Published in final edited form as:

Int J Tuberc Lung Dis. 2015 December ; 19(12): 1455–1462. doi:10.5588/ijtld.15.0290.

Pott's disease in Moroccan children: Clinical features and investigation of the IL-12/IFN- γ pathway

Safa el Azbaoui^{1,2}, Nidal Alaoui Mrani³, Ayoub Sabri^{1,2}, Zineb Jouhadi⁴, Fatima Ailal⁴, Ahmed Aziz Bousfiha⁴, Jilali Najib⁴, Naima El Hafidi⁵, Caroline Deswarte^{6,7}, Erwin Schurr⁸, Jacinta Bustamante^{6,7,9}, Stéphanie Boisson-Dupuis^{6,7,10}, Jean-Laurent Casanova^{6,7,10,11,12}, Laurent Abel^{6,7,10}, and Jamila EL Baghdadi^{1,*}

¹Genetics Unit, Military Hospital Mohamed V, Hay Riad, Rabat, Morocco

²Faculty of Science-Kenitra, Ibn Tofail University, 14000 Kenitra, Morocco

³Department of Pediatric Surgery, Rabat Children Hospital, Medical and Pharmacy School of Rabat, Mohamed V University, Rabat, Morocco

⁴Department of Pediatric Infectious Diseases, Hospital-University Center Ibn Rochd, King Hassan II University, Casablanca, Morocco

⁵Department of Pediatrics, Rabat Children Hospital, Medical and Pharmacy School of Rabat, Rabat, Morocco

⁶Laboratory of Human Genetics of Infectious Diseases, Necker Branch, Institut National de la Santé et de la Recherche Médicale, INSERM U1163, Paris, France

⁷Paris Descartes University, Imagine Institute, Paris, France

⁸McGill international TB centre, the research institute of the McGill University health centre, Montreal, Quebec, Canada H3G 1A4

⁹Center for the Study of Primary Immunodeficiencies, Assistance Publique-Hôpitaux de Paris AP-HP, Necker Hospital for Sick Children, Paris, France

¹⁰St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, New York, NY, USA

¹¹Howard Hughes Medical Institute, NY, USA

¹²Pediatric Hematology-Immunology Unit, Necker Hospital for Sick Children, Paris, France

Abstract

Setting—Tuberculosis spondylodiscitis (TS) or Pott's disease is an extra-pulmonary form of TB that is rare and difficult to diagnose in children. Some cases of severe TB in children were recently explained by inborn errors of immunity affecting the IL-12/IFN- γ axis.

*Corresponding author, Jamila El Baghdadi, baghdadijamila@yahoo.fr, Genetics Unit, Military Hospital Mohamed V, Hay Riad, Rabat, Morocco.

All authors declare no conflict of interest

Objective—To analyze the clinical data for Moroccan children with TS, and to perform immunological and genetic explorations of the IL-12/IFN- γ axis.

Design—We studied nine children with TS diagnosed between 2012 and 2014. We investigated the IL-12/IFN- γ circuit by both whole-blood assays and sequencing of the coding regions of 14 core genes of this pathway.

Results—TS diagnosis was based on a combination of clinical, biological, histological, and radiological data. QuantiFERON TB Gold in Tube results were positive in 75% of patients. Whole-blood assays showed normal IL-12 and IFN- γ production in all but one patient, who displayed impaired decreased response to IL-12. No candidate disease-causing mutations were detected in the exonic regions of the 14 genes.

Conclusions—The diagnosis of TS in children remains challenging, and is based largely on imaging. Further investigations of TS in children are required to determine the role of genetic defects in pathways that may or may not be related to the IL-12/IFN- γ axis.

Keywords

Pott's disease; Tuberculosis; spondylodiscitis; interferon gamma release assay; IL-12; IFN- γ

INTRODUCTION

Tuberculosis (TB) remains a major public health problem, as *Mycobacterium tuberculosis* infects an estimated one third of the world's population, with ~9 million new cases of TB and 1.5 million deaths from TB in 2013¹. The burden of TB in children is also very high, with ~550,000 new cases and 80,000 deaths reported in 2013¹. However, only a small fraction of infected individuals develop clinical TB^{2,3}. Most subjects (~95%) contain the initial infection, and a minority of latently infected subjects (~5%) subsequently develop active disease after an interval of many years, typically due to the reactivation of pulmonary TB⁴. The remaining infected subjects (~5%) develop clinical TB within two years of infection, either without latency or after a very short latent phase. This "primary" TB is particularly common in children, runs an acute course and is often associated with extrapulmonary disease due to dissemination of the bacillus in the bloodstream². BCG vaccination provides some protection against severe disseminated TB in childhood, but this protection is incomplete⁵. There is now accumulating evidence that disseminated forms of childhood TB are controlled, at least in part, by host genetic factors²⁻⁴. In particular, some cases of severe TB have been shown to be explained by single-gene inborn errors of immunity affecting the IL-12/IFN- γ axis^{2,3,6}.

Tuberculous spondylodiscitis (TS), also known as Pott's disease, is one of the disseminated extrapulmonary forms of TB. It consists of infection of vertebral body by *M. tuberculosis*, with or without spinal cord involvement⁷. TS accounts for about 10% of extrapulmonary cases of TB and 1–5% of all TB cases^{8,9}. It is thought to result from the hematogenous dissemination of the bacillus from a primary infection site, which is often asymptomatic. In younger patients, the disc is primarily involved because of its high degree of vascularization. TS generally occurs in young adults between 30 and 40 years of age, and is rather rare in children⁹. Its diagnosis is challenging because of the insidious and variable onset of the

disease, and the common lack of specific symptoms and bacteriological confirmation^{9,10}. Diagnosis is usually based on a combination of epidemiological, clinical, biological and radiological features. However, early diagnosis and adequate treatment are necessary to prevent bone deformities and neurological deficits^{9,10}. Blood interferon (IFN)- γ release assays (IGRAs), such as the QuantiFERON TB Gold in Tube (QFT-GIT) test, are increasingly being used for the diagnosis of latent TB and suspected active TB^{11,12}. The accuracy and impact of this immunological test in pediatric TB remain a matter of debate^{11,12}.

TB is still highly endemic in Morocco, with ~28,000 new TB cases in 2013, including 2051 (7%) in children under the age of 15 years¹. A few studies have reported the various clinical aspects of Pott's disease in children^{13–15}, including one in Morocco¹⁶, but the IL-12/IFN- γ pathway was not investigated in detail in any of these studies. Our aim in this study was to report the clinical, biological and radiological features of Moroccan children with TS, and to assess the performance of the QFT-GIT test in these patients. We also explored the IL-12/IFN- γ circuit in more detail, by carrying out functional tests assessing the production of, or the response to IFN- γ , and sequencing the coding regions of 14 core genes from this pathway.

STUDY POPULATION AND METHODS

Patients

We performed a prospective study of nine patients followed at various pediatric departments of the university hospitals of Rabat and Casablanca between 2012 and 2014. Pott's disease was diagnosed on the basis of a combination of epidemiological, clinical, biological and radiological data. Hospital records were reviewed to obtain clinical, laboratory, and radiological (plain X rays, computed tomography (CT) scans, magnetic resonance imaging (MRI) scans) data. Microbiological investigations included microscopy with Ziehl-Neelsen staining and cultures of sputum samples and gastric lavage from all patients, and of the pus from the drained abscesses of four patients, and were performed before any TB treatment in an accredited hospital bacteriology laboratory. Histological analyses were carried out on biopsy specimens from the bone lesions of two patients. Blood was drawn for QFT-GIT and for the immunologic and genetic exploration of the IL-12/IFN- γ axis. The collection of these samples from all patients was approved by the appropriate ethics committees and/or institutional review boards, and written informed consent was obtained from the parents of all children.

TST and QuantiFERON TB Gold In Tube (QFT-GIT)

The TST was performed with 2 U of standardized PPD solution (Tuberculin PPD RT23, Denmark), by the Mantoux method. The criterion for a positive TST result was an induration of at least 10 mm in diameter 72 h after injection.

The QFT-GIT tests were performed according to the manufacturer's recommendations (*Cellestis Ltd., Carnegie, Victoria, Australia*). Briefly, 1 ml of peripheral blood was drawn directly into each of three blood collection tubes under vacuum: the nil tube, or the negative

control; the mitogen tube, or the positive control, containing phytohemagglutinin; and the TB-antigen tube, containing specific antigens (ESAT-6, CFP-10, and TB7.7) for *M. tuberculosis*. The contents of each tube were mixed and the tubes were incubated for 16 to 24 h at 37°C. They were then centrifuged at 3500 rpm for 15 min at room temperature. The supernatant was then stored at -20°C. The concentration of IFN- γ in each sample was determined with the QFT-GIT ELISA kit. Results were calculated with the QFT-GIT software provided by the manufacturer.

Whole-blood assays

The whole-blood assays carried out to assess the response to, and the production of IL-12 and IFN- γ were conducted as previously described¹⁷; heparin-treated blood samples in cell culture plates with mixed with medium alone (RPMI 1640, PAA), or stimulated with BCG alone (*M. bovis BCG*, Pasteur sub-strain), BCG plus recombinant human IL-12 (20 ng/mL; R&D Systems), or BCG plus IFN- γ (5,000 IU/ml; Imukin, Boehringer Ingelheim). The unstimulated and stimulated blood samples were incubated for 48 h at 37°C, under an atmosphere containing 5% CO₂. The blood samples were then collected and centrifuged at 1700 rpm for 10 min at 4°C. The supernatant was stored at -80°C until analysis¹⁷. The IFN- γ and IL-12p40 present in the cell-culture supernatants were quantified by ELISA with the Human Quantikine IFN- γ and IL-12p40 kits, according to the manufacturer's recommendations (R&D Systems). The IL-12 response was considered normal if the amount of IFN- γ produced in response to stimulation with BCG+IL-12 was at least 10 times that produced in response to stimulation with BCG alone. The IFN- γ response was considered normal if the amount of IL12-p40 produced in response to stimulation with BCG+ IFN- γ was at least twice that produced in response to stimulation with BCG alone¹⁷. As positive controls for these assays, we used 41 unrelated healthy adult subjects (age range: 28–54 years) who had been vaccinated with BCG. All the experiments were performed in the same laboratory, and at least two healthy controls were tested at the same time as a TS patient. Written informed consent was obtained from all participating healthy controls.

Genomic DNA extraction and whole-exome sequencing

DNA was extracted from peripheral blood by the phenol-chloroform method. The whole-exome sequencing method (WES) used has been described elsewhere^{18,19}. Exome capture was performed with the Agilent SureSelect Human all-exon kit 71 MB (V4+UTR) (Agilent Technologies). For this study, we restricted our analysis to 14 core genes of the IL-12/IFN- γ axis: (*IFNG*, *IFNGR1*, *IFNGR2*, *IL12A*, *IL12B*, *IL12RB1*, *IL12RB2*, *IL23A*, *IL23R*, *JAK1*, *JAK2*, *STAT1*, *STAT4* and *TYK2*)²⁰. All calls with a read coverage $\geq 4\times$ and a phred-scaled SNP quality of ≥ 30 were filtered out. Variant frequencies were filtered based on data from the exome variant server (<http://evs.gs.washington.edu/EVS/>) and 1000 Genomes (<http://browser.1000genomes.org/index.html>). All the variants were annotated with ANNOVAR²¹.

RESULTS

Demographics and clinical characteristics

Nine children were recruited: seven girls and two boys. They had a mean age of 8.5 years (range: 2–15 years). All patients had been vaccinated with BCG at birth, with no reported

side effects, and all subjects had a visible BCG scar. Recent contact with pulmonary TB was reported for two children. Three patients were born to consanguineous parents. The duration of symptoms before diagnosis ranged from 15 days to five months. Table 1 describes the various clinical symptoms recorded in each patient. Six patients had paraparesis, including two with back pain, and the remaining three patients had back pain without paraparesis. Kyphosis was found in two patients. All patients had systemic symptoms, mostly consisting of fever, and/or weight loss (Table 1), and none were malnourished at the time of diagnosis.

Radiology data

Plain X rays and computed tomography (CT) and magnetic resonance imaging (MRI) scans were obtained for all patients. The CT scan was useful for the detection of vertebral disc lesions and paravertebral abscesses (Figure 1), whereas MRI was particularly informative about the spread of the disease to the soft tissues and the extent of spinal cord involvement. The distribution of the disease along the vertebral column was variable, but the involvement of multiple vertebrae was common (Supplementary Table 1), with the lumbar spine most frequently affected (6 patients). All levels of the spine were involved in P1 (Figure 1) and P9, who also had osteolysis of the pelvis (Supplementary Figure 2).

Isolated TS was found in four patients: at the cervical level in P4 and P5, the thoracic level in P3 and the lumbar level in P6. The involvement of two contiguous segments of the spine was seen in P2, in the dorso-lumbar region (Figure 2), and in P7, in the lumbosacral region. Specific features of TS, such as the formation of paraspinal fluid collections, psoas abscesses, epidural and spinal cord compression were found in all patients, and were key elements guiding the diagnosis.

Chest X rays were abnormal for three patients, consistent with associated pulmonary TB. P5 had an apical lesion of the right lung, P8 displayed bilateral pneumonia with pleurisy. The chest CT scan of P9 showed a basal lesion of the right lung, with micronodules and pleurisy (Supplementary Figure 1). P9 also had cerebral TB, with a tuberculoma detected in the left frontal lobe on CT scan.

Biological, microbiological and histological data

An inflammatory syndrome was present in seven cases, as attested by a C-reactive protein (CRP) concentration > 20 mg/l and/or an erythrocyte sedimentation rate (ESR) > 10 mm/h (Table 2). In these patients, CRP concentrations were between 68.7 mg/l and 196.5 mg/l and ESR was between 32 and 84 mm/h. Blood cell counts revealed anemia (hemoglobin concentration < 11.5 g/dl) in six patients, neutrophilia ($> 8 \times 10^9/l$) in five patients and lymphopenia ($< 0.5 \times 10^9/l$) in one patient (Table 2). Children with TB are routinely screened for HIV infection. The nine patients studied here were tested, and all were found to be HIV-negative. The conventional bacteriological examinations carried out, including microscopy with Ziehl-Neelsen staining and the culture of sputum samples and gastric lavage from all patients and of the pus of drained abscesses for four patients, were all negative. Histological examinations of biopsy specimens were carried out for two patients and showed granulomatous osteitis with caseous follicles for P1 and granulomatous and epithelioid

osteitis with caseous necrosis for P2. The biopsy specimens studied also gave negative results for bacteriological examinations.

Tuberculin skin test (TST) and QuantiFERON TB Gold in Tube (QFT-GIT)

The TST was performed in seven patients, four of whom tested positive (induration 10 mm). QFT-GIT was performed in eight patients. It was positive in six (75%), negative in one and yielded indeterminate results in one patient (Figure 3A). We compared the results of QFT-GIT and TST in the seven patients who underwent both tests (Figure 3B). Three of the six patients with positive QFT-GIT results (50%) had concordant positive TST results, whereas the other three patients had negative TST results. The remaining patient had an indeterminate QFT-GIT result and a positive TST result.

Final diagnosis

TS was diagnosed on the basis of epidemiological, clinical, biological and radiological investigations (Table 3). In particular, all patients had typical lesions in the vertebrae and contiguous tissues (bone loss, paraspinal fluid collections, vertebral collapses, epidural and cord compression, psoas abscesses) visible on radiological examination, particularly on CT and MRI scans. Concomitant pulmonary TB was found in three patients, together with cerebral TB in P9. Histologic analysis of biopsy specimens for P1 and P2 showed granulomatous osteitis with caseum. There was a history of close contact with a familial pulmonary TB patient for P2 and P3. All TS patients had a good outcome and clinical improvement was observed on treatment.

Treatment

In accordance with the national TB control program in Morocco, all patients received chemotherapy for 12 months, including two months of quadruple therapy (rifampicin, isoniazid, pyrazinamide and ethambutol), followed by 10 months of treatment with rifampicin and isoniazid. Spinal immobilization, in a corset, was indicated for all patients. Early drainage of paraspinal and psoas abscesses was performed in four patients. Only one patient was treated surgically, by arthrodesis. The outcome was good in all patients, with the regression of clinical signs, weight regain, and the disappearance of fluid collections. Bone deformities persisted in two patients.

Exploration of the IL-12/IFN- γ circuit

In seven patients, we assessed the production of IL-12p40 and IFN- γ after the stimulation of whole blood with live BCG alone, or with BCG plus IFN- γ (IL-12p40 production) or BCG plus IL-12 (IFN- γ production), as previously described¹⁷. Normal levels of IL-12p40 production were detected in all patients (Figure 4A). Normal IFN- γ production was observed in all patients except P1 (Figure 4B). P1 displayed normal levels of IFN- γ production in response to BCG stimulation, but the levels of this cytokine only doubled after co-stimulation with IL-12, whereas a normal response would consist of at least a ten-fold increase.

Whole-exome sequencing analysis

WES was performed on six patients (P1, P2, P3, P4, P5, and P6). For this study, we focused on the analysis of 14 core genes of the IL-12/IFN- γ axis (see methods)²⁰. After quality control, 79 high-quality variants were identified in these 14 genes in the six patients. The substitutions and insertions/deletions (indels) identified were then screened with the following criteria: (a) predicted to have one of the following functional impacts: nonsense, missense, splice-site mutation, or coding indels; (b) homozygous with a frequency <0.01 in public databases (EVS and 1000 Genomes); (c) heterozygous with a frequency <0.001 in public databases. None of the 79 variants fulfilled these criteria. There were therefore no interesting candidate disease-causing mutations in the exonic regions covered by the Agilent SureSelect human exome kit 71 MB for the 14 genes investigated.

DISCUSSION

Bone involvement remains a rather rare complication of TB, particularly in children, and TS accounts for up to 50% of osteo-articular TB cases⁹. TS may lead to severe deformity and late neurological complications, especially in children, due to a delayed diagnosis. The signs and symptoms are usually nonspecific, and variable, with an insidious, indolent and slow progression. In our study, the signs of alarm leading to diagnosis included fever, weight loss, anorexia, and weakness associated with paraparesis and back pain. Consistent with other studies^{13,14,16}, the neurological deficit and back pain were the most common symptoms attesting to spinal involvement, due to the late presentation of patients at the hospital. An association of systemic and neurological signs for a period of more than three weeks should raise the alarm¹³, particularly in countries in which TB is endemic. In our study, the duration of symptoms before diagnosis ranged from 15 days to five months depending on the localization of the lesions, the stage of the disease and the signs of associated TB. TB at other sites might facilitate diagnosis, and has been reported in previous studies^{8,22}. Three of the nine patients in our series had pulmonary TB, including one who also had cerebral TB.

TS diagnosis thus remains a challenge in children and is generally based on a combination of clinical, biological, histological, and radiological data. Microbiological investigations are commonly negative in TS patients, due to the paucibacillary nature of childhood extra-pulmonary TB. TST is of limited diagnostic value, particularly in areas of endemic TB with high coverage rates for BCG vaccination²³, as in Morocco. Positive TST results were obtained for only four of the seven patients tested. In our study, histological examination was possible for two patients, and provided strong evidence in favor of TB, with the presence of granulomatous reactions with caseum. However, the most informative evidence was obtained by imaging, by CT and MRI in particular, which showed typical lesions in bones and contiguous tissues in all cases (bone loss, paraspinal fluid collections, vertebral collapses, epidural and cord compression, psoas abscesses). Treatment was administered and compliance was good, resulting in a favorable outcome in all patients, providing further support for the initial diagnosis of TS.

Improvements to the methods used for diagnosis, to facilitate the earlier detection of pediatric TS cases, especially for severe forms, are required to prevent bone loss, neurological complications and damage. In this context, IGRAs, such as QFT-GIT, are

increasingly being used to improve the diagnosis of TB^{12,24}. We found that six of eight patients (75%) tested positive in the QTF-GIT test. This proportion is consistent with the results of another study of 14 children with TS tested with the QFT Gold test, in which nine children (64%) tested positive¹³. A higher proportion of positive results (84%) was reported for a sample of 51 adult patients with TS²⁵. A number of factors, such as young age, previous BCG vaccination, fever, inflammatory syndrome, and nutritional status may favor indeterminate or negative QFT-GIT results in children^{11,26,27}. The results obtained for large series of children with TB suggest that QFT-GIT tests are of limited utility for the diagnosis of active childhood TB¹.

The IL-12/IL-23/IFN- γ circuit plays a critical role in the immune response to mycobacterial infections in humans^{2,3,6}. In particular, mutations of nine genes of this circuit (*IFNGR1*, *IFNGR2*, *STAT1*, *IRF8*, *CYBB*, *IL12B*, *IL12RB1*, *NEMO* and *ISG15*) have been shown to be responsible for the syndrome of Mendelian susceptibility to mycobacterial diseases (MSMD)²⁸. Some cases of severe TB have also been shown to be due to single-gene inborn errors of immunity affecting some of these genes^{2,3,6}. The most common genetic defect reported to date in patients with severe TB is complete IL-12R β 1 deficiency²⁹, which has been diagnosed in several Moroccan children^{30,31}. This finding has important medical implications, as children carrying such defects can be treated with recombinant IFN- γ ³². No underlying genetic defect has yet been identified in any patient with TS. In patients with MSMD, partial IFN- γ R1 deficiency is often associated with osteomyelitis due to BCG or environmental mycobacteria³³.

We investigated the IL-12/IFN- γ pathway in TS patients, by assessing, in seven patients, the production of, and the response to IFN- γ in whole blood. The peripheral blood cells of the patients produced normal amounts of IL-12p40 in response to stimulation with IFN- γ plus BCG, and of IFN- γ in response to stimulation with IL-12 plus BCG, with the exception of P1, who displayed an impaired response to IL-12. P1 had a strong response to BCG stimulation and a positive QTF-GIT result. We explored the role of the IL-12/IFN- γ axis further, by conducting WES and specifically analyzing 14 core genes of this pathway, to search for rare coding mutations potentially associated with disease susceptibility. No rare variants that could be considered reasonable candidate disease-causing mutations were identified for these genes, in any of the patients, including P1. Thus, none of the six TS patients investigated by WES carried a potential disease-causing mutation of 14 genes from this circuit.

CONCLUSIONS

The diagnosis of TS in children remains challenging and is highly dependent on imaging techniques, such as computed tomography and magnetic resonance imaging in particular. We found no strong evidence of functional impairment of the IL-12/IFN- γ axis in our patients. No candidate disease-causing mutations were found in the exonic regions of the 14 genes of this axis investigated in this study. Further analyses of the WES data for a larger sample of patients are underway, to investigate, genome-wide, the role of mutations of genes from pathways that may or may not be related to the IL-12/IFN- γ axis.

Acknowledgments

We thank all patients and family members from Morocco for their participation in this study. This work was supported by grants from the French National Agency for Research (ANR, grant number ANR-14-CE14-0007-01), EU-grant HOMITB (HEALTH-F3-2008-200732), the European Research Council (ERC-2010-AdG-268777), the St. Giles Foundation, the Rockefeller University grant number 8ULTR000043 from the National Center for Research Resources and the National Center for Advancing Sciences (NCATS) of the National Institute of Health, the Rockefeller University, the National Institute of Allergy and Infectious Diseases grant numbers R01AI089970, and U01AI088685.

References

1. WHO. Global tuberculosis report Geneva. World Health Organization; 2014.
2. Abel L, El-Baghdadi J, Bousfiha AA, Casanova JL, Schurr E. Human genetics of tuberculosis: a long and winding road. *Philos Trans R Soc Lond B Biol Sci*. 2014; 369:20130428. [PubMed: 24821915]
3. Alcais A, Fieschi C, Abel L, Casanova JL. Tuberculosis in children and adults: two distinct genetic diseases. *J Exp Med*. 2005; 202:1617–1621. [PubMed: 16365144]
4. O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MP. The immune response in tuberculosis. *Annu Rev Immunol*. 2013; 31:475–527. [PubMed: 23516984]
5. Maartens G, Wilkinson RJ. Tuberculosis. *Lancet*. 2007; 370:2030–2043. [PubMed: 17719083]
6. Boisson-Dupuis S, Bustamante J, El-Baghdadi J, et al. Inherited and acquired immunodeficiencies underlying tuberculosis in childhood. *Immunol Rev*. 2015; 264:103–120. [PubMed: 25703555]
7. Thwaites G, Fisher M, Hemingway C, Scott G, Solomon T, Innes J. British Infection Society guidelines for the diagnosis and treatment of tuberculosis of the central nervous system in adults and children. *J Infect*. 2009; 59:167–187. [PubMed: 19643501]
8. Garg RK, Somvanshi DS. Spinal tuberculosis: a review. *J Spinal Cord Med*. 2011; 34:440–454. [PubMed: 22118251]
9. Trecarichi EM, Di Meco E, Mazzotta V, Fantoni M. Tuberculous spondylodiscitis: epidemiology, clinical features, treatment, and outcome. *Eur Rev Med Pharmacol Sci*. 2012; 16(Suppl 2):58–72. [PubMed: 22655484]
10. Cormican L, Hammal R, Messenger J, Milburn HJ. Current difficulties in the diagnosis and management of spinal tuberculosis. *Postgrad Med J*. 2006; 82:46–51. [PubMed: 16397080]
11. Pollock L, Basu Roy R, Kampmann B. How to use: interferon gamma release assays for tuberculosis. *Arch Dis Child Educ Pract Ed*. 2013; 98:99–105. [PubMed: 23580543]
12. Sester M, Sotgiu G, Lange C, et al. Interferon-gamma release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. *Eur Respir J*. 2011; 37:100–111. [PubMed: 20847080]
13. Eisen S, Honywood L, Shingadia D, Novelli V. Spinal tuberculosis in children. *Arch Dis Child*. 2012; 97:724–729. [PubMed: 22734017]
14. Jain AK, Sreenivasan R, Mukunth R, Dhammi IK. Tubercular spondylitis in children. *Indian J Orthop*. 2014; 48:136–144. [PubMed: 24741133]
15. Kumar R. Spinal tuberculosis: with reference to the children of northern India. *Childs Nerv Syst*. 2005; 21:19–26. [PubMed: 15459785]
16. Benzagmout M, Boujraf S, Chakour K, Chaoui Mel F. Pott's disease in children. *Surg Neurol Int*. 2011; 2:1. [PubMed: 21297923]
17. Feinberg J, Fieschi C, Doffinger R, et al. Bacillus Calmette Guérin triggers the IL-12/IFN- γ axis by an IRAK-4- and NEMO-dependent, non-cognate interaction between monocytes, NK, and T lymphocytes. *Eur J Immunol*. 2004; 34:3276–3284. [PubMed: 15384045]
18. Bogunovic D, Byun M, Durfee LA, et al. Mycobacterial disease and impaired IFN- γ immunity in humans with inherited ISG15 deficiency. *Science*. 2012; 337:1684–688. [PubMed: 22859821]

19. Byun M, Abhyankar A, Lelarge V, et al. Whole-exome sequencing-based discovery of STIM1 deficiency in a child with fatal classic Kaposi sarcoma. *J Exp Med*. 2010; 207:2307–2312. [PubMed: 20876309]
20. Sabri A, Grant AV, Cosker K, et al. Association study of genes controlling IL-12-dependent IFN-gamma immunity: STAT4 alleles increase risk of pulmonary tuberculosis in Morocco. *J Infect Dis*. 2014; 210:611–618. [PubMed: 24610875]
21. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010; 38:e164. [PubMed: 20601685]
22. Pigrau-Serrallach C, Rodriguez-Pardo D. Bone and joint tuberculosis. *Eur Spine J*. 2013; 22(Suppl 4):556–566. [PubMed: 22711012]
23. Rigouts L. Clinical practice: diagnosis of childhood tuberculosis. *Eur J Pediatr*. 2009; 168:1285–1290. [PubMed: 19396462]
24. Bianchi L, Galli L, Moriondo M, et al. Interferon-gamma release assay improves the diagnosis of tuberculosis in children. *Pediatr Infect Dis J*. 2009; 28:510–514. [PubMed: 19504735]
25. Kumar R, Das RK, Mahapatra AK. Role of interferon gamma release assay in the diagnosis of Pott disease. *J Neurosurg Spine*. 2010; 12:462–466. [PubMed: 20433293]
26. Jenum S, Selvam S, Mahelai D, et al. Influence of age and nutritional status on the performance of the tuberculin skin test and QuantiFERON-TB gold in-tube in young children evaluated for tuberculosis in Southern India. *Pediatr Infect Dis J*. 2014; 33:e260–269. [PubMed: 24797993]
27. Li T, Bao L, Diao N, et al. Influential factors of the performance of interferon-gamma release assays in the diagnosis of childhood tuberculosis. *Clin Exp Med*. 2014 24925641.
28. Bustamante J, Boisson-Dupuis S, Abel L, Casanova JL. Mendelian susceptibility to mycobacterial disease: Genetic, immunological, and clinical features of inborn errors of IFN-gamma immunity. *Semin Immunol*. 2014; 26:454–470. [PubMed: 25453225]
29. de Beaucoudrey L, Samarina A, Bustamante J, et al. Revisiting human IL-12Rbeta1 deficiency: a survey of 141 patients from 30 countries. *Medicine (Baltimore)*. 2010; 89:381–402. [PubMed: 21057261]
30. Altare F, Ensser A, Breiman A, et al. Interleukin-12 receptor beta1 deficiency in a patient with abdominal tuberculosis. *J Infect Dis*. 2001; 184:231–236. [PubMed: 11424023]
31. Boisson-Dupuis S, El Baghdadi J, Parvaneh N, et al. IL-12Rbeta1 Deficiency in Two of Fifty Children with Severe Tuberculosis from Iran, Morocco, and Turkey. *PLoS One*. 2011; 6:e18524. [PubMed: 21533230]
32. Alangari AA, Al-Zamil F, Al-Mazrou A, et al. Treatment of disseminated mycobacterial infection with high-dose IFN-gamma in a patient with IL-12Rbeta1 deficiency. *Clin Dev Immunol*. 2011; 2011:691956. [PubMed: 21234109]
33. Jouanguy E, Lamhamedi-Cherradi S, Lammas D, et al. A human IFNGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. *Nat Genet*. 1999; 21:370–378. [PubMed: 10192386]

Figure 1.
CT Scan of the spine of P1. (A) Sagittal and (B) transversal sequences showing bone destruction at lumbo-sacral level of the spine

Figure 2.
Contrast-enhanced T2-weighted sagittal MRI of P2 showing vertebral collapse of D12-L1, posterior angulation, cord compression and epidural extension.

Figure 3. Results of QFT-GIT assays. (A) IFN- γ levels observed in nil-tubes, TB-antigen-tubes, and mitogen-tubes in 8 TS patients. (B) IFN- γ levels observed in TB-antigen-tubes according to the results of TST in 7 patients (circles denote a positive QFT-GIT and a square an indeterminate QFT-GIT result).

Figure 4. Cytokine production (A: IL-12p40, B:IFN- γ) in the supernatants of whole blood cells from patients with TS (n=7) and healthy controls (n=41), not stimulated (Medium alone) or stimulated by BCG alone or BCG plus cytokine.

Table 1

Clinical features of the nine patients on diagnosis of TS.

Patient	Sex	Age of TB diagnosis (year)	Clinical Characteristics							
			Paraparesia	Back pain	Fever	Night sweats	Weight loss	Anorexia	Cough	Kyphosis
P1	F	15	Y			Y	Y	Y		
P2	F	8	Y	Y	Y		Y	Y		Y
P3	M	3	Y		Y					Y
P4	F	2	Y				Y			
P5	M	11		Y	Y		Y	Y	Y	
P6	F	2	Y	Y	Y					
P7	F	14	Y				Y			
P8	F	13		Y	Y		Y	Y		
P9	F	13		Y	Y		Y	Y	Y	

Y= yes

Table 2

Biological data in the nine TS patients

	Normal values	P1	P2	P3	P4	P5	P6	P7	P8	P9
CRP protein (mg/l)	<5	110.9	5.3	NA	2.2	8.1	68.7	106.5	196.5	191
ESR (mm/h)	3–13	NA	NA	32	10	50	84	NA	57	75
Haemoglobin (g/dl)	10.5–15.5	8.5	10	12.6	13.4	12.1	5.8	10.3	9.2	9.6
Neutrophilia 10000/ μ l	1.5–7	10.4	3.7	4.2	7.18	4.15	14.03	8.92	8.17	20.5
Lymphocytes 1000/ μ l	1.5–7	3.4	2.4	3.4	NA	3.7	NA	0.5	2.6	3.3

NA= not available

Table 3

Summary of criteria leading to TS diagnosis in the nine patients

Patient	Age of TB diagnosis	TB contact	TST	Radiology	Histology	Favorable outcome under anti-TB treatment	Other localization	QFT TB GIT
P1	15		Negative	Y	Y	Y	Psoas abscess	Positive
P2	8	Y	Negative	Y	Y	Y		Positive
P3	3	Y		Y		Y		Not done
P4	2		Positive	Y		Y	Psoas Abscess	Positive
P5	11		Positive	Y		Y	Pulmonary TB	Positive
							Vertebral abscess	
P6	2		Positive	Y		Y	Psoas abscess	Positive
P7	14			Y		Y	Psoas abscess	Negative
P8	13		Positive	Y		Y	Psoas abscess	Indeterminate
							Pulmonary TB	
P9	13		Negative	Y		Y	Pleuropulmonary TB	Positive
							Cerebral TB	

Y= yes