

The novel *IFNGR1* mutation 774del4 produces a truncated form of interferon- γ receptor 1 and has a dominant-negative effect on interferon- γ signal transduction

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Background: Patients with interferon- γ receptor 1 (IFN γ R1) deficiency show selective susceptibility to intracellular pathogens such as mycobacteria. IFN γ R1 deficiency is an inherited immunodeficiency disorder, which can be either recessive or dominant. Dominant forms of IFN γ R1 deficiency are known to be associated with mutations that introduce a premature stop codon in the intracellular domain of IFN γ R1. One such mutation, 818del4, is believed to be the most common type. Although these mutations are presumed to exert a dominant-negative effect on IFN γ signal transduction, the underlying molecular mechanism is unresolved. **Objective:** We characterised the 774del4 mutant of IFN γ R1 using a gene-expression system to examine the effects of this mutation on IFN γ signal transduction.

Results: We identified a novel dominant mutation in *IFNGR1*, designated 774del4, which produced a truncated form of IFN γ R1 in a patient with recurrent mycobacterial infections. IFN γ R1 was overexpressed on the surfaces of CD14-positive cells from the peripheral blood of this patient, and STAT1 phosphorylation in response to high doses of IFN γ was partially deficient. We expressed two truncated forms of IFN γ R1, 774del4 and 818del4, in HEK 293 cells using transient transfection and found that these mutants overexpressed IFN γ R1 on the cell surface because of impaired receptor stability, which resulted in a dominant-negative effect on IFN γ signal transduction.

Conclusion: Like the 818del4 mutation, 774del4 produces a truncated form of IFN γ R1, which has a dominant-negative effect on IFN γ signal transduction through altered receptor stability.

Mendelian susceptibility to mycobacterial diseases (MSMD; OMIM 209950) is a rare congenital disorder characterised by susceptibility to infection by poorly virulent intracellular pathogens such as bacillus Calmette-Guérin (BCG) and non-tuberculosis mycobacterium (NTM), without any other marked immunodeficiency.¹ MSMD is caused by an underlying genetic defect in the IL-12/23-IFN γ circuit that enhances the pathogenesis of BCG and NTM. To date, six genes (*IFNGR1*, *IFNGR2*, *IL12B*, *IL12RB1*, *STAT1*, and *NEMO*) are known to be associated with this disorder.^{2–11} Interferon- γ receptor 1 (IFN γ R1) deficiency, caused by mutations in *IFNGR1*, is included in this disease category and is clinically characterised by disseminated BCG and severe NTM infections. This inherited immunodeficiency exists in both recessive and dominant forms, with a high degree of allelic heterogeneity.^{2–4} The dominant forms of IFN γ R1 deficiency are frequently associated with a mutation in the intracellular domain of *IFNGR1*; in particular, the mutant known as 818del4 involves a 4 bp deletion in exon 6. In addition, the 811del4, 817insA, 818delT and G832T mutations have been reported.^{12–15} In this study, we identified a novel dominant mutation in *IFNGR1*, designated 774del4, in a patient with multifocal osteomyelitis due to mycobacterial infection. Although these mutations are presumed to exert a dominant-negative effect on IFN γ signal transduction, the exact molecular mechanism is unknown. Therefore, we used a gene-expression system in heterologous cells to examine the effects of the mutant receptors on IFN γ signalling. We found that the truncated forms of IFN γ R1 are overexpressed on the cell surface because of altered receptor stability, resulting in a dominant-negative effect on IFN γ signal transduction.

MATERIALS AND METHODS

Case report

The subject was a Japanese girl who was vaccinated with BCG at 4 months of age. She developed regional lymph node enlargement 2 months after vaccination, and lymph-node biopsies revealed a tuberculoid granuloma. She was diagnosed with BCG lymphadenitis and treated with isoniazid for 3 years. At 12 years of age, she developed pain in her left knee and back, and persistent low-grade fever. Radiography indicated multifocal osteomyelitis. Bone biopsies revealed tuberculoid granulomas, and PCR detected the presence of *Mycobacterium avium*. The patient was treated with isoniazid, rifampicin, and clarithromycin or azithromycin, and her symptoms gradually improved.

We obtained blood samples from the patient, her relatives and healthy adult controls, after obtaining informed consent. This study was approved by the ethics committee and internal review board of Hiroshima University.

Molecular genetics

Genomic DNA was extracted from peripheral blood leucocytes. Exon 6 of *IFNGR1* and its flanking introns were amplified by PCR using the following primers: sense 5'-GTGTTCTTAA AACTCTGGCC-3' and antisense 5'-TGGTAGACTGACTGATTG

Abbreviations: APC, antigen-presenting cell; BCG, bacillus Calmette-Guérin; FCS, fetal calf serum; IFN γ R1, interferon gamma receptor 1; LPS, lipopolysaccharide; M-CSF, macrophage colony-stimulating factor; MSMD, mendelian susceptibility to mycobacterial diseases; NTM, non-tuberculosis mycobacterium; PBMC, peripheral blood mononuclear cell; STAT1, signal transducer and activator of transcription 1; TNF, tumour necrosis factor; WT, wild type

ATG-3'. The products were sequenced directly and then further analysed by cycle sequencing (BigDye Terminator v3.1 cycle sequencing kit; Applied Biosystems, Foster City, California, USA) and run on an automated analyser (ABI PRISM 310 Genetic Analyzer; Applied Biosystems).

Total RNA was extracted from the peripheral blood mononuclear cells (PBMCs) using Isogen extraction (Nippon Gene Co., Tokyo, Japan), and cDNA was synthesised from 5 µg of total RNA using a first-strand synthesis system for RT-PCR (SuperScript; Invitrogen, Carlsbad, California, USA). PCR of the wild-type (WT) and 774del4 mutant alleles was performed with primers that span the entire coding region of IFN γ R1 (5'-GGATCCCGCAGGCGCTCGGGGTTGGA-3' and 5'-CTCGAGGAAAACGTGCCAGGAAAATCAGACT-3'). The PCR products were cloned into pGEM-T Easy vector (Promega, Madison, Wisconsin, USA). To generate the 818del4 mutant, we performed PCR-based mutagenesis of the WT construct using the following mismatched PCR primers: sense 5'-TTTATATTAAGAAAATCCATTGAAGGAAAA-3' and antisense, 5'-TTTTCCTCAATGGATTTCCTTAATATAAA-3'. These fragments were subcloned into the *Bam*HI and *Xho*I sites of a mammalian expression vector pCDNA (Invitrogen).

Flow cytometry

PBMCs were stimulated with 20 µg/L lipopolysaccharide (LPS) (Sigma-Aldrich, St. Louis, Missouri, UK) and 100 µg/L macrophage colony-stimulating factor (M-CSF) (Kyowa Hakko Kogyo, Tokyo, Japan) for 48 h. Portions of the cells were stained with phycoerythrin-conjugated monoclonal anti-human IFN γ R1 antibody (GIR-94; Santa Cruz Biotechnology, Santa Cruz, CA) or the isotype control, and analysed by gating in a FACS Calibur apparatus (Becton Dickinson, Franklin Lakes, New Jersey, USA). The remaining cells were recultured for an additional 18 h under starvation conditions and then left untreated, or treated with 50 µg/L LPS and 10 000 U/mL IFN γ for 30 min at 37°C. After stimulation, the cells were analysed by flow cytometry using an anti-Stat1 antibody (BD Phosflow anti-Stat1 (pY701); Becton Dickinson).

Analysis of gene expression

The HEK 293 cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (FCS)

(HyClone, Logan, Utah, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin. At 24 h before transfection, the cells were harvested by trypsinisation and replated at a density of 1×10^5 cells/mL in 100 mm culture dishes. Plasmid DNA (10 µg per plate) carrying the WT, 774del4 or 818del4 alleles of *IFNGR1* was introduced into the HEK 293 cells by calcium phosphate-mediated transfection.

At 24 h after transfection, a portion of the cells was used for Western blotting¹⁶ to detect the IFN γ R1 protein using anti-human IFN γ R1 antibodies. The remaining cells were treated with 20 µg/mL cycloheximide for 3 h and analysed by flow cytometry and Western blotting.

Primary cell separation and cytokine measurements

CD14-positive cells and CD3-positive cells were purified from PBMCs using a cell separation system (IMag; Becton-Dickinson) according to the manufacturer's instructions. The purity of the CD14-positive cell population was >90%, and that of the CD3-positive cell population was >97% (data not shown). The CD14-positive cells were cultured for 48 h in RPMI 1640 (Sigma-Aldrich) supplemented with 15% FCS, with the addition of 500 ng/mL LPS and various concentrations (10^2 – 10^5 IU/mL) of IFN γ (Genentech, South San Francisco, California, USA). The cell-culture supernatants were harvested, and the concentration of tumour necrosis factor (TNF)- α was measured in duplicate using a human TNF α antibody bead kit (Biosource International, Camarillo, California, USA) and Luminex system (Luminex, Austin, Texas, USA). The CD3-positive cells were incubated for 48 h with 1 µg/mL phytohaemagglutinin (PHA) (Sigma-Aldrich) and various concentrations (1 – 10^5 pg/mL) of IL-12. The concentration of IFN γ was measured as described above using Luminex.

RESULTS

Sequence analysis

High molecular weight DNA was extracted from the peripheral blood samples, and the exons and flanking introns of *IFNGR1* were amplified by PCR. Sequence analysis revealed that the patient had a heterozygous mutation, 774del4 (TCTA), in exon 6 of *IFNGR1* (figure 1A). This mutation was not detected in the subject's parents or in 30 healthy Japanese controls (figure 1B).

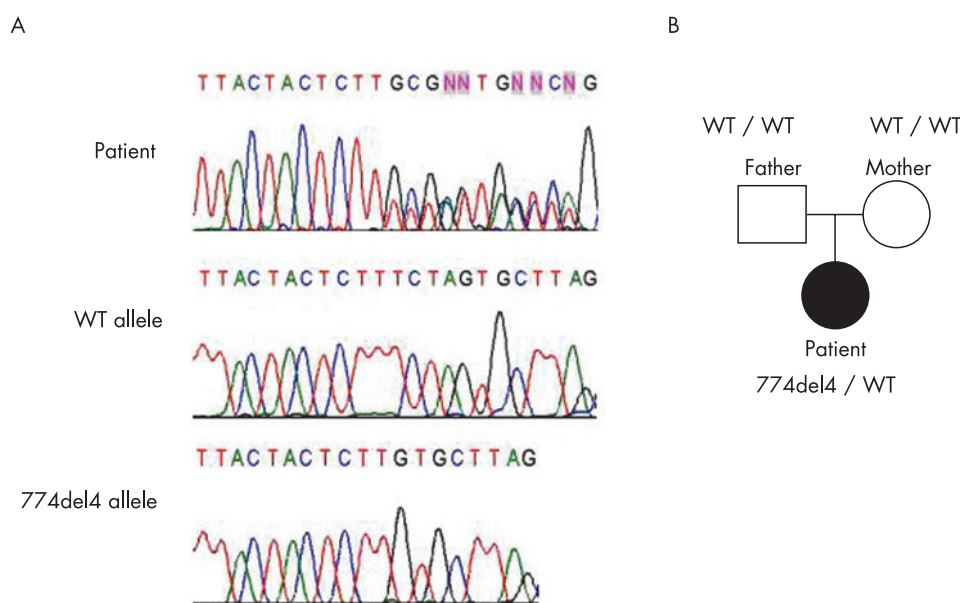


Figure 1 Sequence analysis of exon 6 of *IFNGR1*. (A) The genomic DNA of *IFNGR1* exon 6 from the patient was amplified by PCR, and the sequence was analysed by direct sequencing. The PCR product was cloned into the pGEM-T Easy vector. Sequencing analysis detected the presence of the wild-type (WT) and 774del4 alleles and that the patient had a heterozygous mutation, 774del4 (TCTA), in exon 6 of *IFNGR1*. (B) Pedigree of this family with the dominant form of IFN γ R1 deficiency. Filled symbol, affected person; open symbols, healthy family member. The 774del4 mutation was not detected in either of the parents.

Response to IFN γ

To ascertain the cellular response to IFN γ , we purified CD14-positive cells from the PBMCs of the patient and controls. The CD14-positive cells from the patient produced a lower level of TNF α in response to IFN γ stimulation, regardless of the dose (figure 2A). Once activated, the IFN γ receptor induces STAT1 homodimerisation via phosphorylation of tyrosine residue 701, and these homodimers enter the nucleus, where they act as transcription factors. Therefore, STAT1 Tyr⁷⁰¹ phosphorylation (p-Y701-STAT1) in response to IFN γ was examined in PBMCs from the patient. PBMCs were incubated with M-CSF and LPS for 48 h and then recultured for an additional 18 h under starvation conditions. The cells were then treated with or without LPS and IFN γ for 30 min or left untreated, and the level of p-Y701-STAT1 was analysed by flow cytometry. Although STAT1 phosphorylation was detected in the patient, it was significantly reduced (figure 3B).

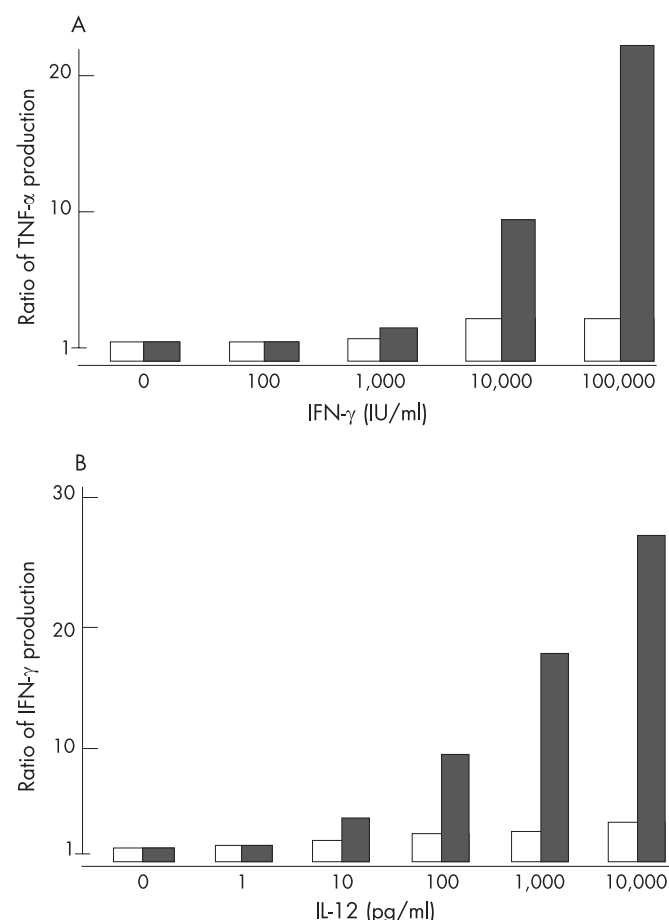


Figure 2 Analysis of cytokine production. (A) CD14-positive mononuclear cells in the peripheral blood samples of a patient and normal controls were stimulated with LPS and various concentrations of interferon (IFN)- γ for 48 hours. The purity levels of the CD14-positive cell populations were >90%. Cell-culture supernatants were harvested, and the concentrations of tumour necrosis factor (TNF)- α were measured by Luminex. The data represent the ratios of the levels of TNF α produced in response to LPS plus IFN γ to the levels of TNF α produced in response to LPS alone. Open columns, data from the patient; filled column, representative data from control subjects. (B) CD3-positive mononuclear cells in the peripheral blood samples of a patient and normal controls were stimulated with purified hemagglutinin (PHA) 1 μ g/ml and various concentrations of interleukin (IL)-12 for 48 hours. The purity levels of CD3-positive cells populations were >95%. Cell-culture supernatants were harvested, and the concentrations of IFN γ were measured by Luminex. The data represent the ratios of IFN γ produced in response to PHA plus IL-12 to the levels of IFN γ produced in response to PHA alone. Open columns, data from the patient; filled columns, representative data from the control subjects.

Expression of the IFN γ R1 mutant proteins

The 774del4 mutation was presumed to cause a frameshift that would lead to a premature stop codon at nucleotide positions 827–829, thereby producing a truncation in the cytoplasmic domain of the receptor.^{4 17–19} To characterise the 774del4 mutant protein, PBMCs from the patient and healthy controls were isolated and stimulated with LPS and M-CSF for 48 h, and surface expression of IFN γ R1 on the activated CD14-positive mononuclear cells was analysed using flow cytometry. Increased expression of IFN γ R1 was detected in the patient (figure 3A).

To characterise the 774del4 and 818del4 mutant versions of IFN γ R1 further, we cloned the cDNAs for these mutants into pcDNA and transiently transfected each construct into HEK 293 cells, using calcium phosphate-mediated transfection. The gene products were analysed by Western blot analysis (figure 4). The 774del4 and 818del4 mutant proteins had apparent molecular weights in the range of 45–60 kDa, in contrast to the WT molecular weight of 80–95 kDa. These results indicate that the 774del4 and 818del4 mutant alleles of *IFNGR1* encode truncated forms of IFN γ R1 that accumulate on the cell surface, as predicted by the primary structures of the mutants.

IFN γ R1 stability

As cell-surface overexpression of the 774del4 truncated form of IFN γ R1 was detected in the patient, we established HEK 293 cells that transiently expressed either 774del4 or 818del4 and examined the stability of the truncated proteins. HEK 293 cells transiently transfected with either of the vectors were treated with cycloheximide for 3 h, and IFN γ R1 expression was analysed by Western blotting (figure 5B) and flow cytometry (figure 5A). The 774del4 and 818del4 mutant versions of IFN γ R1 were consistently detected, even after the inhibition of translation by cycloheximide (see supplementary figure, available online at <http://jmg.bmj.com/supplemental>). This result indicates that these mutant proteins are stably expressed on the cell surface due to alteration of receptor stability.

Cellular response to IL-12

To examine the cellular response to IL-12, we purified CD3-positive cells from the PBMCs of the patient and the controls. Compared with the CD3-positive cells of the controls, those of the patient produced very little IFN γ in response to IL-12 (figure 2B).

DISCUSSION

IFN γ and IL-12 play important roles in initiating and sustaining the Th1 cytokine cascade. Antigen-presenting cells (APCs), including macrophages and dendritic cells, produce IL-12 in response to mycobacterial infection. IL-12 stimulates T cells and natural killer cells through its receptor (a heterodimer of IL-12 receptors β 1 and β 2) to produce IFN γ . IFN γ acts through its receptor (a heterodimer of IFN γ R1 and IFN γ R2) on macrophages to activate the defence against mycobacteria. IFN γ signalling in APCs increases microbicidal activity and upregulates IL-12 and TNF α production through STAT1 activation. Thus, IFN γ and IL-12 work synergistically to induce cellular immunity,^{20 21} and impairment of the IL-12/23-IFN γ circuit results in MSMD. Recently, mutations were identified in the *IFNGR1*, *IFNGR2*, *IL12B*, *IL12RB1*, *STAT1*, and *NEMO* genes of patients with MSMD.^{2–11} In fact, 13 different genetic disorders associated with MSMD have been shown to be caused by mutations in these six genes.¹¹ In particular, defects in *IFNGR1*, *IFNGR2*, and *STAT1* are associated with impaired cellular responses to IFN γ , and defects in *IL12B*, *IL12RB1*, and *NEMO* are associated with IL-12-dependent IFN γ production.¹¹ Further, an X-linked recessive form of MSMD was recently

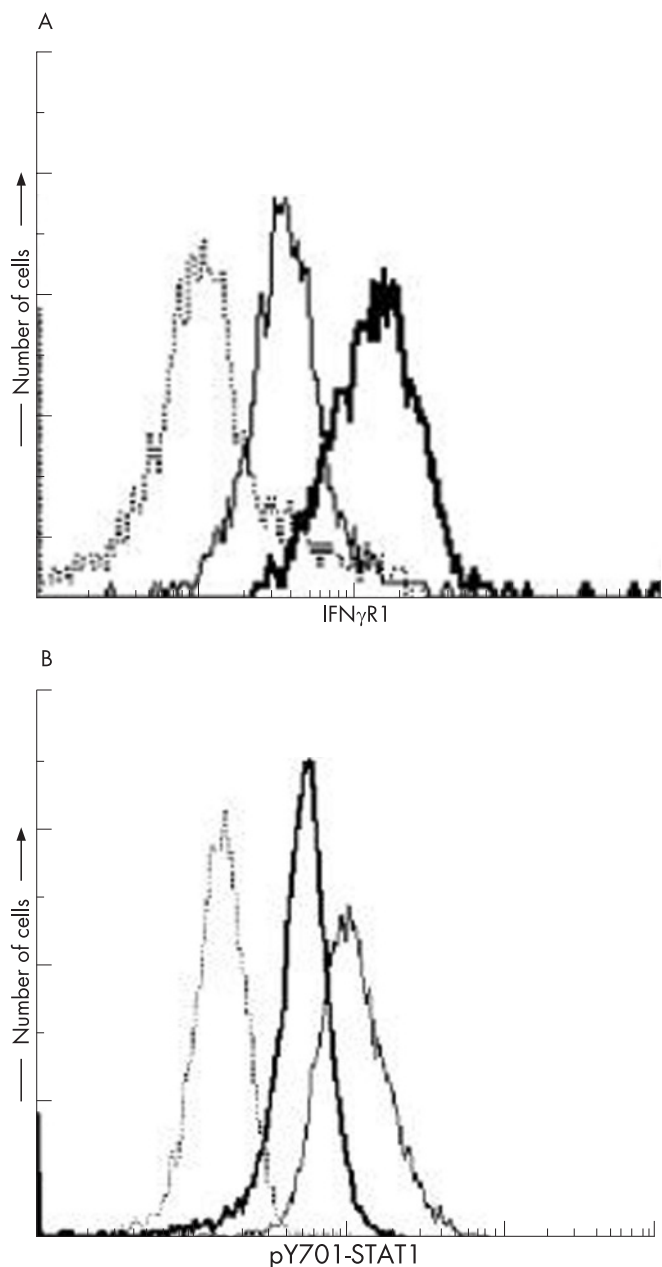


Figure 3 Analysis of cell surface IFN γ R1 and STAT1 phosphorylation in response to IFN γ stimulation. (A) Peripheral blood mononuclear cells were cultured with RPMI 1640 containing 15% FCS, 50 μ g/l LPS and 100 μ g/l M-CSF for 48 hours. The cells were stained with phycoerythrin (PE)-conjugated anti-interferon γ receptor 1 (IFN γ R1) antibody and fluorescein isothiocyanate (FITC)-conjugated anti-CD14 antibody and analysed by flow cytometry. The data represent the levels of expression of IFN γ R1 on CD14-positive cells. Bold line, patient; solid line, control. The dotted line indicates binding of the appropriate isotype control antibody. (B) Peripheral blood mononuclear leukocytes were incubated in RPMI 1640 that was supplemented with 15% fetal calf serum (FCS), 100 μ g/l macrophage colony-stimulating factor (M-CSF), and 20 μ g/l lipopolysaccharide (LPS) for 48 hours. The harvested cells were washed and then cultured for an additional 18 hours in RPMI 1640 medium alone. Then cells were treated with or without 50 μ g/l LPS and 10 000 U/ml IFN γ for 30 minutes, and the cells were then stained with anti-STAT1 (pY701) antibody and analysed by flow cytometry. Bold line, patient sample with cytokine stimulation; solid line, normal subject sample with cytokine stimulation; dotted line, normal subject sample without cytokine stimulation.

identified and has been mapped to two candidate regions, Xp11.4-Xp21.2 and Xq25-Xq26.3.²² Patients bearing these mutant alleles share certain clinical features, including selective susceptibility to mycobacteria, *Salmonellae* and some viral

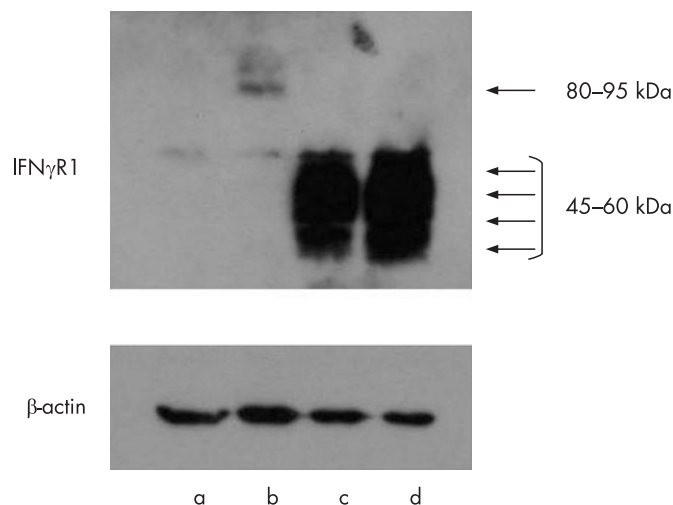


Figure 4 Expression of 774del4 and 818del4 mutant IFN γ R1 proteins by transfected cells. Expression constructs carrying the wild-type (WT), 774del4, and 818del4 forms of *IFNGR1* were transiently transfected into HEK 293 cells using the calcium phosphate method. IFN γ R1 protein was detected by Western blotting. The WT IFN γ R1 (B) was detected with an apparent molecular weight of 80–95 kDa. The 774del4 (C) and 818del4 (D) mutant forms of IFN γ R1 gave strong signals with an apparent molecular weight of 45–60 kDa; this band was not detected in the WT-transfected cell lysate. IFN γ R1 was not detected in the total lysate of cells transfected with the mock vector (A).

infections, although with various sensitivity. In addition, they show no marked immune deficiencies against other pathogens.

In this study, we identified a novel mutation in *IFNGR1*, designated 774del4, in a patient with MSMD. Consistent with the results of a previous report,²³ the CD14-positive cells from this patient exhibited impaired production of TNF α in response to LPS and IFN γ , and the patient's CD3-positive cells did not produce IFN γ effectively in response to PHA and IL-12. It seems likely that the IFN γ R1 deficiency not only impairs IFN γ signaling but also abolishes the Th1 cytokine cascade.

Based on these results, we examined the molecular basis for the dominant forms of IFN γ R1 deficiency. We identified a 4 bp deletion, designated 774del4, in the *IFNGR1* gene of a patient with multiple osteomyelitic lesions due to *M. avium* infection. This was considered to be a sporadic case, as the same mutation was not detected in either of the patient's parents. The deletion causes a frameshift that introduces a premature stop codon (TGA) at nucleotides 827–829; the resulting truncated version of IFN γ R1 lacks the expected intracellular domain. The 818del4 allele, which is the most frequent mutation observed in patients with the dominant form of IFN γ R1 deficiency, also introduces a frameshift and premature stop codon at nucleotides 827–829.⁴ Although the truncated protein encoded by 774del4 differs from that encoded by 818del4 in terms of the last 14 amino acids at the C-terminal end, patients with these mutations have similar clinical features.⁵ Multifocal osteomyelitis caused by BCG or NTM in the absence of susceptibility to other infectious agents appears to be a characteristic feature of patients with either 818del4-related or 774del4-related IFN γ R1 deficiency.

The protein encoded by the 774del4 allele of *IFNGR1* was overexpressed on the cell surface. To characterise this truncated version of IFN γ R1, we cloned the WT allele and the 774del4 and 818del4 alleles of *IFNGR1* into a mammalian expression vector. Cycloheximide treatment enabled us to assess the stability of IFN γ R1 expression by flow cytometry and Western blotting. In contrast to the WT protein, which disappeared rapidly even after cycloheximide treatment, the 774del4 and 818del4 mutant proteins were stably retained on the cell surface. These

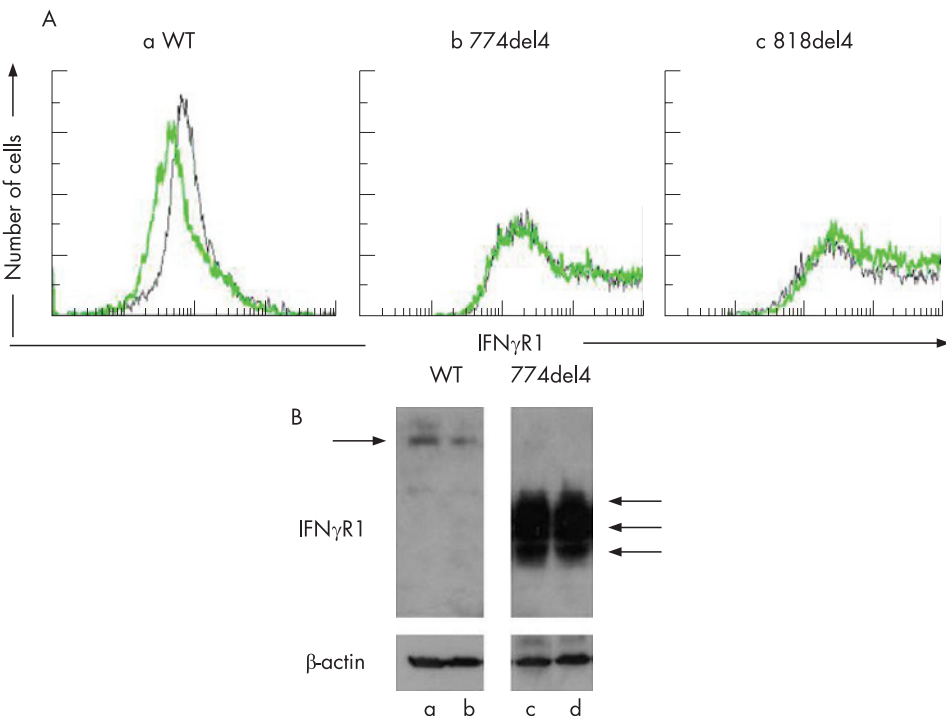


Figure 5 Stability of interferon γ receptor 1 (IFN γ R1) in HEK 293 cells transfected with the wild-type (WT), 774del4 or 818del4 forms of *IFNGR1*. The WT, 774del4 and 818del4 *IFNGR1* cDNAs were introduced into the HEK 293 using the calcium phosphate method. At 24 hours after transfection, the cells were treated with 20 μ g/ml cycloheximide for 3 hours. The expression levels of IFN γ R1 were analysed by flow cytometry and Western blotting using monoclonal mouse antibodies against human IFN γ R1. (A) IFN γ R1 analysed by flow cytometry. (a) WT; (b) 774del4; (c) 818del4; solid line, cycloheximide-treated cells; dotted line, untreated cells. (B) The lysates of cells transfected with WT and 774del4 IFN γ R1 were analysed by Western blotting. The cells were treated with CHX (a, c) or untreated (b, d).

observations suggest that both the 774del4 and 818del4 mutant forms of IFN γ R1 are overexpressed on the cell surface, due to impaired receptor degradation. The intracellular domain of IFN γ R1 contains two regions that are important for the receptor's function: the YDKPH sequence (residues 440–444), which is located near the C-terminus of IFN γ R1 and is required for IFN γ -dependent cellular responses through STAT1 binding,

and the membrane proximal region (residues 256–303), which is required for both receptor-mediated ligand internalisation and subsequent degradation, and for the induction of the biological response.^{24, 25} The importance of these regions is supported by radioligand binding analysis using both ¹²⁵I-labelled recombinant IFN γ and a series of IFN γ R1 deletion mutants lacking the cytoplasmic region.²⁶ Without this region,

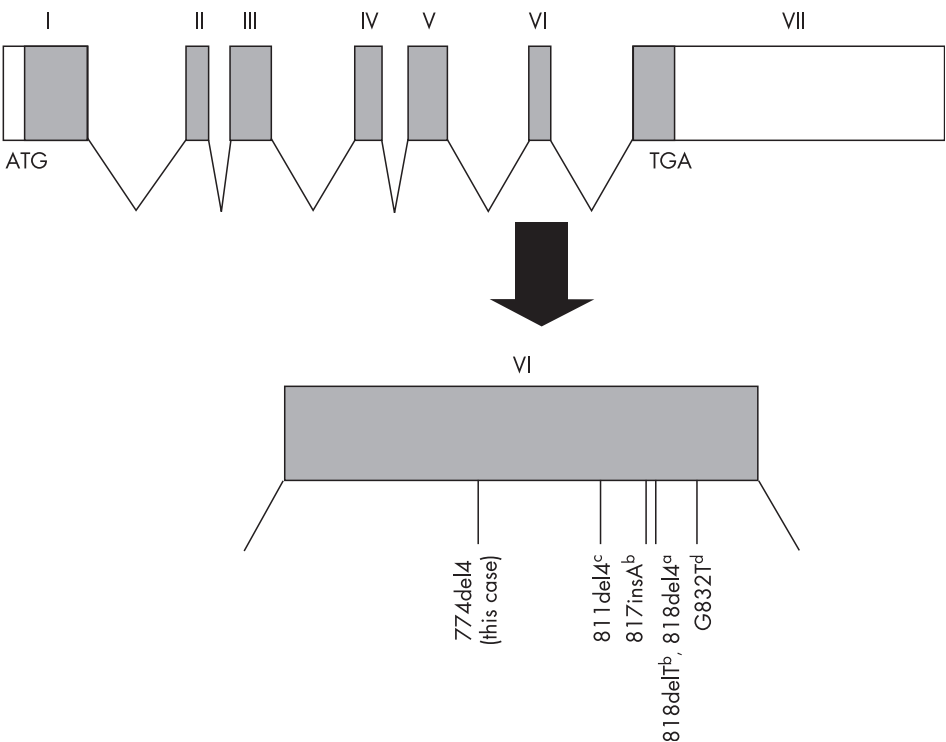


Figure 6 Summary of mutations in *IFNGR1* associated with dominant forms of IFN γ R1 deficiency. The primary genomic structure of *IFNGR1*, including its seven exons, is shown, and all of the mutations previously shown to be associated with dominant forms of IFN γ R1 deficiency are indicated. Specifically, the 774del4 mutation described in this report is depicted, along with the alleles described by Jouvanguy *et al.*^a, Dorman *et al.*^b, Sasaki *et al.*^c, and Villella *et al.*^d.^{4, 12–15} All of the mutations are localised in exon 6. Unfilled boxes, non-coding regions; filled boxes, *IFNGR1* coding region.

both the internalisation and degradation of IFN γ is impaired, and it accumulates at the cell surface.¹⁸ Further analysis has revealed that a leucine-isoleucine motif (residues 270–271) near this region also plays an important role in receptor-mediated ligand internalisation/degradation and that the LPKS sequence (residues 266–269) is required for biological responses through JAK-1 binding.^{24–27, 28}

Jouanguy *et al.*⁴ have suggested that the 818del4 mutation impairs the recycling of IFN γ R1 because of the lack of the membrane proximal region, which results in cell-surface overexpression. This mutant receptor, which lacks both the LPKS and YDKPH motifs, is thought to impair IFN γ signalling via a dominant-negative effect; however, this assumption has not yet been examined at the molecular level. In this study, we examined the molecular mechanisms through which the 774del4 and 818del4 mutants exert dominant-negative effects on IFN γ signalling. In general, an mRNA with a premature stop codon tends to be rapidly degraded by the nonsense-mediated mRNA decay pathway.²⁹ However, both WT and mutant cDNAs were detected at similar ratios in the total RNA extracted from the peripheral blood of the patient, who carries both the WT and 774del4 mutant alleles (data not shown). Furthermore, we directly showed that the 774del4 and 818del4 mutant versions of IFN γ R1 were stably overexpressed on the cell surface owing to alteration of receptor stability. Additionally, the CD14-positive cells from the patient showed a weaker response to IFN γ than did those from the healthy controls. Similarly, STAT1 phosphorylation in response to IFN γ was significantly impaired in cells derived from the patient. Together, these observations suggest that the 774del4 version of IFN γ R1 is non-functional and has a dominant-negative effect on IFN γ signal transduction. To our knowledge, all of the mutations identified to date that cause dominant forms of IFN γ R1 deficiency are localised in exon 6 of *IFNGR1* and lead to a premature stop in the intracellular domain (figure 6).

IFN γ R1 deficiency is a primary immunodeficiency characterised by severe mycobacterial infections. Multifocal osteomyelitis without other organ involvement is an outstanding clinical feature of individuals with this deficiency.⁵ Several patients with IFN γ R1 deficiency who developed multiple osteomyelitis were initially misdiagnosed as having accompanying Langerhans cell histiocytosis and were treated with cytotoxic chemotherapy.³⁰ Of 50 previously reported patients with dominant forms of IFN γ R1 deficiency, most were found to have the 818del4 mutation.^{4–11} Sasaki *et al.* reported four Japanese patients with dominant forms of *IFNGR1* deficiency from three unrelated families.¹³ One of these patients was heterozygous for the 811del4 mutation, and the other three were heterozygous for 818del4. The novel 774del4 mutation described in this study is the fifth allele shown to cause a dominant form of *IFNGR1* deficiency in Japan. Given that adjunctive therapeutic administration of IFN γ is beneficial for patients with dominant forms of IFN γ R1 deficiency, more attention should be paid to this deficiency, especially when treating patients with recurrent mycobacterial infections.



Supplementary figure available on JMG website—<http://jmg.bmj.com/supplemental>

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Competing interests: None declared.

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Correction

Okada S, Ishikawa N, Shirao K, Kawaguchi H, Tsumura M, Ohno Y, Yasunaga S, Ohtsubo M, Takiyama Y, Kobayashi M. The novel *IFNGR1* mutation 774del4 produces a truncated form of interferon- γ receptor 1 and has a dominant-negative effect on interferon- γ signal transduction. *J Med Genet* 2007;**44**:485–91.

The authors apologise for an error in the legend of figure 5. The last sentence should read: “The cells were treated with CHX (b, d) or untreated (a, c).”

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