Missense splice variant (g.20746A>G, p.Ile183Val) of interferon gamma receptor 1 (*IFNGR1*) coincidental with mycobacterial osteomyelitis - a screen of osteoarticular lesions

Agnieszka Bińczak-Kuleta[1], Aleksander Szwed[2], Mark R. Walter[3], Maciej Kołban[2], Andrzej Ciechanowicz[1], Jeremy S. C. Clark[1*]

[1]Department of Clinical & Molecular Biochemistry, Pomeranian Medical University in Szczecin, Szczecin, Poland, [2]Department of Orthopedics & Child Traumatology, Pomeranian Medical University in Szczecin, Szczecin, Poland, [3]Department of Microbiology, Bevill Biomedical Research Building, University of Alabama at Birmingham, Birmingham, AL, USA

ABSTRACT

Previously, dominant partial interferon-gamma receptor 1 (IFN-γ-R1) susceptibility to environmental mycobacteria was found with *IFNGR1* deletions or premature stop. Our aim was to search for *IFNGR1* variants in patients with mycobacterial osteoarticular lesions. Biopsies from the patients were examined for acid-fast bacilli, inflammatory cell infiltration, and mycobacterial niacin. Mycobacterial rRNA was analyzed using a target-amplified rRNA probe test. Peripheral-blood-leukocyte genomic DNA was isolated from 19 patients using the QIAamp DNA Mini Kit, and all *IFNGR1* exons were sequenced using an ABIPRISM 3130 device. After the discovery of an exon 5 variant, a Polish newborn population sample (n = 100) was assayed for the discovered variant. Splice sites and putative amino acid interactions were analyzed. All patients tested were positive for mycobacteria; one was heterozygous for the *IFNGR1* exon 5 single-nucleotide-missense substitution (g.20746A>G, p.Ile183Val). No other variant was found. The splice analysis indicated the creation of an exonic splicing silencer, and alternatively, molecular graphics indicated that the p.Ile183Val might alter beta-strand packing (loss of van der Waals contacts; Val183/Pro205), possibly altering the IFN-γ-R1/IFN-γ-R2 interaction. The probability of non-deleterious variant was estimated as <10%. Heterozygous *IFNGR1*:p.Ile183Val (frequency 0.003%) was found to be coincidental with mycobacterial osteomyelitis. The small amount of variation detected in the patients with osteoarticular lesions indicates that screens should not yet be restricted: Intronic variants should be analyzed as well as the other genes affecting Type 1 T-helper-cell-mediated immunity.

KEY WORDS: Bacilli strain Calmette-Guerin; environmental mycobacteria; interferon gamma receptor 1; Mendelian susceptibility to mycobacterial disease; *Mycobacterium bovis* bacillus Calmette-Guérin; osteoarticular lesions
DOI: http://dx.doi.org/10.17305/bjbms.2016.1232 *Bosn J Basic Med Sci. 2016;16(3):215-221. © 2016 ABMSFBIH*

INTRODUCTION

Susceptibilities to mycobacterial osteoarticular infections are thought to result from disorders in Type 1 T-helper-cell-mediated immune inflammation, resulting in deficient production and/or action of interleukin 12 (IL12) and interferon gamma (IFN-γ) [1]. Genetic disorders associated with inborn errors in IFN-γ-dependent immunity include those which impair production of IFN-γ (*IL12B*, *IL12RB1*, *IRF8*, *ISG15*, *NEMO*) and those which impair response to IFN-γ (*IFNGR1*, *IFNGR2*, *STAT1*, *IRF8*, *CYBB*) [2]. Note that, perhaps "Familial" (rather than "Mendelian") "susceptibility to mycobacterial disease" might be a more appropriate term to describe many of these disorders, as "most of these inborn errors do not show complete clinical penetrance for the case-definition phenotype of Mendelian susceptibility to mycobacterial diseases" [2].

Variants in *IFNGR1* often result in susceptibility to environmental mycobacteria (e.g., *Mycobacterium avium*; immunodeficiency 27a), *M. bovis* bacilli strain Calmette-Guerin (BCG), as well as to *Helicobacter pylori* [3], *Salmonella* (~5%), *Listeria*, herpes virus, cytomegalovirus, and *Histoplasma* [4]. Further, diseases/infections associated with missense variants in *IFNGR1* include Epstein-Barr virus infection; disseminated

*Corresponding author: Jeremy S. C. Clark, Department of Clinical & Molecular Biochemistry, Pomeranian Medical University in Szczecin, ul. Powstancow Wlkp. 72, 70-111 Szczecin, Poland.
Fax: +004891 4661492, Tel.: 004891 4661490,
E-mail: jeremyclarkbio@gmail.com

Submitted: 24 March 2016/Accepted: 05 May 2016

M. tuberculosis infection (p.Gly219Arg [5]); allergies [6], dermatitis, and lymphadenitis; and variants of *IFNGR1* are thought to be associated with susceptibility to *Schistosoma mansori* and *M. tuberculosis* [7]. Promoter variants have been associated with atopic cataracts, protection against cerebral malaria [8], protection from tuberculosis [9], and with the clinical outcome of HBV infection in Chinese adults [10].

The signaling complex which binds IFN-γ is formed from a dimer of IFN-γ-receptor 1 (IFN-γ-R1) which then binds two IFN-γ-R2 molecules (note that the official short names for these proteins, P15260 and P38484, respectively, both have two hyphens according to UniProtKB, e.g., http://www.uniprot.org/uniprot/P15260); IFN-γ-R1 also interacts with JAK1, JAK2, and STAT1 (minimum evidence level 2 [11]). On binding to the signaling complex, IFN-γ induces antiviral activity, increases expression of the class II major histocompatibility complex, and causes B cell maturation and release of mediators of inflammation ([12]; IFN-γ is ubiquitously expressed and highly expressed in plasma/pancreas). IFN-γ itself is highly conserved: No variants of IFN-γ have ever been found [13]. It is, therefore, perhaps not surprising that the receptor gene *IFNGR1* is also highly conserved, illustrated by the fact that only one missense polymorphism (defined as having a frequency >1% worldwide; p.Leu467Pro; frequency 5%) was found by the Exome Aggregation Consortium (ExAC) in 60,706 unrelated individuals [14]. In this study, three missense low-frequency (defined as frequency <1%) variants were found with world frequencies 0.1-1%: p.Val14Met, p.Gly180Arg, and p.His335Pro; and nine with 0.01-0.1%: p.Met11Leu, p.Thr31Ile, p.Val46Ile, p.Val61Ile, p.Asn79Ser, p.Pro148Arg, p.Glu197Lys, p.Thr189Lys, and p.Pro431Leu. The variant subject of this paper, p.Ile183Val, was found at 0.003%, only in the European (non-Finnish) population.

Tolerance to missense variants with frequencies <1% should be questioned, even if predicted to be "benign" or "tolerated" *in silico*, because of the likelihood of negative selection maintaining low frequency. Note that even the polymorphism p.Leu467Pro (which is probably a marker for another frequent causal variant [15]) is associated with allergies [6] and *Helicobacter* susceptibility [3] (p.Leu467Pro is listed in this reference as Leu450Pro, i.e., minus the signal peptide).

Two of the three missense low-frequency variants found with the highest frequency >0.1% have been found to be associated with deleterious effects: p.His335Pro and p.Val14Met. Although the p.His335Pro transcript assessment by ExAC shows polyphen output "benign" ("most likely lacking any phenotypic effect") and SIFT output "tolerated" ("not deleterious"), p.His335Pro is associated with susceptibility to *H. pylori* infection [3]. p.Val14Met (polyphen: "Possibly-damaging," SIFT: "Tolerated") is associated with the greatest risk for development of systemic lupus erythematosus in the Japanese population in heterozygous state [12]. From those with a frequency 0.1-0.01%, p.Val61Glu was found in a compound heterozygote together with a deletion, resulting in recessive complete susceptibility to mycobacterial infection ([4]; note here, this variant was incorrectly described as p.Val61Ile and corrected in the Leiden Open Variation Database [LOVD] to p.Val61Glu).

The LOVD (www.lovd.nl/IFNGR1; Fokkema et al. [16]) listed 9 missense low-frequency variants of *IFNGR1* associated with disease (accessed November 2015; Table 1). All of these were associated with autosomal recessive immunodeficiency-27A, with susceptibility to mycobacteria (most often to *M. avium*).

IFNGR1 variants associated with mycobacterial infections were extensively studied by Dorman et al. [4] using a large cohort of patients worldwide, and clinical features of recessive and dominant *IFNGR1* disorders were assessed. All dominant disorders found were associated with either c.819_822del, 818delT, or 832G>T (p.Glu278*); all producing truncated IFN-γ-R1 proteins. Most (79%) of the dominant partial patients had mycobacterial infections; one-third had osteomyelitis with no other organs apparently affected.

The aim of our study was to search for *IFNGR1* variants by sequencing all exons, in a cohort of Polish patients with osteoarticular lesions.

MATERIALS AND METHODS

Patients

From child patients hospitalized at the Clinic of Orthopedics and Child Traumatology at the Pomeranian Medical University in Szczecin, Poland, between 2005 and 2010, 20 consecutive patients (11 girls; 9 boys; mean admission age 12 years; age range 7-17 years) were diagnosed with osteoarticular lesions and enrolled at a children's clinic. Written informed consent was obtained from all legal guardians. The study protocol and DNA screen were approved by the Pomeranian Medical University Bioethics Committee in compliance with the Helsinki Declaration (2013 revision). Note that all patients had been inoculated using the BCG vaccine before age one.

Methods

Biopsies (open or needle biopsies from bone or arthroscopic synovial biopsies) were examined by a histopathologist for inflammatory cell infiltration, acid-fast bacilli, and with the Niacin test. A target-amplified mycobacterial rRNA probe test (Amplified MTD Test, Hologic, Wiesbaden, Germany) was used. One patient was omitted from the sequencing study, but at a later date, mycolic acid

TABLE 1. Deleterious (and putative deleterious) missense variants of *IFNGR1* with estimated frequencies and association with disease

Variant	Allele frequency (%) [14]		Mode and disease association	Source
	World	European (non-Finnish)		
p.Leu467Pro	5.2	0.21	Dominant: Allergies	[6]
			Dominant: *Helicobacter* susceptibility	[3]
			Dominant: Risk for systemic lupus erythematosus	[12]
p.Val14Met	0.18	0.0038	Recessive: mycobacteria	[4]
			Dominant: Atopic dermatitis+eczema herpeticum	[7]
p.His335Pro	0.13	0.0030	Dominant: Helicobacter susceptibility	[18]
p.Val61Glu	0.092	0.012	Compound heterozygote: Mycobacteria	[4]
p.Ile183Val	0.0033	0.0061	Dominant: Mycobacteria	This article
p.Ile87Thr	0.0017	<0.0015	Recessive: Mycobacteria	[8]
p.Ile352Met	0.0016	0.0030	Recessive: Mycobacteria	[9]
p.Ser485Phe	0.00083	0.0015	Recessive: Mycobacteria	[16]
p.Tyr397Cys	<0.0008	<0.0015	Dominant: Atopic dermatitis+eczema herpeticum	[7]
p.Gly219Arg	<0.0008	<0.0015	Recessive: Mycobacteria, EBV, *M. tuberculosis*	[5]
p.Met1Lys	<0.0008	<0.0015	Recessive: Mycobacteria	[28]
p.Val61Glu	<0.0008	<0.0015	Recessive: Mycobacteria	[16]
p.Val63Gly	<0.0008	<0.0015	Recessive: Mycobacteria	[16]
p.Tyr66Cys	<0.0008	<0.0015	Recessive: Mycobacteria	[16]
p.Cys71Tyr	<0.0008	<0.0015	Recessive: Mycobacteria	[29]
p.Cys77Tyr	<0.0008	<0.0015	Recessive: Mycobacteria	[16]
p.Cys77Phe	<0.0008	<0.0015	Recessive: Mycobacteria	[16]
p.Cys85Tyr	<0.0008	<0.0015	Recessive: Mycobacteria	[16]
p.Ser149Leu	<0.0008	<0.0015	Recessive: Mycobacteria	[15]

Note that association does not imply causality (as several, e.g., p.Leu467Pro) are only markers for putative unknown linked variants. *IFNGR1*: Interferon gamma receptor 1

high-performance liquid chromatography was used to identify *M. fortuitum* in this patient. All patients received multiple drug anti-mycobacterial treatment with good result (mean observation time: 4 years).

Peripheral-blood-leukocyte genomic DNA from 19 patients was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Using primer selection software (Lasergene v8.0, DNASTAR, Madison, Wisconsin, USA), primers were designed for all exons of *IFNGR1*:GenBank: NG_007394 (chromosome 6q23); sequencing was carried out using ABIPRISM 3130, Sequencing Analysis Software v5.4 (Applied Biosystems, Life Technologies Polska, Warsaw, Poland).

Following the discovery of an exon 5 variant, a population group (100 Polish newborns randomly chosen from the Newborn DNA Repository at the Department of Clinical and Molecular Biochemistry at the Pomeranian Medical University in Szczecin [17]) was screened by sequencing exon 5.

Database search included the ExAC Browser [18]; Leiden Open Variation Database (www.lovd.nl/IFNGR1; [16]); UCSC Genome Browser [19]; Seattle SNPs [20]; NCBI ClinVar database [21]; and ENSEMBL [22]. Probability of finding a non-deleterious missense variant (n = 87), i.e., not including deletions, frameshifts, stop codons or intronic splice region variants, at random in a European (non-Finnish) sample was estimated from ExAC Browser data by 1 - ((product (1 - *frequency of each variant*)) ^ 38) for 38 haploid genotypes (i.e., 19 patients).

An open-source molecular graphics system (Pymol, version 1.3rl [23]) was used to visualize amino acid interactions. Polyphen and SIFT designations for the variant found were taken from ENSEMBL. Splice variant analysis used the Human Splicing Finder [24].

RESULTS

All patients screened (n = 20) suffered from osteoarticular pathologies (Table 2). For all patients the analysis of biopsy specimens using the target-amplified nucleic acid probe test detected mycobacterial rRNA, indicating species from the *M. tuberculosis* complex (i.e., either *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. microti*, *M. canetti*, or *M. pinnipedii*). One patient (number 1, Table 2) tested positive with niacin and acid-fast bacilli tests, suggesting *M. tuberculosis* itself. The histopathological examination for acid-fast bacilli or inflammatory cell infiltration gave only several positive results (Table 2), indicating perhaps that in most patients mycobacterial infections were latent.

The analysis of sequencing chromatograms (n = 19) identified the missense low-frequency variant *IFNGR1*: GenBank: NG_007394:g.20746A>G (corresponding to GenBank: NM_000416.2:c.547A>G; dbSNP: rs752756474) in exon 5 from one allele (male; patient number 12; admission age 17 years; Figure 1). This patient had: The right 2nd metatarsal bone inflamed (Figure 2); no other inflammatory lesions;

TABLE 2. Characteristics and treatment of the patients with osteoarticular lesions whose *IFNGR1* exons were sequenced

Age (years)	Patient number	Gender	Osteoarticular lesion site	Treatment	Biopsy analyses			
					rRNA	AF	HP	N
7	8	M	Spine L5, endometrial inflammation	Cons., surg.	+	–	–	–
7	10	F	Knee joint synovial hypertrophy	Cons.	+	–	+	–
9	3	M	Femur	Cons., surg.	+	+	–	–
11	7	F	Knee joint synovial hypertrophy	Cons.	+	–	–	–
11	9	M	Foot-soft tissue tumor	Cons., surg.	+	+	–	–
11	13	F	Active chronic inflammation of clavicle proximal end	Cons.	+	–	+	–
11	17	F	Tibia stem and epiphyses	Cons.	+	–	–	–
12	4	F	Spine L3/L4, endometrial inflammation	Cons., surg.	+	–	–	–
12	5	M	Tibia stem and epiphyses	Cons., surg.	+	–	–	–
12	6	M	Spine L4, intervertebral disc	Cons., surg.	+	–	–	–
12	18	F	Tibia stem and epiphyses	Cons., surg.	+	+	–	–
13	14	M	Ankle	Cons.	+	+	+	–
13	1	F	Active chronic inflammation of clavicle proximal end	Cons.	+	+	–	+
14	19	M	Tibia stem and epiphyses	Cons.	+	–	–	–
15	11	M	Ankle	Cons.	+	–	–	–
15	15	F	Tibia stem and epiphyses	Cons., surg.	+	–	–	–
16	16	F	Knee joint synovial hypertrophy	Cons.	+	–	+	–
17	2	F	Tibia stem and epiphyses	Cons., surg.	+	+	–	–
17	12*	M	Osteosclerotic metatarsal bone tumor	Cons.	+	–	–	–

Age (years) is age at first admission. *Patient 12: Variant *IFNGR1*:c.547A>G identified by sequencing. M: Male; F: Female; Cons.: Conservative; Surg.: Surgical; rRNA: Mycobacterial rRNA test (Hologic amplified MTD); AF: Microscopic test for presence of acid-fast bacilli; HP: Histopathological examination for inflammatory cell infiltration; N: Niacin test; +: Positive result; –: Negative result; *IFNGR1*: Interferon gamma receptor 1

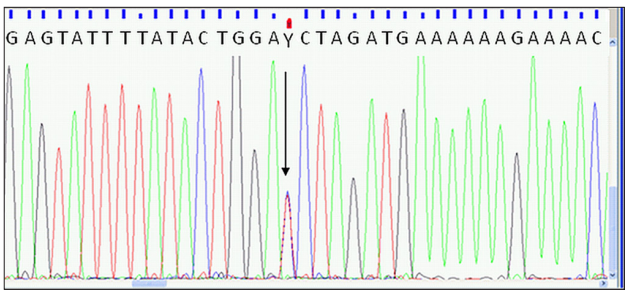

FIGURE 1. Chromatogram showing the interferon gamma receptor 1 (*IFNGR1*):p.Ile183Val low-frequency variant (antisense sequence of part of exon 5). Arrow indicates the NG_007394:g20746A>G (p.Ile183Val) variant.

no apparent autoimmune disease; was well-nourished; pain increased over 6 months with diagnosis of a fracture.

The low-frequency variant gave ATC>GTC (p.Ile183Val) at codon 183. No other variants were found in the patients or in the population group subsequently analyzed for this low-frequency variant.

This low-frequency variant was previously found in two European (non-Finnish) people from worldwide exome analysis of ~61,000 unrelated individuals [18], i.e., at 0.003% frequency. *IFNGR1* is highly conserved, and it is possible to estimate the probability of finding a non-deleterious variant at random from the *IFNGR1* entry in the ExAC browser [18]. Note that this analysis does not include other categories of mutation, e.g., intronic splice variants, frameshifts, stop codons, etc., and that no other missense exonic splice variants have been found. This estimate for finding any European (non-Finnish), non-deleterious, missense exonic variant (n = 87) at random in 19 patients was 0.095. However, we know that it is likely that many variants are held at low frequencies by negative selection (it is also likely that so-far undetected variants also have very low frequency), and many have not been investigated sufficiently to confirm the lack of disease association, and this is therefore likely an overestimate. We, therefore, submit that the p.Ile183Val low-frequency variant, with 0.003% frequency, is here associated with mycobacterial disease.

Polyphen and SIFT analyses for this variant gave "tolerated" and "benign" respectively (ENSEMBL). However, molecular graphics (Pymol [23]; Figure 3) indicated that, although structural changes would appear to be minor, p.Ile183Val could alter beta-strand packing due to loss of van der Waals contacts between Val183 and Pro205. It should also be noted that the variant is found at the first nucleotide of exon 5, and is, therefore, the only missense exonic splice variant to have been identified. Analysis via the Human Splicing Finder indicated that the variant alters an exonic splicing enhancer and creates an exonic splicing silencer, thereby potentially altering splicing - perhaps more likely for a possible deleterious effect.

DISCUSSION

Our study has identified a new low-frequency variant *IFNGR1*:c.547A>G (p.Ile183Val) found with mycobacterial osteomyelitis. The infective agent was very likely environmental mycobacteria or possibly *M. bovis* BCG (from tuberculosis vaccination).

While it was not possible to eliminate the possibility of mere coincidence of a non-deleterious variant with this disease, the chances of this are low (<10%), and note that, the

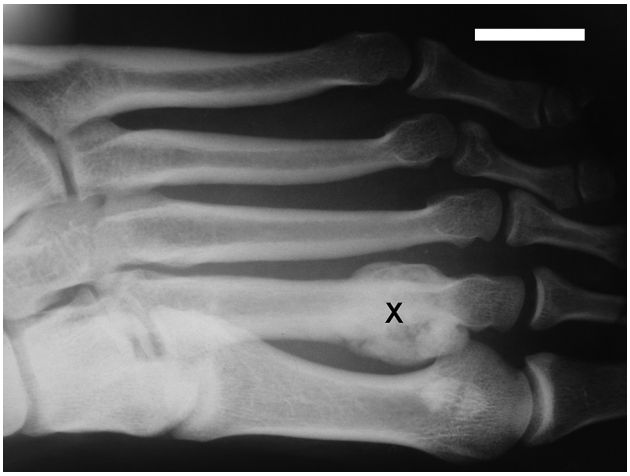

FIGURE 2. Mycobacterial lesion. Right foot showing inflammatory lesion (X) of the 2nd metatarsal bone of the patient 12 (in which the low-frequency variant interferon gamma receptor 1 (*IFNGR1*):p.Ile183Val was identified). White bar length = 2.5 cm.

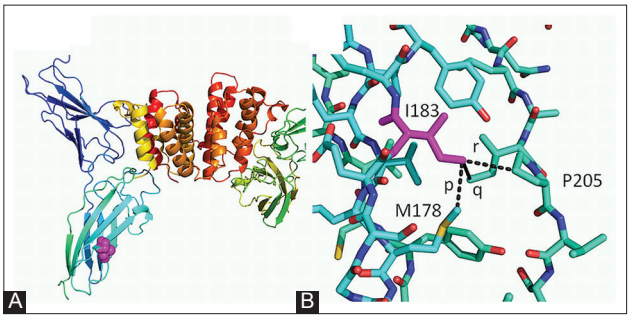

FIGURE 3. Molecular interactions at site of interferon gamma receptor 1 (*IFNGR1*):p.Ile183Val low-frequency variant. (A) Ribbon diagram of the IFN-γ/IFN-γ-R1 complex. Ile183 is colored magenta; positioned near the putative binding site of the IFN-γ-R2 chain, (B) Close-up view of residues surrounding Ile183 in the crystal structure of IFN-γ-R1. The Ile183Val low-frequency variant could alter the packing of the beta-strands due to loss of van der Waals contacts between Val183 and Pro205 (black lines:p: 0.31 nm; q: 0.55 nm; r: 0.34 nm), possibly altering interaction with IFN-γ-R2.

low-frequency variant detected is very rare (with 0.003% world frequency) and might be deleterious. We therefore feel that, together with the creation of an exonic splicing silencer (or the loss of van der Waals contacts) and with the patient characteristics, this result should be presented to the scientific community. This is the first case of a non-synonymous exonic splice variant to be found, and would be the first case in which a missense low-frequency variant (rather than a deletion/premature stop codon) is associated with dominant susceptibility to mycobacterial osteomyelitis.

The limitations of the study were as follows:

1. Biochemical data were not, unfortunately, obtained which could have provided evidence that disruption in IFN-γ-R1 action was the cause of susceptibility to mycobacteria.
2. Only one case was found, in which the *IFNGR1*:c.547A>G variant was associated with mycobacterial susceptibility.
3. The screen of the patients with osteoarticular lesions did not include adults (only children were enrolled at the clinic).

It is of some significance that our study, with all exons of *IFNGR1* sequenced, did not find any other low-frequency variant previously associated with mycobacterial infection. A similar study in 93 South Korean patients [25] also did not identify previously reported low-frequency variants in *IL12RB1* and *IFNGR1*, found with mycobacterial infection, despite sequencing all exons (although further changes: Three polymorphisms, one silent and two missense low-frequency variants were identified). As mentioned in the introduction, this indicates that further genes involved in the production or response to IFN-γ should be analyzed, and that, although the study of *IFNGR1* and mycobacterial infection by Dorman et al. [4] was very comprehensive, it was not complete.

It is, therefore, of some importance that screening groups do not narrow the search for *IFNGR1* low-frequency variants prematurely where cost allows (e.g. Quispel et al. [26] only studied exons 2, 3, and 6). A similar observation was made by Fraser et al. [7], who suggested that sequencing all exons and introns of *IFNGR1* might identify previously "unidentified allelic variations in the *IFNGR1* gene (that) might elevate or decrease the risk in this ethnic population (Croatian), as a part of the multigenic predisposition to tuberculosis." (Also note that, IFN-γ-R1 has at least 11 splice variants and thorough analyses of these, and the consequences of variation, has yet to be achieved, although it is thought that only one splice variant predominates).

Dorman et al. [4] identified 38 patients worldwide with a dominant partial deficiency in IFN-γ-R1, 22 patients with recessive complete IFN-γ-R1 deficiency, and 2 patients with recessive partial deficiency. The mean age of those with dominant partial deficiency was 13.4 years, and 32% of patients had mycobacterial infection of bone with no other organs affected (as in our patient number 12 with p.Ile183Val; although note the median number of bone lesions was five, whereas the patient 12 only had one bone lesion). With dominant partial deficiency, the penetrance by environmental mycobacterial disease was 45% by age 10 years in the Dorman et al. [4] study. Of importance here is that, in this study, all dominant partial cases were heterozygous for *IFNGR1* low-frequency variants c.819_822del, 818delT, or 832G>T - all producing premature translation stop, resulting in IFN-γ-R1 proteins truncated in the extracellular domain; lacking intracellular domains, JAK1, and STAT1 binding sites. In addition, lack of a recycling motif leads to the accumulation of non-functional IFN-γ-R1 proteins (at ~10x normal levels) on the cell membrane, which bind IFN-γ to the same extent as reference IFN-γ-R1 proteins [13,26] but do not transmit a signal. This is thought to result in impedance

of reference proteins produced by the other allele (especially as inactive dimers of affected plus reference proteins will be formed), giving diminished cellular responsiveness to IFN-γ, i.e. dominant partial deficiency results from a dominant negative effect rather than from decrease in gene dosage, and only very high administered concentrations of IFN-γ can compensate for this deficiency.

In dominant partial deficiency very low plasma levels of IFN-γ are found, as in healthy patients, probably indicating sufficient clearance via (lower) levels of receptor binding. In dominant partial deficiencies, IFN-γ does not accumulate to the high levels found in recessive complete deficiency, where there is impaired clearance together with lack of receptor binding [27]. In the latter cases, severe infection with mycobacterial diseases often occurs [13], with a familial preponderance for high IFN-γ levels [27], both of which are not common with dominant partial deficiencies.

Note that in our case, the affected amino acid (183) is found in the extracellular domain and the p.Ile183Val low-frequency variant could alter beta-strand packing due to loss of van der Waals contacts between Val183 and Pro205, altering interactions with IFN-γ-R2 (but this idea would have to be validated experimentally). It would be interesting to now carry out an experiment similar to that by van de Wetering et al. [15], in which the IFN-γ-responsive cell line THP-1 was transduced with *IFNGR1* gene variant p.Tyr66Cys (previously a IFN-γ-R1 deficient cell line transduced with *IFNGR1*:p.Tyr66Cys had given no STAT1 phosphorylation). This resulted in the expression of p.Tyr66Cys at the cell surface but no hampering of action of the reference IFN-γ-R1 with no reduction in response to IFN-γ (with a dominant p.Ile183Val low-frequency variant this would be expected to reduce response to IFN-γ).

In contrast to recessive complete forms, dominant partial deficiency is seldom fatal [27] and can usually be treated, as in our case. Fieschi et al. [27] recommended that, along with genetic counseling, "undetectable or low levels of IFN-γ should lead to the child being treated with subcutaneous IFN-γ while searching for mild low-frequency variants of *IFNGR1* and *IFNGR2*, or null low-frequency variants of *IL12B* and *IL12RB1*". Patients with such low-frequency variants should not be vaccinated with the *M. bovis* BCG vaccine.

It remains to be seen whether the low-frequency variant p.Ile183Val affects IFN-γ-R1 function (and whether/how this low-frequency variant affects the various IFN-γ-R1 splice variants). The cause of mycobacterial osteoarticular infection in most patients in this study could not be explained by exonic low-frequency variants of *IFNGR1*, and therefore, the other genes mentioned earlier should also be analyzed in order to evaluate further mechanisms for susceptibility to mycobacterial osteoarticular infections.

CONCLUSION

1. Coincidence of heterozygous low frequency (0.003%) variant *IFNGR1*:g.20746A>G (p.Ile183Val) was found with susceptibility to mycobacterial osteomyelitis. This is the first missense exonic splice variant to be found and also is the first time that mycobacterial osteomyelitis has been found together with an *IFNGR1* missense variant in dominant mode. The probability of detecting a non-deleterious variant was estimated at <10%.
2. Lack of other low-frequency variants found with all exons of *IFNGR1* sequenced, in the screen of patients with osteoarticular lesions, indicates that screening should not yet be restricted to those *IFNGR1* exons, in which deleterious low-frequency variants have so far been found; plus other genes involved in Type 1 T-helper-cell-mediated immune inflammation should also be analyzed.

ACKNOWLEDGMENTS

This study was supported by Research Grant No. N N403 583338 from the Polish Ministry of Science and Higher Education. This funding body had no role in design, in the collection, analysis, and interpretation of data; in the writing of the manuscript and in the decision to submit the manuscript for publication.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

REFERENCES

[1] Cottle LE. Mendelian susceptibility to mycobacterial disease. Clin Genet 2011;79(1):17-22. http://dx.doi.org/10.1111/j.1399-0004.2010.01510.x.

[2] Bustamante J, Boisson-Dupuis S, Abel L, Casanova JL. Mendelian susceptibility to mycobacterial disease: Genetic, immunological, and clinical features of inborn errors of IFN-γ immunity. Semin Immunol 2014;26(6):454-70. http://dx.doi.org/10.1016/j.smim.2014.09.008.

[3] Thye T, Burchard GD, Nilius M, Müller-Myhsok B, Horstmann RD. Genomewide linkage analysis identifies polymorphism in the human interferon-gamma receptor affecting *Helicobacter pylori* infection. Am J Hum Genet 2003;72(2):448-53. http://dx.doi.org/10.1086/367714.

[4] Dorman SE, Picard C, Lammas D, Heyne K, van Dissel JT, Baretto R, et al. Clinical features of dominant and recessive interferon gamma receptor 1 deficiencies. Lancet 2004;364(9451):2113-21. http://dx.doi.org/10.1016/S0140-6736(04)17552-1.

[5] Tesi B, Sieni E, Neves C, Romano F, Cetica V, Cordeiro AI, et al. Hemophagocytic lymphohistiocytosis in 2 patients with underlying IFN-[gamma] receptor deficiency. J Allergy Clin Immunol 2015;135(6):1638. http://dx.doi.org/10.1016/j.jaci.2014.11.030.

[6] Aoki M, Matsui E, Kaneko H, Inoue R, Fukao T, Watanabe M, et al.

A novel single-nucleotide substitution, Leu 467 Pro, in the interferon-gamma receptor 1 gene associated with allergic diseases. Int J Mol Med 2003;12(2):185-91.
DOI: 10.3892/ijmm.12.2.185

[7] Fraser DA, Bulat-Kardum L, Knezevic J, Babarovic P, Matakovic-Mileusnic N, Dellacasagrande J, et al. Interferon-gamma receptor-1 gene polymorphism in tuberculosis patients from Croatia. Scand J Immunol 2003;57(5):480-4.
http://dx.doi.org/10.1046/j.1365-3083.2003.01253.x.

[8] Matsuda A, Ebihara N, Kumagai N, Fukuda K, Ebe K, Hirano K, et al. Genetic polymorphisms in the promoter of the interferon gamma receptor 1 gene are associated with atopic cataracts. Invest Ophthalmol Vis Sci 2007;48(2):583-9.
http://dx.doi.org/10.1167/iovs.06-0991.

[9] Cooke GS, Campbell SJ, Sillah J, Gustafson P, Bah B, Sirugo G, et al. Polymorphism within the interferon-γ/receptor complex is associated with pulmonary tuberculosis. Am J Respir Crit Care Med 2006;174(3):339-43.
http://dx.doi.org/10.1164/rccm.200601-088OC.

[10] Zhou J, Chen DQ, Poon VK, Zeng Y, Ng F, Lu L, et al. A regulatory polymorphism in interferon-γ receptor 1 promoter is associated with the susceptibility to chronic hepatitis B virus infection. Immunogenetic 2009;61(6):423-30.
http://dx.doi.org/10.1007/s00251-009-0377-8.

[11] Chatr-Aryamontri A, Breitkreutz BJ, Oughtred R, Boucher L, Heinicke S, Chen D, et al. The BioGRID interaction database: 2015 update. Nucleic Acids Res 2015;43:D470-8.
http://dx.doi.org/10.1093/nar/gku1204.

[12] Tanaka Y, Nakashima H, Hisano C, Kohsaka T, Nemoto Y, Niiro H, et al. Association of the interferon-γ receptor variant (Val14Met) with systemic lupus erythematosus. Immunogenetics 1999;49(4):266-71.
http://dx.doi.org/10.1007/s002510050492.

[13] Haverkamp MH, van de Vosse E, van Dissel JT. Nontuberculous mycobacterial infections in children with inborn errors of the immune system. J Infect 2014;68(Suppl 1):S134-50.
http://dx.doi.org/10.1016/j.jinf.2013.09.024.

[14] Stephens ZD, Lee SY, Faghri F, Campbell RH, Zhai C, Efron MJ, et al. Big data: Astronomical or genomical? PLoS Biol 2015;13(7):e1002195.
http://dx.doi.org/10.1371/journal.pbio.1002195.

[15] van de Wetering D, de Paus RA, van Dissel JT, van de Vosse E. Functional analysis of naturally occurring amino acid substitutions in human IFN-γammaR1. Mol Immunol 2010;47(5):1023-30.
http://dx.doi.org/10.1016/j.molimm.2009.11.016.

[16] Fokkema IF, Taschner PE, Schaafsma GC, Celli J, Laros JF, den Dunnen JT. LOVD v.2.0: the next generation in gene variant databases. Hum Mutat 2011;32(5):557-63.
http://dx.doi.org/10.1002/humu.21438.

[17] Loniewska B, Clark JS, Kaczmarczyk M, Adler G, Bińczak-Kuleta A, Kordek A, et al. Possible counter effect in newborns of 1936A>G (I646V) polymorphism in the AKAP10 gene encoding A-kinase-anchoring protein 10. J Perinatol 2012;32(3):230-4.
http://dx.doi.org/10.1038/jp.2011.85.

[18] UniProt Consortium. UniProt: A hub for protein information. Nucleic Acids Res 2015;43:D204-12.
DOI: 10.1093/nar/gku989.

[19] Karolchik D, Barber GP, Casper J, Clawson H, Cline MS, Diekhans M, et al. The UCSC genome browser database: 2014 update. Nucleic Acids Res 2014;42:D764-70.
DOI: 10.1093/nar/gkt1168.

[20] SNPs S. NHLBI Program for Genomic Applications. Vol. 564. Seattle, WA: UW-FHCRC; 2007. p. 565-6.
Available from: http://www.pga.gs.washington.edu. [Last accessed on 31 Aug 2015].

[21] Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, et al. ClinVar: Public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res 2014;42:D980-5.
DOI: 10.1093/nar/gkt1113.

[22] Kersey PJ, Allen JE, Armean I, Boddu S, Bolt BJ, Carvalho-Silva D, et al. Ensembl genomes 2016: More genomes, more complexity. Nucleic Acids Res 2016;44(D1):D574-80.
DOI: 10.1093/nar/gkv1209

[23] DeLano WL. The PyMOL Molecular Graphics System. Palo Alto, CA, USA: Scientific; 2002.

[24] Desmet FO, Hamroun D, Lalande M, Collod-Béroud G, Claustres M, Béroud C. Human splicing finder: An online bioinformatics tool to predict splicing signals. Nucleic Acids Res 2009;37(9):e67.
DOI: 10.1093/nar/gkp215.

[25] Lee SB, Kim BC, Jin SH, Park YG, Kim SK, Kang TJ, et al. Missense mutations of the interleukin-12 receptor beta 1(IL12RB1) and interferon-gamma receptor 1 (IFNGR1) genes are not associated with susceptibility to lepromatous leprosy in Korea. Immunogenetics 2003;55(3):177-81.
http://dx.doi.org/10.1007/s00251-003-0573-x.

[26] Quispel WT, Stegehuis-Kamp JA, Santos SJ, van Wengen A, Dompeling E, Egeler RM, et al. Intact IFN-γR1 expression and function distinguishes langerhans cell histiocytosis from Mendelian susceptibility to mycobacterial disease. J Clin Immunol 2014;34(1):84-93.
http://dx.doi.org/10.1007/s10875-013-9959-1.

[27] Fieschi C, Dupuis S, Picard C, Smith CE, Holland SM, Casanova JL. High levels of interferon gamma in the plasma of children with complete interferon gamma receptor deficiency. Pediatrics 2001;107(4):E48.

[28] Kong XF, Vogt G, Chapgier A, Lamaze C, Bustamante J, Prando C, et al. A novel form of cell type-specific partial IFN-γammaR1 deficiency caused by a germ line mutation of the IFNGR1 initiation codon. Hum Mol Genet 2010;19(3):434-44.
http://dx.doi.org/10.1093/hmg/ddp507.

[29] Noordzij JG, Hartwig NG, Verreck FA, De Bruin-Versteeg S, De Boer T, Van Dissel JT, et al. Two patients with complete defects in interferon gamma receptor-dependent signaling. J Clin Immunol 2007;27(5):490-6.
http://dx.doi.org/10.1007/s10875-007-9097-8.