medium (Pan Biotech) with 10% FBS and 2 mM L-glutamine (Invitrogen). All cells were incubated at 37°C, under an atmosphere containing 5% $CO_2$.

*Transfection*

*Saimiri* herpesvirus-transformed T cells were transfected with a wild-type pEGFPN1-IL12RB1 vector or with one of the various missense mutants. We transfected 5 x $10^6$ cells with 2 μg of DNA, using the Cell Line Nucleofector Kit V (VCA-1003 from Amaxa) and Y-001. We assessed receptor expression at the cell surface and IL-12 binding 48 hours after transfection.

*Flow cytometry*

PHA-T-cell blasts or EBV-B cell lines were washed in PBS and dispensed into a 96-well plate for labeling. The cells were incubated with an anti-IL-12Rβ1 antibody (1:100 dilution of the 2.4E6 or 2B10 clone, BD Biosciences) or an equivalent concentration of isotype-matched control mAb (MOPC-21 and/or R35-95, BD Biosciences) in 2% FBS in PBS, on ice, for 20 minutes. The cells were then washed twice with cold 2% FBS in PBS and incubated on ice for 20 minutes with Alexa Fluor 488-conjugated goat anti-mouse or goat anti-rat antibody (A-11029 or A-11006 from Invitrogen). Cells were then washed twice with 2% FBS in PBS and analyzed with a FACScan machine, using Cellquest software (Becton Dickinson).

*Fluorescent IL-12 binding*

IL-12 fluorescence-binding experiments were performed as follows: $5x10^5$ transfected cells were incubated in 25 µl of phosphate-buffered saline (PBS) with or without 50 ng/ml IL-12p70 (R&D Systems) for 30 minutes at 4°C, and then with mouse anti-human IL-12p40-p70 IgG1 (Pharmingen) and, finally, with PE-conjugated goat anti-mouse antibody (Invitrogen). Stained cells were analyzed with a FACScan machine, using Cellquest software (Becton Dickinson).

*Genetic analysis*

Human genomic DNA was isolated from the pellets obtained after the Ficoll-Paque Plus gradient purification of PBMC, or from whole blood or cell lines. The cells were lysed in extraction buffer (10 mM Tris, pH 7.4, 0.1 M EDTA, 0.5% SDS, and 10 mg/ml proteinase K) and incubated overnight at 37°C. The DNA was isolated by phenol/chloroform extraction, precipitated in ethanol and resuspended in 10 mM Tris, pH 7.4, 1 mM EDTA. RNA was isolated from EBV-B cell lines or PHA-T-cell blasts with Trizol reagent (Invitrogen), according to the manufacturer's instructions. RNA was reverse transcribed by Superscript II reverse transcriptase (Invitrogen) with oligo-dT. The first-strand cDNA was stored at -20°C. PCR amplification was carried out with the AmpliTaq DNA polymerase (Applied Biosystems) and the GeneAmp PCR system 9700 (Applied Biosystems). The primers and conditions used for PCR amplification of the coding exons, including the flanking intron sequences, or the cDNA of *IL12RB1* are available upon request. Amplified PCR products were checked by electrophoresis in a 1% agarose gel and were purified by centrifugation through Sephadex G-50 Superfine resin (Amersham GE) on multiscreen MAHV-N45 (Millipore) filter plates. PCR products were sequenced by dideoxynucleotide termination, with the BigDye Terminator kit v1.1 (Applied Biosystems) and appropriate primers.

Sequencing products were purified by centrifugation through Sephadex G-50 Superfine resin and analyzed on an ABI Prism 3100 or 3130xl apparatus (Applied Biosystems). Sequences files and chromatograms were analyzed with GENALYS Software from CNG, France (62).

*Statistical methods*

Infection-free status, survival and penetrance curves as a function of age were estimated by the Kaplan-Meier method and, when necessary, curves were compared by log-rank tests. Penetrance curves for IL-12Rβ1 deficiency were obtained from the data for the siblings of index cases, through the use of two strategies. The first strategy was based on the use of data for siblings with identified IL-12Rβ1 mutations only (n=28). The second strategy was based on the assumption that non genotyped siblings suffering from MSMD-related infections were also IL-12Rβ1-deficient, leading to the inclusion of these siblings in the estimation of penetrance (N=10). However, we avoided bias due to the addition of clinically affected siblings only, by also including non genotyped healthy siblings as follows: 1) we calculated the proportion of genotyped healthy siblings with genetically confirmed IL-12Rβ1 deficiency: 0.08 (8/100); 2) we assumed that the same proportion of the 57 non genotyped healthy siblings would be IL-12Rβ1-deficient (i.e. 5 siblings); 3) we randomly selected five follow-up periods for the 57 healthy siblings, such that the mean duration of follow-up for these five siblings did not differ significantly from the overall mean follow-up period for all 57 healthy siblings (i.e. 12.78 +/- 13.70 years). All calculations were carried out and curves plotted with R software (http://cran.r-project.org/).

## Results

*Clinical features and mutation analysis in 102 index cases*

Children and young adults with clinical disease caused by BCG or EM, or with salmonellosis or tuberculosis and suspected IL-12Rβ1 deficiency were referred to our laboratory. By sequencing the 17 *IL12RB1* coding exons and flanking intron regions, we identified 102 IL-12Rβ1-deficient index cases from 30 countries (Table 1; Figure 1 and 2). We identified 54 mutant alleles, including nonsense (n = 11), missense (n = 14) and splice (n = 10) mutations, small insertions (n = 2), small deletions (n = 9), large deletions (n = 3), deletions/insertions (n = 4), and one duplication (Table 1; Figure 4). Missense mutations were not found among the polymorphisms reported in the NCBI and Ensembl databases. Furthermore, none of the missense mutations were found in 50 healthy control individuals. All predicted splice mutations had a major impact on the structure of the *IL12RB1* mRNA, with no full-length mRNAs detected, as determined by RT-PCR (data not shown). All but two of the deletions and insertions resulted in frame shifts (see below) and the 14 missense mutations tested compromised protein expression (see below). The index cases were typically homozygous (n = 87) or, in rare cases, compound heterozygous (n = 14). The overall clinical spectrum of infectious diseases in index cases was as follows: isolated BCG disease was present in 43 patients, isolated salmonellosis in 15 patients, isolated EM disease in 6 patients, and isolated TB in 2 patients. A combination of BCG disease and salmonellosis was reported in 18 cases, BCG and EM disease in only 1 case, BCG disease and TB in 2 cases, and BCG and EM disease plus salmonellosis in 3 cases. A combination of salmonellosis and EM disease was diagnosed in 7 cases, and salmonellosis and TB in 1 case. One of the four remaining probands presented EM disease and TB, the second presented nocardiosis and klebsiellosis, the third presented granulomatous disease of unknown origin, and the fourth

suffered from salmonellosis and a mycobacterial disease of unknown origin (Figure 3). Among the 102 index cases, the first clinical infections typically occurred in childhood (mean 2.38 years, SD +/-4.86 years, range 2 weeks to 31.72 years). Sixty-seven of the 86 BCG-vaccinated probands developed BCG disease (78%).

*Abolition of cellular responses to IL-12 and IL-23*

Whole-blood responses to IL-12 were investigated in 65 patients carrying two mutant *IL12RB1* alleles (47 index cases and 18 relatives; see below). We measured the production of IFN-γ in whole blood in response to stimulation with BCG alone (partly resulting from BCG-dependent, endogenous IL-12 production) and in response to BCG plus exogenous recombinant IL-12, as previously described (22, 24). All patients tested had an impaired response to IL-12 in this assay (Figure 5). The whole-blood phenotype of the patients was, therefore, functional IL-12Rβ1 deficiency. We then assessed IL-12Rβ1 expression on the surface of T-cell blasts and/or EBV-B cell lines, by flow cytometry with two specific antibodies recognizing different epitopes on the extracellular domain of IL-12Rβ1. No IL-12Rβ1 molecules were detected on the surface of cells from patients carrying 47 alleles tested, except for four patients from two Israeli families (kindreds 10 and 43) carrying the same, large, in-frame deletion 700+362_1619-944del as described in a previous study (23). This deletion led to the generation of a truncated IL12Rβ1 protein, which was present at the cell surface but was non functional, resulting in complete IL-12Rβ1 deficiency. The C198R mutation has been described elsewhere and is thought to confer residual responsiveness to IL-12 (42). We have identified another patient with the C198R mutation. However, neither cell surface IL-12Rβ1 expression on the patient's PHA-T-cell blasts (Supplementary Figure 1) nor IFN-γ production by these cells in response to IL-12 stimulation was detected (data not shown). Four of the remaining six homozygous alleles not tested by flow cytometry abolished

IFN-γ production in response to the IL-12 stimulation of whole blood. The other two alleles were predicted to result in a loss of expression due to the creation of a premature stop codon. Finally, we have shown that IL-12Rβ1-deficient patients do not respond to IL-23 in terms of IFN-γ production by T-cell blasts (23). Consistent with these data, we have subsequently shown that IL-12Rβ1-deficient patients had a smaller proportion of IL-17-producing T cells *ex vivo*, and that their T-cell blasts did not express IL-17 in response to stimulation with IL-23 *in vitro* (14). Overall, the blood cells from the patients tested displayed an impaired response to both IL-12 and IL-23 (14), strongly suggesting that these patients had complete IL-12Rβ1 deficiency.

*Missense mutations responsible for IL-12Rβ1 deficiency*

Missense mutations were common (14 of the 54 alleles, 26%; 27 of 102 probands, 26 %). Unlike mutations causing a premature termination of translation, it is difficult to predict whether missense and other in-frame mutations (n=2, found in 3 index cases) are intrinsically deleterious. They may be in linkage disequilibrium with a causal mutation elsewhere, particularly in the broad *IL12RB1* regulatory regions not sequenced in the patients. The 14 *IL12RB1* missense mutations found are not polymorphisms, as they were not found in a panel of 50 healthy controls studied. Most are clustered in fibronectin domain 2 (FD2, 9 mutations), although some are found in FD1 (2 mutations), FD4 (2 mutations) and the transmembrane domain (TM, 1 mutation). The four known missense polymorphisms are also found in FD2 and FD4 (Figure 6A). We predicted the impact of the 18 amino acid substitutions with PolyPhen, which classifies the impact as benign, possibly damaging, or probably damaging (53). Three of the four polymorphisms were predicted to have a benign impact, with only R156H being possibly damaging. By contrast, 12 of the 14 rare mutations were classified as probably (n=12) damaging, and two were classified as possibly damaging (n=2) (Figure 6A).

ClustalX assessments of the phylogenic conservation of *IL12RB1* in several species revealed considerable variation in *IL12RB1*, particularly in FD2 (32). However, 10 of the 14 rare mutations affected conserved residues, whereas the residues affected by the four polymorphisms were not conserved residues (Figure 6C). We further investigated the function of 13 of the 14 rare missense alleles, by transient transfection of an IL-12Rβ1-deficient *Saimiri* herpesvirus-transformed T cell line. We assessed the cell-surface expression of IL-12Rβ1 with two antibodies recognizing different epitopes (24). We also assessed the ability of the encoded receptors to bind IL-12 in the same assay, because these missense mutations might affect the epitope recognized by the antibodies. Neither the surface expression of IL-12Rβ1 nor IL-12 binding was detected for the 13 missense alleles tested (Figure 6B). Cells from patients carrying the remaining missense mutation were unresponsive to IL-12 (see above). Thus, the rare missense *IL12RB1* alleles found in our patients resulted in a loss of both expression and function.

*Relatives of the index cases*

The 102 probands had a total of 208 siblings, 174 of whom were alive and 34 of whom had died (Supplementary Figure 2). Genotyping was carried out for 116 of the 174 living siblings. The other 58 siblings were not genotyped. We found that 92 of the genotyped siblings were wild-type or heterozygous for the *IL12RB1* mutation, whereas another 24 siblings carried mutations in both alleles. Sixteen of these siblings with mutations in both alleles presented unusual infections, whereas the remaining eight were asymptomatic. Fifty-seven of the 58 non genotyped living siblings had not suffered from diseases caused by mycobacteria or *Salmonella*. The remaining non genotyped sibling presented disseminated BCG disease (96.II.2). Thirty of the 34 siblings that had died had not been genotyped. The other four dead siblings had carried homozygous mutations in *IL12RB1* and had died from

BCG (n=3) and EM (n=1) diseases. Nine of the 30 non genotyped dead siblings had died from infections caused by mycobacteria or *Salmonella* (see below). No unusual infections were reported for the remaining non genotyped siblings that had died (Supplementary Figure 2). We identified two *IL12RB1* null alleles in 28 of the 38 siblings affected clinically or genetically. Genetic analysis was not possible for one sibling with BCG disease, and the molecular defect was considered probable but not documented in nine of the siblings with symptoms that had died. These nine siblings died from BCG-osis (n=5, 26.II.1, 58.II.1, 68.II.3, 68.II.4, 74.II.1), *S. enteritidi*s disease (n=2, 30.II.5, 31.II.6), *M. avium* disease (n=1, 4.II.2), and disseminated tuberculosis (n=1, 61.II.1). Eight of the 28 genetically identified siblings displayed no known MSMD infectious phenotype at last follow-up. This group of siblings lacking MSMD symptoms presented the same cellular phenotype as their clinically affected IL-12Rβ1-deficient siblings. Fifteen of the 20 genetically affected symptomatic siblings had been vaccinated with BCG and 11 developed BCG disease, which was the first clinical manifestation of MSMD in all of these cases (n=10 BCG alone, and n=1 BCG plus *Salmonella*). In the other four vaccinated patients, salmonellosis was the first clinical manifestation in two cases, with EM disease and TB being the first clinical manifestation in one individual each. The five remaining genetically affected siblings that had not been vaccinated with BCG developed salmonellosis (n=2), EM disease (n=1), disease due to *Mycobacterium* spp. and salmonellosis (n=1), and TB and salmonellosis (n=1) (Figure 7). Age at first infection could be evaluated in only 26 of the 30 symptomatic patients, and did not differ from that of index cases (mean 2.4 years +/- 4 years, range from 5 days to 18 years). The duration of follow-up for these siblings was also similar to that for the index cases (mean 7.91 years, +/- 6.92 years range 0.51-28 years). The infectious phenotype of these 30 siblings was thus similar to that of the 102 index cases, in terms of the nature of the infectious diseases suffered and the age at which they occurred. In total, 161 parents of index cases were

genotyped. One mother (46.I.2) was found to be homozygous for an *IL12RB1* mutation, but neither she nor any of the other parents presented any symptoms. In total, at least 141 individuals from 102 kindreds, including 132 individuals actually carrying two *IL12RB1* mutant alleles and nine related individuals identified on the basis of their clinical presentation, probably suffered from autosomal recessive IL-12Rβ1 deficiency (Figure 1, Table 1). One of the major conclusions to be drawn from the analysis of the genetically affected relatives is that the clinical penetrance of IL-12Rβ1 deficiency is incomplete.

*Mycobacterial diseases in 132 symptomatic patients*

Mycobacterial diseases were the most frequent infections, diagnosed in 109 of the 132 symptomatic patients (83%; Supplementary figure 3). We first analyzed the individuals developing case-definition opportunistic infections caused by weakly virulent mycobacteria (BCG and EM). We found that 108 of the 132 patients had been vaccinated with BCG and 84 patients developed BCG disease (localized, n = 17; disseminated, n = 63; not known, n = 4). By contrast, only 21 of the 132 patients developed EM disease due to *M. avium* (n = 10); *M. avium*, *M. triplex* and *M. genavense* (n = 1); *M. genavense* (n = 1); *M. avium*, *M. chelonae* and *M. fortuitum* (n=1); *M. chelonae* (n = 1), *M. simiae* (n = 1), *M. avium-intracellulare* (n=1) and undefined *M.* spp. (n= 5). Two of these patients suffered from multiple EM diseases, with one patient in particular presenting successive infections with *M. avium*, *M. triplex* and *M. genavense* (n = 1). Another patient presented infection with *M. chelonae* and *M. fortuitum*, followed by an infection with *M. avium*, (n=1). One patient suffered from both BCG and EM disease and three patients presented infections with BCG, EM and *Salmonella*. Two patients suffered from mycobacterial infections caused by unidentified *M.* spp. and *Salmonella* infection. In total, nine patients presented tuberculosis, with four of these patients developing disease due to *M. tuberculosis* alone, one developing disease due to *M. tuberculosis* in

combination with *M. avium*, *M. chelonae* and *M. fortuitum*, and two patients developing disease due to *M. tuberculosis* and *Salmonella*. The remaining two cases of tuberculosis occurred in combination with BCG disease. Thus, 109 of the 132 symptomatic patients presented mycobacterial disease, but two different mycobacterial species were involved in only nine of these cases (8%). Multiple mycobacterial diseases are thus rare, consistent with our previous observations (24). Finally, up to 36 of the 42 deaths (among 132 patients) could be attributed to mycobacterial disease (BCG = 23, EM = 11, TB = 1, EM+Salmonella = 1; see below). We defined recurrence as a subsequent episode of infection with the same microorganism after a period free from clinical symptoms and treatment. However, reliable systematic data on the complete absence of clinical symptoms and treatment, and bacteriological identification of the pathogen responsible for the new clinical episode were often lacking. Based on the available data, recurrent BCG infection was diagnosed in 15 cases (18% of all patients with BCG disease), and recurrent EM in only three cases (14% of all patients with EM infections).

*Impact of BCG vaccination on other mycobacterial diseases*

The rarity of multiple mycobacterial diseases (8%) was also consistent with the rarity of recurrence (18% for BCG and 14% for EM). This finding may reflect the protective role of primary infection against the reactivation of a latent organism or secondary infection. The protective effects of primary infections with EM and TB are difficult to assess, but precise information about BCG vaccination was available for most patients. We thus determined the impact of BCG vaccination and BCG disease on the clinical phenotype of 129 patients. Only four of the 84 patients with BCG disease developed EM diseases, with a mean age of EM disease onset at 4.5 years, suggesting that BCG vaccination may prevent EM disease. Seven of the 24 patients resistant to BCG (vaccinated with BCG but without BCG infection) suffered

from EM disease, with late onset of the disease (mean age 10.83 years +/- 12.02 years; range 5 days to 31.72 years). By contrast, up to 10 of the 21 symptomatic patients (48%) who had not been vaccinated with BCG suffered from EM diseases, with an early onset of EM disease (mean age 5.08 years +/- 3.48 years range 0.83 to 12.05 years). The difference in incidence of EM disease between the three groups of patients (patients with both BCG and EM disease, patients resistant to BCG with EM disease and patients not vaccinated with BCG with EM disease) was highly significant ($p = 8x10^{-5}$, Figure 8). This difference in EM disease incidence was also significant if patients with BCG disease were compared with patients not inoculated with BCG ($p = 1.55x10^{-5}$). The difference in incidence between patients resistant to BCG and non vaccinated patients was also statistically significant ($p = 0.045$). Finally, the difference in EM disease incidence between BCG-vaccinated (with or without BCG disease) and non vaccinated patients was highly significant ($p = 4.83x10^{-5}$). However, this pattern was not observed for TB ($p = 0.25$; Supplementary figure 4). The difference in the onset of salmonellosis was barely significant between these three groups ($p = 0.03$, Supplementary figure 5). These data confirm our previous description of a strong protective effect of BCG vaccination (24), preventing EM disease in IL-12Rβ1-deficient patients. This observation can probably be extended to account for the rarity of recurrences and multiple mycobacterial diseases in patients. Human IL-12Rβ1 seems to be essential for protective immunity to primary infection, but not to secondary infection or reactivation by mycobacteria.

*Salmonellosis in the 132 symptomatic patients*

Salmonellosis occurred in up to 57 of the 132 symptomatic patients (43%) (Figure 3, Figure 7 and Supplementary figure 3) and was the only infectious disease in 21 patients (16%). The remaining 36 patients with salmonellosis also had TB (n=2), EM disease (n=8), BCG disease (n=21), EM and BCG disease (n=3), or mycobacterial disease caused by

unidentified *M.* spp (n=2). Four patients died from salmonellosis (10 %). Various serotypes of non-typhoidal *Salmonella* (*S. enteritidis, S. typhimurium, S. dublin, S. hadar, S. typhi O and typhi H, S. group B and D, S. portland, S. paratyphi*) were isolated from the 57 patients. Two patients were diagnosed with typhoid fever (patients 9.II.3 and 77.II.1), caused by *S. typhi* and/or *S. paratyphi*. Up to 6 of the 57 patients (11%) suffered from salmonellosis caused by two or more serotypes (3.II.3, 9.II.3, 30.II.6, 53.II.2, 73.II.2 and 77.II.1). Multiple salmonellosis was more frequent than multiple mycobacteriosis (8 % versus 11%, respectively), however not all clinical episodes were confirmed bacteriologically. Recurrent salmonellosis was diagnosed in 22 patients (39% of all patients with salmonellosis), but the *Salmonella* species responsible for the recurrence was not identified bacteriologically. Recurrent *Salmonella* infection was more frequent than recurrent infection with BCG and EM (18% and 14%, respectively), consistent with our previous findings suggesting that IL-12 and IL-23 are required to mount an efficient immune response to primary, latent, and secondary *Salmonella* infections (24).

*Infections caused by agents other than mycobacteria and Salmonella*

We recently found that 32 (24%) of the 132 symptomatic patients for whom information was available presented mucocutaneous disease caused by *Candida albicans* (Supplementary figure 3). The vast majority of patients had recurrent oral thrush, even in the absence of antibiotic treatment. The clinical features of candidiasis in IL-12Rβ1-deficient patients will be reported elsewhere (Rodriguez-Gallego C. *et al.*, manuscript in preparation). One patient developed recurrent visceral leishmaniasis at the age of five years (71.II.2) (57). Another suffered from disseminated paracoccidioidomycosis at the age of 21 years [29.II.1, (47)]. Two patients had posterior uveitis due to toxoplasmosis (19.II.1 and 29.II.1). One patient suffered from disseminated histoplasmosis at the age of five years (54.II.1). Five patients suffered from

*Klebsiella pneumoniae* infection (66.II.1, 78.II.2, 79.II.5, 89.II.2 and 98.II.1). One patient developed sepsis and meningitis due to *Citrobacter freundii* at three months of age, recovering fully on treatment (35.II.4). Patient 89.II.2 presented with simultaneous *Klebsiella pneumoniae* and *Nocardia nova* infections in the absence of mycobacterial or *Salmonella* infection (Picard *et al*., manuscript in preparation). The occurrences of klebsiellosis and salmonellosis may be linked, because these two species are phylogenetically related (44). Symptoms of vasculitis were reported in three patients. Vasculitis was considered secondary to *S. enteritidis* or mycobacterial infection in two patients (34, 58), but no histological examination was available for the third patient, so other causes of vasculitis could not be ruled out. IL-12Rβ1-deficient patients therefore seem to be susceptible to *Candida* and *Klebsiella*, and to intracellular microbes with pathogenesis and immune control similar to those of mycobacteria, such as *Nocardia*, *Paracoccidioidomyces*, *Histoplasma*, and *Leishmania*. Patients with unusually severe disease caused by these and, possibly, other microorganisms should be investigated for IL-12Rβ1 deficiency. This is particularly important for children with disseminated disease.

*Age at onset of infections in the 129 symptomatic patients*

We then focused our analysis on the 126 symptomatic patients for whom relevant information was available: 100 index cases and 26 siblings. The age at onset of the first infection was typically in early childhood. The mean age at onset of first infection was 2.4 years (range: 1 week to 31.7 years, SD 4.9 years; Figure 9). In most cases, the first infection was due to live BCG (regional BCG-itis or disseminated BCG-osis). It occurred at ages between 2 weeks and 7.1 years, with a mean of 0.6 years +/- 0.9 years (from 1 week to 3.2 years after vaccination, with a mean at 0.4 years after vaccination +/- 0.4 years). In 75 cases (96%), BCG disease occurred within a year of vaccination. Salmonellosis (range: 3 months to

12.5 years, mean: 4 years, SD: 3 years) and EM disease (range: 1 week to 31.7 years, mean: 6.9 years, SD: 7.7 years) occurred at a similar age. TB occurred later, at ages of 2.5 to 31 years, with a mean age at TB onset of 11 years +/- 8.9 years. The earlier onset of BCG, EM and *Salmonella* disease than of TB may be accounted for by earlier exposure to these microorganisms.

*Survival analysis of IL-12Rβ1-deficient patients*

The mortality rate among symptomatic patients was 32% (42 of the 132 symptomatic patients) (Table 1), which is somewhat higher than the value previously reported for a series of 41 patients (15%) (24). Global mortality, including asymptomatic patients, was 30 (24). The date of birth and date of death were known for 40 of the 42 patients that died. The mean age at death was 7.5 years in the 40 patients that died (range: 1.2-37.7 years and SD: 8.1 years). The cause of death was BCG-osis (n = 23, 27% of patients with BCG disease) in most of the patients that died, with smaller numbers of patients dying from EM disease (n = 11, up to 52% of patients with EM disease), TB (n = 1, 11% of patients with TB), or salmonellosis (n = 4, only 7% of patients with salmonellosis). One patient died from concurrent *M. avium* and *Salmonella* infections (patient 39.II.2) and another patient died from a severe electrolyte disorder following diarrhea. However, it is unknown whether this diarrhea was related to salmonellosis in this patient. One patient died from esophageal carcinoma (patient 30.II.6) (Rodriguez-Gallego C. *et al.*, manuscript in preparation). Clinical outcome depends largely on the infectious agent concerned, with mortality ranging from 7% (*Salmonella*) to 52% (EM) (Figure 10). However, there is an ascertainment bias, as discussed above, with fewer asymptomatic siblings investigated than in the previous study. The clinical outcome of this defect is directly related to the therapeutic approach used. IL-12Rβ1-deficient individuals were commonly treated with prolonged courses of antibiotics and exogenous IFN-γ. More

rarely, they underwent surgical resection of the affected areas (abdominal in particular) and, in very rare cases, hematopoietic stem cell transplantation (HSCT) was carried out. However, there are no comprehensive data available to evaluate the impact of treatment options at the moment. We are currently collecting data about both the treatment and preventive management of these patients.

*Incomplete clinical penetrance*

Eight of the 28 known genetically affected siblings were free of MSMD-related or other unusual infections at their last follow-up visit. We first estimated clinical penetrance by focusing on these 20 symptomatic (follow-up from 1.3 to 28 years, mean = 9.2 years, SD = 7.8 years) and eight asymptomatic (follow-up from 0.7 to 21.5 years, mean = 12.7 years, SD = 8.4 years) genetically affected siblings. The overall penetrance of infections was found to increase rapidly to 0.63 (95% confidence interval (CI): 0.39-0.77) at the age of five years, increasing slowly thereafter to reach 0.79 (0.51-0.91) by the age of 20 years (Figure 11). Eleven (65%) of the 17 BCG-vaccinated siblings developed BCG-osis. EM disease, salmonellosis and tuberculosis disease occurred in 2 (7%), 10 (36%), and 2 (7%) genetically affected siblings, respectively. These proportions are higher than reported in the previous series (24). However, 58 (33%) of the 174 living siblings had not been genotyped, whereas only 9% of the living siblings had not been genotyped in the smaller series studied in 2003 (24). We therefore also estimated clinical penetrance by including 15 non genotyped symptomatic and healthy siblings (see Patients and Methods). This second curve provided estimates of penetrance very similar to those for the first curve generated from data for genotyped siblings only. We cannot rule out the possibility of an ascertainment bias, with genetically affected asymptomatic relatives being underdiagnosed, but both estimation

strategies indicate that IL-12Rβ1 deficiency may have a higher penetrance than initially thought.

**Discussion**

We describe here 141 patients with IL-12Rβ1 deficiency. The patients originate from 30 countries in the Americas, Europe, Africa and Asia and comprise individuals from various ethnic groups [Africans, Amerindians, Arabs, Chinese, Europeans, Indians, Iranians, Japanese, Jews, and Turks, (41)]. Consistent with the considerable geographic and ethnic heterogeneity of the patients, we also observed substantial genetic heterogeneity, with up to 54 mutant alleles in 102 kindreds. In all but two kindreds (23, 59), the patients suffered from IL-12Rβ1 deficiency with no expression of the receptor on the cell surface. The cells of all patients have an impaired response to IL-12 and IL-23, resulting in the impaired production of IFN-γ and IL-17. A diagnosis of partial, as opposed to complete, IL-12Rβ1 deficiency was proposed in a child homozygous for the C198R mutation (42). However, we detected no IL-12Rβ1 expression at the surface of the patient's PHA-T-cell blasts, and no IFN-γ was produced upon IL-12 stimulation. Similarly, an IL-12Rβ1-independent T-cell response to IL-12 has been proposed as a general compensatory mechanism (66), but this hypothesis was not confirmed in our assays [(8, 23, 24, 48, 60, 65) and this report]. In all patients tested, including patients with IL-12Rβ1 expression on the cell surface, no cellular response to IL-12 was detected in our whole-blood assay(22). Despite the varying clinical presentation as well as substantial genetic heterogeneity, the cellular defect was complete. In any event, the large number of kindreds from different ethnic groups, bearing different mutant alleles, identified in this study strongly suggests that IL-12Rβ1 deficiency will be diagnosed in many other families worldwide, particularly as awareness of the clinical features of MSMD and IL-12Rβ1 deficiency increases. Furthermore, many of these patients come from countries with high prevalence of consanguinity, as well as with the national coverage of BCG vaccination during the first days of life. The latter increases the probability for this autosomal recessive defect to

occur, whereas the former accounts for the high prevalence of BCG infection in such patients. Low consanguinity and more restricted BCG vaccination policy may explain why, there were no North American and Australian patients diagnosed. It is difficult to speculate on whether the lack of patients diagnosed in Africa is attributed to the under-reporting, early death from infectious disease, or some other yet unrecognized factor. This study should help to increase awareness of these conditions, thereby improving the diagnosis, and clinical management of these patients worldwide, as well as incite the critical reappraisal of risks and benefits of the BCG vaccination in a global context.

The uniform cellular phenotype is associated with a highly heterogeneous clinical phenotype, ranging from early death in infancy to an asymptomatic course until adulthood. The information provided by this article is invaluable, because it reflects the natural course of this condition in 141 patients originating from highly diverse ethnic backgrounds (41) and exposed to highly diverse microbial flora (26). Mycobacterial infections predominated in these patients, affecting 83% of symptomatic patients and 77% of all patients. The high proportion of mycobacterial diseases, and of infections due to BCG and EM disease in particular, may reflect an ascertainment bias, as most of the subjects studied for defects in the IL-12Rβ1 chain are patients with MSMD. However, similar proportions were obtained for affected relatives of probands (80% of symptomatic relatives). We also report four cases in which tuberculosis was the sole clinical manifestation (6, 8, 48). The *IL12RB1* gene may be considered to be the first Mendelian gene for susceptibility to TB to have been discovered (4). The prevalence of TB in IL-12Rβ1-deficient patients is lower than that of disease due to BCG or EM infection in these patients, probably because patients are less frequently exposed to *M. tuberculosis* than to BCG vaccines (85% vaccination coverage worldwide) and the almost ubiquitous EM. It is less likely to be due to an initial mycobacterial infection protecting against TB. Indeed, two patients presented with BCG-osis and TB. In IL-12Rβ1-deficient

patients, BCG seems to confer greater protection against EM disease than against TB, despite the close phylogenetic relationship between BCG and *M. tuberculosis*, presumably because *M. tuberculosis* is more virulent than EM. In any event, children with severe forms of TB should be tested for IL-12Rβ1 deficiency.

Salmonellosis is the second most common infection in these patients, affecting 43% of symptomatic IL-12Rβ1-deficient patients. It was the only infection in 37% of salmonellosis cases (21/57) and in 15% of all 141 patients. The remaining patients suffered from both mycobacteriosis and salmonellosis. Our study highlights the need to consider IL-12Rβ1 deficiency in patients with a pure phenotype of salmonellosis particularly in cases of extra-intestinal non-typhoidal salmonellosis (typhoid fever was diagnosed in only two patients). We suspect that more of the patients may have suffered from *Salmonella* infection, but the complex, “noisy” clinical setting of concomitant mycobacterial infection and the use of broad-spectrum antibiotics for treatment may have led to underdiagnosis. Furthermore, salmonellosis was not accompanied by an overt inflammatory syndrome in some patients. Infections other than those caused by mycobacteria and salmonella are also increasingly being diagnosed in these patients. Klebsiellosis has been diagnosed in five IL-12Rβ1 patients, *Klebsiella* being closely phylogenetically related to *Salmonella* (44). Toxoplasmosis was diagnosed in two patients, and histoplasmosis, paracoccidioidomycosis, leishmaniasis, and nocardiosis were each diagnosed in one patient. These organisms are intra-macrophagic pathogens, consistent with a possible role of IL-12Rβ1 deficiency in the pathogenesis of these infections. Moreover, one child with nocardiosis has been reported to suffer from IL-12p40 deficiency (51) and a patient with IFN-γR1 deficiency and histoplasmosis has been reported (73). These findings tend to implicate IL-12Rβ1 deficiency in these infections, but the diagnosis of a larger number of cases is required to confirm this hypothesis. More surprisingly, mild forms of chronic mucocutaneous candidiasis have been diagnosed in up to

33 patients (23%, Rodrigues-Gallego C. *et al.*, manuscript in preparation). Over the last few years, IL-12Rβ1 has been implicated in the human IL-23-IL-17 axis (1, 10, 21, 68), as initially described in mice [reviewed in (17, 61)]. Mice with impaired IL-17 immunity are susceptible to *Candida* (12, 31, 38). It has been demonstrated that patients with IL-12Rβ1 deficiency display impairment of the development of IL-17-producing T cells, although this impairment is less pronounced than that in STAT3-deficient patients (14). The high proportion of patients with candidiasis may therefore reflect changes in the IL-23-IL-17 axis. The impairment of IL-23-IL-17 immunity may also account for the higher frequency of salmonellosis in IL-12p40- and IL-12Rβ1-deficient (43%) patients than in IFN-γ receptor-deficient patients [(7%, (43)], and the small number of cases of klebsiellosis reported here [Pedraza S. *et al.*, in preparation]. Indeed, in mice and primates, the IL-23-IL-17 circuit is important for immunity to *Salmonella* and *Klebsiella* (29, 52, 71). In any event, the infectious phenotype of IL-12Rβ1-deficient patients appears to be broader than initially thought.

We confirm that the penetrance of MSMD in IL-12Rβ1 deficiency is not complete for either BCG or EM disease. The penetrance of susceptibility to salmonellosis also seems to be incomplete, although it is difficult to determine which patients have been exposed to *Salmonella*. This problem also makes it difficult to assess the penetrance of susceptibility to tuberculosis, as it is likely that only a small fraction of patients have been exposed to *M. tuberculosis*. The larger number of patients in this study than in our 2003 survey (141 versus 41) resulted in a higher penetrance of MSMD (including salmonellosis) in this study (72%) than in the previous study (45%) at the age of 20 years. If we include TB, global penetrance reaches 79% at this age. However, this revised penetrance value is probably overestimated, because the proportion of asymptomatic siblings tested was much lower than in the 2003 study. Penetrance may also vary between countries, as a function of BCG vaccination policy, tuberculosis burden and the likelihood of being exposed to *Salmonella*. The virulence and

abundance of EM may also vary with geographic region. Thus, even healthy siblings of probands and their more distant relatives in consanguineous kindreds should be investigated. We also confirm that IL-12Rβ1 deficiency mostly begins in childhood. Only three of the 141 patients had a clinical onset after the age of 13 years. Our findings also reveal that the prognosis of IL-12Rβ1 deficiency is not as good as initially thought. Consistent with the higher penetrance, the outcome is much poorer than observed in 2003 in a study of fewer patients. The overall mortality rate for IL-12Rβ1-deficient patients was estimated at 32%, versus only 15% in 2003. There does not seem to be a correlation between mortality rate and country of origin, but the type of infection has a detectable impact, EM disease being associated with a poorer prognosis. Only six deaths were recorded in patients over the age of 13 years. However, the revised mortality rates obtained in this study may reflect the underdiagnosis of asymptomatic siblings. IL-12Rβ1 deficiency is often, but not always, symptomatic. It typically begins in childhood and is lethal in up to a third of patients, particularly in patients with EM disease, and its prognosis seems to improve with age. Both curative and preventive treatment of IL-12Rβ1 deficiency, based on prolonged courses of antibiotics, exogenous IFN-γ treatment, and, in rare cases, surgical resection of affected areas, may influence clinical outcome in these patients. However, we were unable to explore the effects of treatment on clinical outcome because the information available was too limited. We are currently collecting data on the treatments administered to our patients. The description of IL-12Rβ1 deficiency, like that of IL-12p40 deficiency, is essential, not only to improve patient care, but also to improve the quality and safety of monitoring for potential adverse effects, including infectious diseases in particular, in other patients treated with antibodies blocking IL-12p40 or IL-12Rβ1, both of which are currently used to treat various clinical conditions (39, 50).

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**Web Resources**

The URLs for the data presented herein are as follows:

GENALYS Software: http://software.cng.fr

GenBank: http://www.ncbi.nlm.nih.gov/Genbank/

Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov/Omim

ClustalX alignment program: http://bips.u-strasbg.fr/fr/Documentation/ClustalX/

PolyPhen prediction program: http://genetics.bwh.harvard.edu/pph/

Human Genetic of Infectious Diseases website: http://www.hgid.net

Global Infectious Diseases and Epidemiology Online Network: http://www.gideononline.com/features/micro.htm

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## Legends to table and figures

**Table 1: Genetic and clinical features of patients with IL-12Rβ1 deficiency**

**Figure 1: Pedigrees of 102 families with IL-12Rβ1 deficiency.** Each kindred is designated by an integer (1–102), each generation is designated by a roman numeral (I–II), and each individual by an Arabic numeral (each individual studied is identified by these three numbers, organized from left to right). The double lines connecting the parents, and in one case parent and offspring indicate presumed consanguinity. Symbols are divided into two by a horizontal line. The upper part of the symbol indicates mycobacterial infection status (in black, patients with BCG disease or atypical mycobacteriosis; in gray, patients with TB); the lower part of the symbol indicates salmonellosis status, black indicating that the patient has had salmonellosis. The probands are indicated by an arrow. Proband 88.II.1 suffered from granulomatous disease of unknown origin; proband 89.II.2 presented with nocardiosis and klebsiellosis. Individuals whose genetic status could not be evaluated are indicated by the symbol “E?”. Asymptomatic individuals carrying two mutant *IL12RB1* alleles are represented by a vertical line. Kindreds 11 and 63 were related, as were kindreds 12, 13, 36 and 80, kindreds 25 and 30, and kindreds 48 and 51.

**Figure 2: Origin of the kindreds.** Geographical origin of the 141 patients with complete IL-12Rβ1 deficiency. These patients originated from 30 countries (Argentina, Belgium, Bosnia Herzegovina, Brazil, Cameroon, Chile, China, Cyprus, France (mainland and French West Indies), Germany, India, Iran, Israel, Japan, Morocco, Mexico, Netherlands, Pakistan, Poland, Qatar, Saudi Arabia, Slovakia, Spain (mainland and Canaries), Sri Lanka, Taiwan, Tunisia, Turkey, United Kingdom, Ukraine and Venezuela). The numbers indicate the number of patients originating from a given country.

**Figure 3: Distribution of clinical phenotypes for IL-12Rβ1-deficient index cases.** Each patient is classified as a function of mycobacterial infection status (in red, BCG for BCG disease, EM for EM disease, Mtb for TB) and salmonella infection status (in blue, *Salmonella* for *Salmonella* disease). Patients with both mycobacterial infection and salmonellosis are shown in purple. TB is represented as a dotted circle in each group. Infection with unidentified mycobacterial species is presented as a hatched circle.

**Figure 4: Mutated alleles of *IL12RB1* genes.** Schematic representation of the coding region of the IL-12Rβ1 chain containing 17 coding exons encoding a 662-amino acid protein with a peptide leader sequence (exon1, L), extracellular domain (exons 2 to 13, EC), transmembrane domain (exon 14, TM) and an intracellular cytoplasmic domain (exons 15 to 17, IC). Missense mutations are shown in purple, nonsense mutations are shown in red, and complex mutations are shown in brown. Splicing mutations are shown in blue, large deletions are shown in green, insertions are shown in orange, and duplication in magenta. * The 700+362_1619-944del mutation is the only mutation resulting in protein expression at the cell surface.

**Figure 5: Impaired cellular response to interleukin-12.** Production of IFN-γ by whole blood cells from 38 healthy “local” positive controls (fresh blood), from 49 healthy “travel” positive controls and from 65 patients, either unstimulated (-) or stimulated with BCG alone or with BCG plus recombinant IL-12p70. The horizontal bars indicate the median. Individual responses are not indicated due to the large number of patients studied.

**Figure 6: Missense mutations affecting IL-12Rβ1.** (A) Localization of polymorphism (n=4, blue star) and missense mutations (n=14, red stars) in the IL-12Rβ1 gene. (B) Table of the

impact of these mutations as assessed with PolyPhen, and the phenotype (expression of IL-12Rβ1, binding of IL-12) of *Saimiri* herpesvirus-transformed T cells transfected with the various constructs. (C) Table of predicted conservation of residues in various species, based on ClustalX alignment.

**Figure 7: Distribution of the clinical phenotypes of IL-12Rβ1-deficient siblings.** Each patient is classified as a function of their status for mycobacterial (in red, BCG for BCG disease, EM for EM disease, Mtb for TB) and *Salmonella* (in blue, *Salmonella* for *Salmonella* disease) infections. Patients with both mycobacterial infection and salmonellosis are shown in purple. TB is represented as a dotted circle in each group. Infection with unidentified mycobacterial species is presented as a hatched circle.

**Figure 8: Onset of EM disease in symptomatic patients.**

**Figure 9: First onset of infection.**

**Figure 10: Survival.**

**Figure 11: Penetrance of infection.**

**Legends to supplementary figures**

**Supplementary figure 1:** Flow cytometry staining for IL12Rβ1 molecules expressed on the surface of PHA-T-cell blasts from a healthy control (WT/WT) and a patient carrying the homozygous C198R mutation. In the right column, the 2.4E6 antibody specific for IL-12Rβ1 was used. In the left column, the 2B10 antibody specific for IL-12Rβ1 was used. Specific antibiodies are indicated by a solid, non colored line, isotype control antibodies are shown in red and blue, respectively.

**Supplementary figure 2:** Description of the all siblings of IL-12Rβ1-deficient index cases, according to vital status, genotyping status and the presence or absence of clinical symptoms of MSMD.

**Supplementary figure 3: Distribution of clinical phenotypes of all IL-12Rβ1-deficient patients (n=141).** (A) Overall distribution of clinical phenotypes. (B) Proportion of multiple infections due to one or more different families of infectious agents. (C) Distribution of mycobacterial and *Salmonella* diseases. (D) Distribution of salmonellosis. (E) Distribution of mycobacterial diseases. (F) Distribution of environmental mycobacterial diseases. (G) Distribution of candidiasis. (H) Distribution of non vaccinated individuals resistant to BCG, and BCG diseases.

**Supplementary figure 4: Onset of TB disease in symptomatic patients**

**Supplementary figure 5: Onset of salmonellosis in symptomatic patients**