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Clinical implications of interferon-γ genetic and epigenetic variants

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Summary

Interferon-γ (IFN-γ) is an integral and critical molecule of the immune system, with multiple functions, mostly related to the T helper type 1 (Th1) response to infection. It is critical for defence against mycobacterial infection and is of increasing interest in defence against fungi. In this article, we review the genetic and epigenetic variants affecting IFN-γ expression and investigate its role in disease, with an emphasis on fungal diseases such as invasive and chronic pulmonary aspergillosis. Over 347 IFN-γ gene variants have been described, in multiple ethnic populations. Many appear to confer a susceptibility to disease, especially tuberculosis (TB) and hepatitis, but also some non-infectious conditions such as aplastic anaemia, cervical cancer and psoriasis. Several epigenetic modifications are also described, increasing IFN-γ expression in Th1 lymphocytes and reducing IFN-γ expression in Th2 lymphocytes. Recombinant IFN-γ administration is licensed for the prophylaxis of infection (bacterial and fungal) in patients with the phagocyte functional deficiency syndrome chronic granulomatous disease, although the benefits appear limited. Interferon-γ therapy is given to patients with profound defects in IFN-γ and interleukin-12 production and appears to be beneficial for patients with invasive aspergillosis and cryptococcal meningitis, but the studies are not definitive. A high proportion of patients with chronic pulmonary aspergillosis are poor producers of IFN-γ in response to multiple stimuli and could also benefit from IFN-γ administration. The investigation and management of patients with possible or demonstrated IFN-γ deficiency in adulthood is poorly studied and could be greatly enhanced with the integration of genetic data.

Keywords: epigenetics; fungal disease; genetics; interferon-γ; tuberculosis.

Introduction

Interferon-γ (IFN-γ; also known as type II interferon) is a cytokine that is critical in both innate and adaptive immunity in humans. It is a highly pleiotropic cytokine produced by many immune cells in response to interleukin-12 (IL-12) as well as to microbial stimuli such as zymosan, lipopolysaccharide and β-glucan, which acts to stimulate and modulate the immune response by modulating the production or activities of several cytokines and chemokines.[1,2] It is also an important activator of macrophages and one of the key cytokines that distinguishes differentiated T cells as either T helper type 1 (Th1; IFN-γ-producing) or Th2.[1,2] Because of the importance of IFN-γ in human immune responses, it is unsurprising that genetic and epigenetic variations within the IFN-γ gene are associated with a range of diseases. These genetic and epigenetic variations are reviewed here. Several genetic IFN-γ and IL-12 receptor defects are also described, but are not reviewed here. The currently under-studied role of IFN-γ genetic and epigenetic variation in fungal disease is also discussed.

Abbreviations: CPA, chronic pulmonary aspergillosis; IA, invasive aspergillosis; IFN-γ, interferon γ; IL, interleukin; MAF, minor allele frequency; NF-κB, nuclear factor-κB; RCT, randomised control trial; rIFN-γ, recombinant interferon-γ; SNP, single nucleotide polymorphism; STAT, signal transducer and activator of transcription; SUMO, small ubiquitin-like modifier; TB, tuberculosis; Th1, T helper type 1

IFN-γ and host immunity

Interferon-γ is important in the immune response to various pathogens. Recognition of these pathogens by Toll-like receptors or other receptors induces production of IL-12 by macrophages and dendritic cells, which in turn stimulates Th1 responses and production of IFN-γ.[1,3] Thus IFN-γ has many important immunostimulatory and immunomodulatory effects.

Interferon-γ up-regulates antigen presentation by MHC class I and class II by increasing expression of the subunits as well as by increasing the expression and activity of the proteasome.[4] Increased presentation by MHC increases the visibility of the pathogen to the host, and so increases the host ability to recognize and respond to the pathogen. Interferon-γ is also important in activation of macrophages to produce tumour necrosis factor-α, which then acts together with IFN-γ to increase macrophage phagocytosis and microbicidal activity, such as production of reactive nitrogen and oxygen species including superoxide radicals, nitric oxide and hydrogen peroxide.[1,3,5] In addition, IFN-γ enhances lymphocyte recruitment and results in prolonged activation within the tissues, induces components of the complement cascade and the acute phase response, plays a role in IgG class switching, and has direct anti-viral effects.[6,7] Interferon-γ is also key in controlling naive CD4 T-cell differentiation into Th1 effector T cells, critical mediators of cellular immunity against viral and intracellular bacterial infections.[4]

Production of IFN-γ is affected by various other members of the immune response, via the action of various transcription factors which activate or repress its transcription. Interleukin-12 enhances IFN-γ production via activation of signal transducer and activator of transcription 4 (STAT4) and subsequent increased expression of *IFNG*.[8] Interleukin-18, IFN-α, IL-12 and IL-2 also promote IFN-γ production and can augment IL-12-induced IFN-γ production.[4,9] Interleukin-21, IL-18 and IL-15 can act in synergy to enhance IFN-γ production by cells.[9] In addition, IFN-γ strongly up-regulates its own expression.[10] Transforming growth factor-β inhibits IFN-γ expression by inhibiting expression of the transcription factors T-bet and STAT4, which are important for IFN-γ expression.[11] Transforming growth factor-β also induces phosphorylation of SMAD3, which then binds with SMAD4 forming a heterodimer that can bind to the *IFNG* promoter and repress transcription.[12] Interleukin-6 potentiates expression of the suppressor of cytokine signalling-1, which then prevents the phosphorylation and subsequent activation of STAT1.[13] As STAT1 influences IFN-γ expression by potentiating the expression T-bet, prevention of STAT1 activation prevents IFN-γ expression.[13]

Genetic variation in the IFN-γ gene

A number of studies have identified 419 variations in the IFN-γ gene (data from Ensembl website;[14] Table 1, Fig. 1). These fall into different categories, described in Table 1. These variations may or may not affect the expression of the IFN-γ gene or function of the protein, depending on their location within the gene and on their effect on the DNA sequence (Fig. 2).

IFN-γ genetic variation and disease

Many variations within the IFN-γ gene have been shown to be associated with disease (Table 2).[15–46] These associations may be related to expression of the IFN-γ gene.

Table 1. Types and number of variations within the interferon-γ (IFN-γ) gene

Type of variation	Description	Number present in IFN-γ gene
Splice donor variant	A splice variant that changes the two-base region at the 5′ end of an intron	1
Gain of stop variant	A sequence variant whereby a premature stop codon is created, leading to a shortened transcript	1
Loss of stop variant	A sequence variant whereby at least one base of the stop codon is changed, resulting in an elongated transcript	1
Non-synonymous (Missense) variant	A sequence variant, that changes one or more bases, resulting in a different amino acid sequence but where the transcript length is preserved	31
Splice region variant	A sequence variant in which a change has occurred within the region of the splice site, either within one or three bases of the exon or three to eight bases of the intron	7
Synonymous variant	A sequence variant where there is no resulting change to the encoded amino acid or transcript length	10
5′ UTR variant	A variant in the 5′ untranslated region (UTR). This is upstream of the gene	3
3′ UTR variant	A variant in the 3′ untranslated region (UTR). This is downstream of the gene	16
Intron variant	A variant that occurs within an intron	119
Upstream gene variant	A sequence variant located 5′ (upstream) of a gene	119
Downstream gene variant	A sequence variant located 3′ (downstream) of a gene	118

A codon is a group of three bases that code for one amino acid, or start/stop signal. Data from Ensembl website[14].

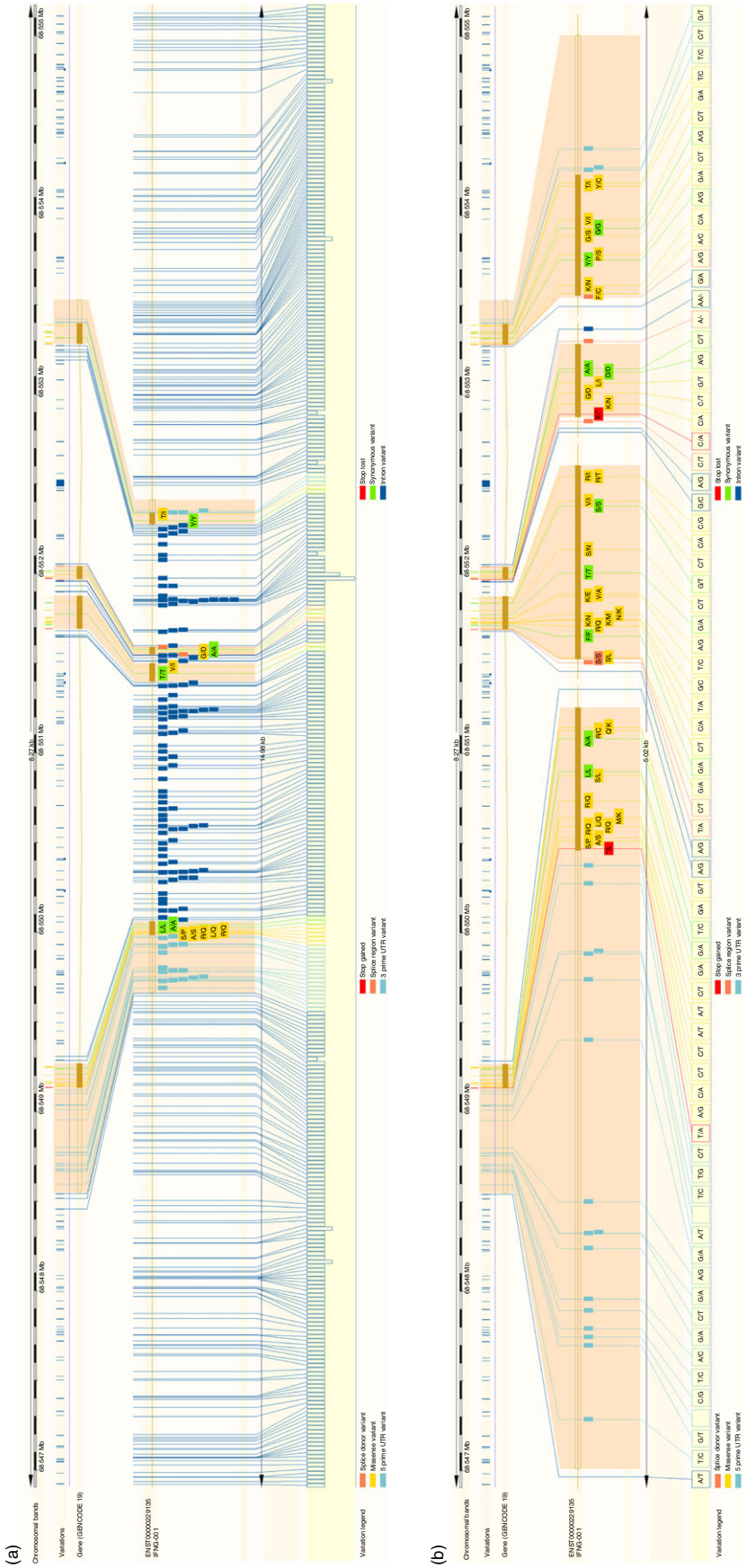

Figure 1. Genetic variation within the interferon-γ gene (*IFNG*). (a) Variation within the IFN-γ gene, plus upstream and downstream regions; (b) variations within the exons and untranslated regions (UTRs), plus 20 bp either side of these. The variations track shows the variations present in the region (each line is a variation site). The exons (brown rectangles), introns (brown lines) and UTRs (unfilled rectangles) are shown. Variations within the exons and UTRs are highlighted with pale orange background throughout the figure, and are detailed using the variation legend shown. Figure adapted from Ensembl website[14].

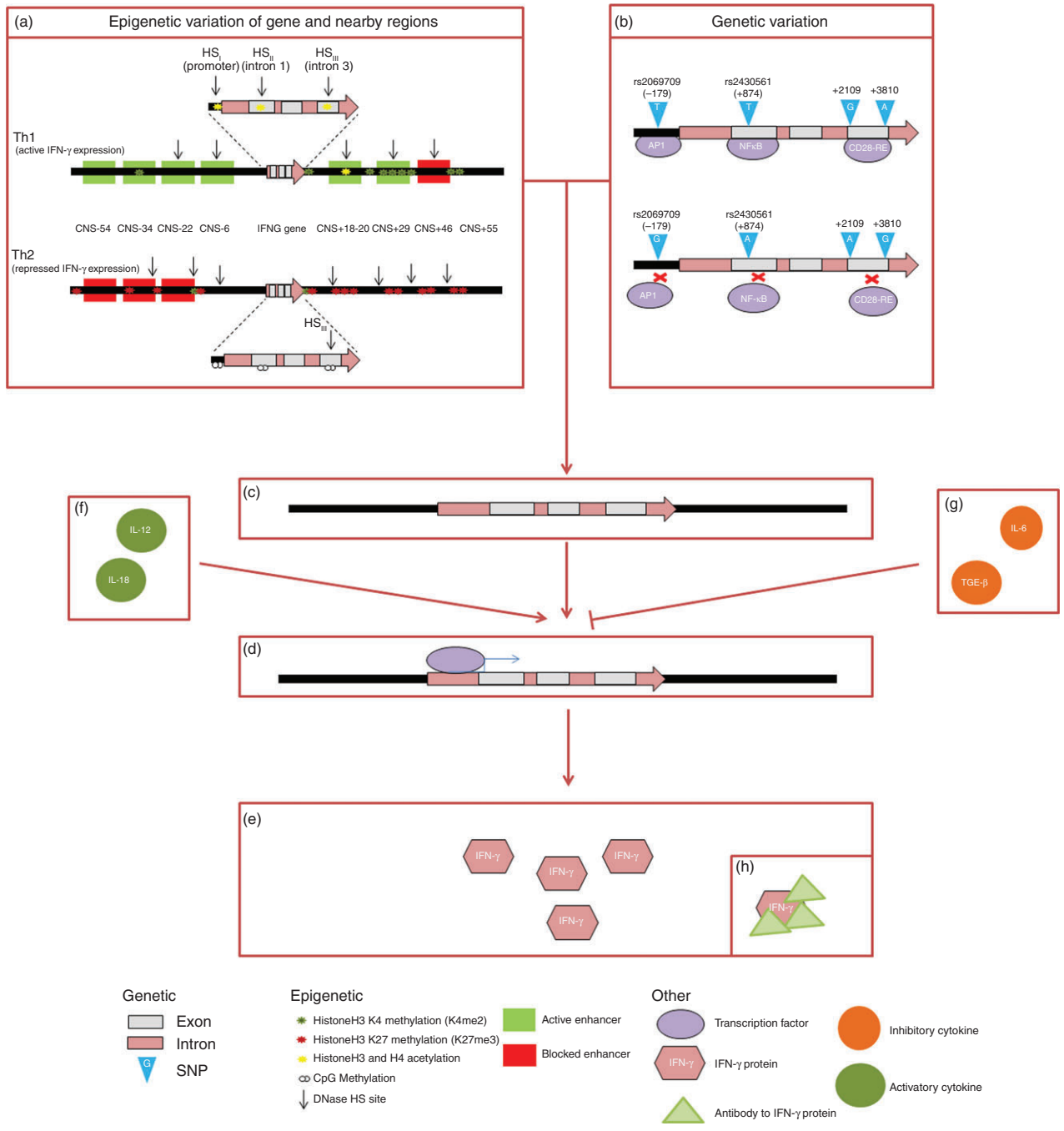

Figure 2. Differential mechanisms to impairment of interferon-γ responses. Epigenetic (a) and genetic (b) variations affect the chromatin structure and result in a specific pattern of variation at the DNA level of the interferon-γ gene (*IFNG*) and surrounding regions (c). Epigenetic variations include histone acetylation and H3K4 methylation, which activate gene expression, and DNA CpG methylation and histone H3K27 methylation, which repress gene expression. Genetic variation includes single nucleotide polymorphisms (SNPs) that affect the presence or functioning of a transcription factor binding site. Rs2430561 (+874T/A) is located in a nuclear factor-κB (NF-κB) binding site and NF-κB binds preferentially to the T allele. Rs2069709 (−179G/T) is located in the promoter. The T may create a putative activator protein 1 (AP-1) binding element or oestrogen-like response element. SNPs in intron 3 (+2109A/G and +3810G/A) may also affect transcription, by altering the binding of protein complexes such as CD28-RE. Binding of transcription factors (d) is affected by this genetic and epigenetic variation as well as by the presence or absence of the transcription factors themselves, and binding of different transcription factors activates or represses expression of the *IFNG* gene, to affect production of IFN-γ protein (e). Cytokines such as interleukin-12 (IL-12) and IL-18 can promote *IFNG* expression (f), while those such as IL-6 and transforming growth factor-β (TGF-β) can prevent expression (g), so altering the production of IFN-γ protein (e). This IFN-γ protein can be prevented from functioning by the presence of IFN-γ antibodies (h).

Table 2. Interferon-γ (IFN-γ) genetic associations with disease

Variation	Association	Ethnicity	References
rs2430561 (+874A/T) rs2069718 (+3234C/T) rs2069705/rs2430561/rs2069718 haplotype	Paediatric tuberculosis, particularly in females	Han Chinese	21
rs3138557 (CA repeat)	Ossification of the posterior longitudinal ligament	Korean	22
rs2430561 (+874T/A)	Increased fatigue during the acute sickness response	Caucasian	23
rs2430561 (+874T/A)	Paroxysmal nocturnal haemoglobinuria	Han Chinese	24
rs3138557/rs2430561 haplotype	Number of vessels affected and severity in coronary heart disease	Korean	25
rs2430561 (+874T/A)	Cortical cataracts and posterior subcapsular cataracts	Indian	26
rs2430561 (+874T/A)	Mild pulmonary tuberculosis	Pakistani	27
rs2430561/IL10 rs1800870/IL6 rs1800795 haplotype	Tuberculosis (mild or advanced)	Pakistani	27
rs2430561 (+874T/A)	Aplastic anaemia	Chinese	28
rs2430561 (+874T/A)	Response to immunosuppressive therapy in aplastic anaemia	Chinese	28
rs2069727 (A/G)	Acute lymphoblastic leukaemia in males	Welsh and Mexican	29
rs2430561 (+874T/A)	Protective against leprosy	Brazilian	30
rs11177074 (C/T)	Cervical cancer	Costa Rican	31
rs2069727/rs2069718/rs2430561/ rs2069705 haplotype	Atopic dermatitis complicated by eczema herpeticum	Mixed African American and Caucasian	32
rs2430561 (+874T/A)	Chagas disease	Colombian	33
rs2430561 (+874T/A)	Tuberculosis (pulmonary and extra-pulmonary)	Brazilian	34
rs2430561 (+874T/A)	Cervical cancer	Swedish	35
rs2069705	Systemic lupus erythematosus	Korean	36
rs2430561 (+874T/A)	Mediterranean spotted fever	Sicilian	37
TNFA rs1800629/IL6 rs1800795/IFNG rs2430561 haplotype	Spontaneous preterm birth	Brazilian	38
rs2430561 (+874T/A)	Cervical cancer	Indian	39
rs2430561 (+874T/A)	Psoriasis vulgaris	Polish	40
rs2069707 (−764C/G)	Recovery from hepatitis C virus infection	American	41
rs3138557 (CA repeat) rs2430561(+874T/A)	Chronic hepatitis B virus infection	Polynesian	42
rs2430561(+874T/A)	Liver cirrhosis in chronic hepatitis C	Taiwanese	43
rs2430561(+874T/A) rs3138557 (CA repeat)	IgA nephropathy	Italian	44
rs2430561 (+874T/A) rs3138557 (CA repeat) rs2430561(+874T/A)	Intrauterine hepatitis B virus infection	Chinese	20
rs2430561(+874T/A)	Tuberculosis	Sicilian	19
−1616G/A	Pulmonary tuberculosis	West African	18
+3234T/C	Pulmonary tuberculosis	West African	18
rs2069709 (−179G/T)	AIDS progression in HIV-positive individuals	African American	17
+2109A/G	Severe hepatic fibrosis in hepatic schistosomiasis	Sudanese	16
+3810G/A	Severe hepatic fibrosis in hepatic schistosomiasis	Sudanese	16
rs2430561(+874T/A)	Tuberculosis	Chinese	15
rs3138557 (CA repeat)	Tuberculosis	Chinese	15
rs3138557 (CA repeat)	Hepatitis E	Indian	45
rs3138557 (CA repeat)	Tuberculosis	Chinese	46
rs2430561(+874T/A)	Tuberculosis	Chinese	46

Gene expression is often affected by single nucleotide polymorphisms (SNPs) in either the promoter region or in nuclear factor-κB (NF-κB) binding regions. In the case of IFN-γ, there are two genetic variations that are known to affect expression. These are a polymorphic CA repeat region, where allele #2 (12 repeats) has been shown to result in high IFN-γ production when cells are stimulated,[47] and a T/A SNP (rs2430561, +874T/A) in intron 1, where the T allele correlates to allele #2 of the CA repeat and high IFN-γ production.[48] This T/A SNP is located in an NF-κB binding site and NF-κB binds preferentially to the T allele; the presence of the A allele reduces NF-κB binding, thereby reducing IFN-γ expression in response to stimuli.[48] Studies have found that the rs2430561 SNP is associated with various diseases, including hepatitis[20,41–43] and TB,[19,21,27,34] in various populations

Table 3. Non-synonymous and loss/gain of stop single nucleotide polymorphisms within the interferon-γ gene

SNP ID	Location (Chr:bp)	Alleles	Type	AA change	AA position	SIFT	PolyPhen
COSM942843	12:68551973	C/A	Stop gain	E/*	61	N/A	N/A
COSM549550	12:68549134	T/A	Stop lost	*/L	167	N/A	N/A
rs372093951	12:68549141	A/G	Missense	S/P	165	tolerated	benign
rs369578383	12:68549144	C/A	Missense	A/S	164	deleterious	benign
rs201359065	12:68549155	C/T	Missense	R/Q	160	tolerated	benign
rs374634889	12:68549161	A/T	Missense	L/Q	158	tolerated	possibly damaging
rs377736305	12:68549179	C/T	Missense	R/Q	152	deleterious	possibly damaging
rs150875052	12:68551842	C/T	Missense	V/I	73	tolerated	benign
rs76012457	12:68551993	C/T	Missense	G/D	54	tolerated	benign
rs371849964	12:68553385	G/A	Missense	T/I	4	deleterious	probably damaging
COSM942837	12:68549155	C/T	Missense	R/Q	160	tolerated	benign
COSM356728	12:68549164	A/T	Missense	M/K	157	tolerated	benign
COSM549549	12:68549203	G/A	Missense	S/L	144	tolerated	benign
COSM1476858	12:68549246	G/A	Missense	R/C	130	deleterious	probably damaging
COSM549548	12:68549249	G/T	Missense	Q/K	129	deleterious	probably damaging
COSM1363841	12:68551694	G/A	Missense	S/L	122	deleterious	probably damaging
COSM942839	12:68551724	C/T	Missense	R/Q	112	tolerated	benign
COSM1210288	12:68551729	C/A	Missense	K/N	110	tolerated	benign
COSM1582160	12:68551730	T/A	Missense	K/M	110	tolerated	benign
COSM1476859	12:68551735	G/C	Missense	N/K	108	tolerated	benign
COSM1210289	12:68551752	T/C	Missense	K/E	103	tolerated	possibly damaging
COSM942840	12:68551754	A/G	Missense	V/A	102	tolerated	benign
COSM942841	12:68551796	C/T	Missense	S/N	88	tolerated	benign
COSM942842	12:68551865	C/A	Missense	R/I	65	tolerated	benign
COSM694678	12:68551865	C/G	Missense	R/T	65	tolerated	benign
COSM942844	12:68551983	C/A	Missense	K/N	57	deleterious	benign
COSM942845	12:68552003	G/T	Missense	L/I	51	tolerated	probably damaging
COSM942846	12:68553283	A/C	Missense	F/C	38	deleterious	possibly damaging
COSM194534	12:68553291	C/A	Missense	K/N	35	deleterious	probably damaging
COSM1705908	12:68553320	G/A	Missense	P/S	26	tolerated	benign
COSM942847	12:68553338	C/T	Missense	G/S	20	tolerated	benign
COSM240197	12:68553353	C/T	Missense	V/I	15	tolerated	benign
COSM1363842	12:68553388	T/C	Missense	Y/C	3	tolerated	possibly damaging

AA, amino acid.
Data from Ensembl[14].

(Table 2). Other, less well studied IFN-γ SNPs also appear to affect IFN-γ expression. Rs2069709 is a G to T transition at position −179 in the promoter region.[49] It has been proposed that the T allele may create a putative activator protein 1 binding element or oestrogen-like response element, and electrophoretic mobility shift analysis has identified a unique complex that binds to the −179T variant but not to the −179G variant.[49,50] Cells transfected with reporter complexes containing the T allele produce up to 13-fold more IFN-γ than those containing the G allele.[49] This is true in T cells and peripheral blood mononuclear cells, but does not have the same effect in lamina propria cells, perhaps because of differences in oestrogen or CD2 signalling within these cells.[49,50] This SNP was identified in HIV-infected individuals, and appears to affect AIDS progression.[17,49] It is rare in Europeans (minor allele frequency 0·001), but is more common in African Americans (minor allele frequency 0·021).[49] Two SNPs in intron 3 (+2109A/G and +3810G/A) of the IFN-γ gene may also affect transcription, by altering the binding of protein complexes, including CD28-RE, which itself binds to the transcription factors nuclear factor of activated T cells and to NF-κB.[16] The +2109 G allele and +3810 A appear to form a DNA/protein complex that was not formed by the respective A and G alleles.[16]

Other SNPs in IFN-γ may affect its function. Of particular interest are SNPs within the exons of the gene, as these are the regions that are made into the final protein. Amino acid changing SNPs, called non-synonymous or missense SNPs, are more likely to have an effect than synonymous SNPs, and, depending on the location, may or may not affect the expression or function of the final protein. The effect of an SNP on protein function can be predicted using the PolyPhen-2[51] and SIFT[52] programs. PolyPhen-2 predicts variation effects based on physical

and comparative considerations, while SIFT predicts variation effects based on sequence homology and the physical–chemical similarity between the alternative amino acids. The non-synonymous variations in the IFN-γ gene, and their predicted effects on protein function, are shown in Table 3 (data from Ensembl website[14]). This table shows that there are many variations in the IFN-γ gene that are predicted to have deleterious or damaging effects on the IFN-γ protein function. In addition to non-synonymous SNPs, SNPs that cause gain or loss of 'stop' signals can result in a truncated or elongated protein, which may affect function. Two such SNPs exist in IFN-γ (Table 3).

Epigenetic variation

Epigenetic modifications are extremely flexible and often reversible inheritable changes that can affect the accessibility of DNA for gene expression without affecting the DNA sequence itself. DNA is stored wrapped around cylinder-like structures called histones to form chromatin fibres. Epigenetic modifications of these histones or of the DNA itself can affect the structure of the chromatin fibre, making the DNA within it more or less accessible to the DNA binding proteins that are required to initiate the process of transcription, consequently affecting production of RNA and protein. Modifications that result in compacted (closed) chromatin lead to gene silencing, while those that result in relaxed (open) chromatin allow for gene expression.

Histone modification occurs predominantly at the N-terminal 'tails' of histones, which can undergo enzymatic post-translational modification including methylation, acetylation, phosphorylation, ubiquitination and sumoylation [addition of a small ubiquitin-like modifier (SUMO) protein].[53] The effect of these varies depending on their location within a gene, and which residue is affected, but generally, histone acetylation causes activation of a gene and increased gene expression, while the effect of histone methylation is dependent on position; histone methylation at positions H3K4, H3K36, H3K79 results in activation and histone methylation at positions H3K9, H3K27, H4K20 results in silencing of a gene.[53] The effects of phosphorylation, ubiquitination and sumoylation are less clearly defined, however, ubiquitination may increase transcriptional elongation and sumoylation appears to antagonize ubiquitination and acetylation to repress transcription.[53]

At the DNA level, the major epigenetic modification is CpG methylation, where the cytosine nucleotide of a CpG dinucleotide (CpG island) is methylated. This prevents recruitment of methyl-sensitive DNA binding proteins, preventing the initiation of transcription, and also generates an inaccessible chromatin structure.[54] Together, these effects prevent gene expression and silence the gene. DNase hypersensitivity sites are areas of chromatin with increased sensitivity to an enzyme called DNase I. These areas of chromatin are highly accessible to DNA binding proteins, resulting in increased transcription and gene expression.

Epigenetic modifications occur naturally (e.g. during development and cellular differentiation), but can also be influenced by environmental factors including diet,[55] smoking[56] and microbial infections.[57,58] The epigenetic modifications caused by these environmental exposures may have disease contributing effects, and may be one explanation for the disease discordance observed in identical twins as they age.[59,60] Many diseases have been shown to involve aberrant epigenetic profiles, including cancer, atherosclerosis and osteoarthritis.[60–63] Environmental exposure may contribute to these. For example, it has been proposed that early stage nutrition can affect CpG methylation levels, in turn affecting susceptibility to chronic disease as an adult,[55] and smoking may cause promoter hypermethylation and silencing of p16, a tumour suppressor gene, possibly increasing susceptibility to oral cancer.[56]

Epigenetic variation and the IFN-γ gene

There is much evidence that the IFN-γ gene is subject to epigenetic modification, and that this modification is

Table 4. Interferon-γ epigenetic modification and disease

Modification of interferon-γ gene	Association	References
Hypermethylation in effector T cells	Asthma (in discordant asthmatic twins)	76
Hypermethylation of the promoter in blood DNA	Diisocyanate induced occupational asthma	71
Hypomethylation	Increased diastolic blood pressure in elderly subjects	72
Hypomethylation of the promoter in T cells	Biliary atresia	73
Hypomethylation in bile duct cells	Biliary atresia	74
Hypomethylation of the promoter	Gingival biopsy samples of sites of chronic periodontitis	77
Hypomethylation	Samples of inflamed dental pulp, compared with healthy dental pulp	78
Hypomethylation in peripheral T cells	Inflammatory bowel disease patients requiring surgery, compared with non-surgical patients	68
Hypomethylation of the promoter in blood DNA	Increasing job seniority in chemical plant workers	69
Hypomethylation of the promoter	Severe acute graft-versus-host disease	70

flexible and reversible. The epigenetic modifications are controlled by various transcription factors, including t-bet and GATA3, and those modifications that occur at promoter regions or conserved non-coding sequence sites are particularly important as these areas are involved in gene expression.[64,65] In particular, much work has been completed investigating the differing patterns of epigenetic modification of the IFN-γ gene in Th1 and Th2 cells.

Interferon-γ is one of the key cytokines that distinguishes differentiated T cells as either Th1 (produce IFN-γ) or Th2 (do not produce IFN-γ). Differentiation of T cells involves various epigenetic changes in a 100-kb region surrounding the IFN-γ gene itself.[66] These epigenetic modifications include gain or loss of histone modifications and changes in DNase hypersensitivity sites and CpG dinucleotide methylation, which activate the IFN-γ gene in Th1 cells and silence the IFN-γ gene in Th2 cells.[66,67]

Th1 cells produce IFN-γ. Within these cells, the IFN-γ gene shows histone H4 acetylation and histone H3 lysine 4 (H3K4) methylation and DNase hypersensitivity sites that are not present in naive T cells, including strong sites within the conserved non-coding sequence regions and the promoter.[65–67] In addition, the CpG methylation seen in specific sites within the IFN-γ gene in naive T cells is largely lost during Th1 differentiation such that Th1 cells show reduced CpG methylation.[66] The promoter is completely non-methylated.[66] These changes in methylation and DNase hypersensitivity are associated with an open chromatin structure and increased production of IFN-γ.[66] Like Th1 cells, natural killer cells produce IFN-γ. The IFN-γ gene within these cells also shows histone H4 acetylation and histone H3K4 methylation.[67]

Th2 cells do not produce IFN-γ. Within these cells, the IFN-γ gene shows histone H3K27 di-methylation and trimethylation and no hyperacetylation.[65,66] These modifications repress gene expression. As with Th1 cells, DNase hypersensitivity sites that are not present in naive T cells are observed, but these are different to the pattern in Th1 cells and fall adjacent to but not within conserved non-coding sequence sites.[66] In contrast to Th1 differentiation, during Th2 differentiation the CpG methylation of naive T cells is largely maintained and the promoter becomes hypermethylated.[66] These modifications silence gene expression.

IFN-γ epigenetic variation and disease

Interferon-γ gene epigenetics has been investigated in a growing number of conditions over recent years, and IFN-γ methylation has now been implicated in diseases from asthma to periodontitis (Table 4).[68–74] Some diseases are associated with decreased (hypo-) methylation of the IFN-γ gene, others with increased (hyper-) methylation.

Interferon-γ methylation, and consequently IFN-γ gene expression in humans, has been shown to be modified by various microbial factors. HIV causes hypermethylation and silencing of the IFN-γ gene, possibly as a method to evade the immune response,[58] while hypomethylation and activation of the IFN-γ gene is found in Epstein–Barr virus-transformed B cells.[57] It has been suggested that low-level microbial exposure during early life can reduce demethylation of the IFN-γ gene in naive T cells, reducing activation of this gene and leading to an increased risk of allergic disease.[75]

Asthma in humans appears to be associated with increased methylation and consequent decreased expression of the IFN-γ gene. Effector T cells from discordant asthmatic twins show increased methylation and decreased expression of the IFN-γ gene, compared with their non-asthmatic twin.[76] T-cell function is also reduced.[76] Similarly, hypermethylation of the IFN-γ promoter has also been found in blood DNA from workers with diisocyanate-induced occupational asthma, suggesting that, in these subjects, diisocyanate exposure may have caused increased methylation of the IFN-γ gene, leading to increased production of IFN-γ and the development of asthma.[71] Interferon-γ promoter methylation status was found to be a sensitive and specific method for identifying diisocyanate asthma workers.[71]

Other diseases are associated with decreased methylation and increased expression of the IFN-γ gene. Diastolic blood pressure is negatively associated with methylation of the IFN-γ gene (as blood pressure increases, methylation decreases), as shown by longitudinal measurements of DNA methylation in elderly subjects.[72] People with high diastolic blood pressure have hypomethylation of the IFN-γ gene. The IFN-γ promoter is also hypomethylated in T cells and bile duct cells from patients with biliary atresia, together with the expected increased gene expression.[73,74] Similar promoter hypomethylation and increased gene expression have been observed in gingival biopsy samples of sites of chronic periodontitis.[77] Reduced methylation was also found in samples of inflamed dental pulp, when compared with healthy dental pulp.[78]

Interferon-γ antibodies

Antibodies to IFN-γ may be found in a few apparently normal individuals, 2–3% in the Netherlands, with slightly higher rates in older adults.[79] Most anti-IFN-γ antibodies are IgG class, but they may or may not be functional. Production of functional anti-IFN-γ antibodies is more common in those of Asian descent, and is closely linked to certain HLA class II types.[80] Over 100 individuals with neutralizing anti-IFN-γ antibodies and serious infection have been described, mostly in Asia, but not exclusively. It is probably more common than has been realized.[81]

Interferon-γ antibodies and disease

The most reported infections associated with anti-IFN-γ antibodies are disseminated or severe non-tuberculous mycobacterial infections, *Mycobacterium tuberculosis*, salmonellosis, varicella zoster reactivation, disseminated *Penicillium marneffei* infection, histoplasmosis, cryptococcosis, listeriosis and meliodosis,[81–83] Unusual skin conditions also appear to be common, notably Sweet syndrome (neutrophilic dermatosis), but also erythema nodosum, pustular psoriasis and exanthematous pustulosis.[83] Therapy with rituximab (anti-CD20) appears to be effective in blocking anti-IFN-γ antibody production.[84]

Assessment of IFN-γ deficits

Interferon-γ production is currently assessed using a whole blood stimulation assay, in which whole blood is stimulated with a variety of stimuli including zymosan, lipopolysaccharide, β-glucan, bacillus Calmette–Guérin and IL-12. The level of IFN-γ is measured after stimulation and this level of IFN-γ production is compared with the level of production by a control sample, so that impaired responses can be identified. As well as being used to investigate the inherent ability of an individual's cells to produce IFN-γ, the IFN-γ release assays have been developed for use in the diagnosis of TB, as an alternative to the tuberculin skin test.[85] Two commercial IFN-γ release assays are available; the ELISA, QuantiFERON-TB Gold Intube, and the ELISPOT, T-SPOT.TB. Both involve measuring IFN-γ release by T cells stimulated by *M. tuberculosis* antigens, which are present in infected individuals but not uninfected individuals or in the bacillus Calmette–Guérin vaccine.[85] T cells that respond to these antigens should therefore only be present in infected individuals, and so only cells from infected individuals should release IFN-γ upon stimulation. However, as has been discussed, differences in expression, either constitutively, or in response to stimulation, can be caused by genetic and epigenetic variations within the IFN-γ gene, independent of the stimulus. This has not been rigorously assessed for TB diagnostic assays as an explanation for false negatives.

IFN-γ and mycobacterial infection

Interferon-γ is key in the immune response to *M. tuberculosis*. Interferon-γ production following recognition of this pathogen is important in macrophage activation and phagocytosis and results in inhibition of growth and death of the mycobacteria.[5] Reduced IFN-γ production, and the resulting reduced Th1 response has been particularly associated with TB, and levels of IFN-γ and IL-12 increase during anti-TB treatment.[86] In addition, adjuvant therapy with IFN-γ may be beneficial in TB patients.[87] TB had been associated with mutations in the IFN-γ gene in many different ethnic groups.[15,18,19,21,27,34,46] Reduced IFN-γ production and reduced Th1 responses have also been observed in non-tuberculosis mycobacterial infections such as those caused by *Mycobacterium malmoense* or *Mycobacterium avium*.[88,89] Genetic associations with the IFN-γ gene have not yet been identified in these groups; however, associations with the IFN-γ receptor have been described, as have associations with anti-IFN-γ antibodies.[90]

IFN-γ and fungal infection

In addition to a role in bacterial infections, IFN-γ may also be important in defence against fungal infections. Cryptococcosis is usually caused by *Cryptococcus neoformans*, occasionally by *Cryptococcus gattii*, the latter notably in non-immunocompromised patients.[91] Following inhalation, the usual manifestation of disease is meningitis, although pneumonia[92] and other forms of disseminated cryptococcosis, notably skin and bone disease, also occur. Patients who fail to mount a directed IFN-γ response in cryptococcal meningitis are much more likely to die than those who do, irrespective of antifungal therapy.[93] Aspergillosis is caused by the fungus *Aspergillus*, usually *Aspergillus fumigatus*.[94] In the majority of individuals, inhaled *A. fumigatus* spores are cleared without causing disease, however, in immunocompromised individuals, *A. fumigatus* can cause an acute and severe disease called invasive aspergillosis (IA), and in overtly immunocompetent individuals *A. fumigatus* can cause chronic pulmonary aspergillosis (CPA).[94]

Invasive aspergillosis is a serious invasive fungal infection that occurs predominantly in the lung but also occasionally in other sites such as the paranasal sinuses, or postoperatively, and which can disseminate if untreated.[95] It occurs in a wide variety of immunocompromised patients, including those undergoing organ transplants, critically ill patients, those receiving high-dose corticosteroid therapy, in liver failure and during neutropenia. Unless diagnosed early, it is associated with extremely high morbidity and mortality, although outcomes have improved in recent years with earlier diagnosis and better antifungal agents, notably voriconazole.[95] Invasive aspergillosis is closely associated with profound neutropenia, monocytopenia and thrombocytopenia, or a blunted immune response (usually mediated by corticosteroid therapy). In addition, genetic susceptibility also plays a role and several mutations in donor or recipient following haematopoietic stem cell transplantation have been shown to affect susceptibility (e.g. ref. 96).

Chronic pulmonary aspergillosis is a serious and debilitating progressive lung condition that involves the formation of a cavity or cavities within the lung, with progressive fibrosis and consequent reduction in lung function and

quality of life.[94,97] A fungal ball, or aspergilloma, may be present.[94] If untreated, CPA can be fatal and has a ≥ 50% 5-year mortality.[98] Long-term treatment with expensive antifungal drugs is required to prevent deterioration; however, even with therapy, many patients do not improve but instead either deteriorate or remain stable, and morbidity and relapse remain high.[99] Unlike in IA, CPA patients have overtly normal immune systems without deficient numbers of immune cells, and the reasons behind development of this disease are unclear. While patients almost invariably have some previous lung disease, such as pulmonary TB or chronic obstructive pulmonary disease, they do not generally have a clinical history of recurrent infection.[100] In addition, the majority of patients with these underlying diseases do not develop CPA. It is likely that an immunogenetic deficiency is involved, and some genes have indeed been implicated (e.g. ref. 101). However, these associations do not explain all of the cases of CPA.

As with mycobacterium infection, Th1 responses and macrophages are important in *Aspergillus* infection. Various studies have suggested that Th1 responses (e.g. IFN-γ, tumour necrosis factor-α, IL-15) are beneficial during infection with *A. fumigatus*, whereas uncontrolled Th2 responses (e.g. IL-4, IL-13) are detrimental.[102,103] Recent reports indicate a role for IFN-γ in immune tolerance to *A. fumigatus*, acting via indoleamine 2,3-deoxygenase and culminating in inhibition of Th17 cell responses and control of inflammation and allergy in *Aspergillus*-related infections.[104] Interferon-γ is therefore important in resistance to CPA and IA. Production of IFN-γ in response to standard stimuli is impaired in IA[105] as well as in CPA.[106,107] Both CPA and IA patients have been treated with recombinant IFN-γ with benefit;[94,108] CPA patients have stable or improved disease when IFN-γ is given in combination with itraconazole, and a replacement dose of rIFN-γ (50 μg subcutaneously three times per week at night) can make a substantial difference to patients. In addition to its use in aspergillosis, exogenous IFN-γ therapy has proved beneficial in other patients with a range of invasive fungal infections (*Ochroconis gallopava*, *Alternaria malorum*, *Pyrenochaeta romeroi*, *Davidiella tassiana* and *Candida albicans*) after kidney transplantation.[108] Several cases of disseminated invasive fungal infections that were refractory to conventional antifungal drug therapy were rapidly cured with IFN-γ therapy.[108]

Clinical trials of gIFN treatment in infection and fungal diseases

Recombinant IFN-γ was originally licensed for infection prophylaxis in patients with chronic granulomatous disease following a randomized controlled trial (RCT) showing a significant reduction of infections and severity of infection in those receiving rIFN-γ.[109] A 12-month double-blind, placebo-controlled RCT of rIFN-γ was suggestive of benefit in HIV-positive patients with low CD4 cell counts, with reduced incidence of mucosal *Candida*, herpes simplex virus and cytomegalovirus infections and a trend towards increased survival (28% compared with 18%).[110] The advent of combination antiretroviral therapy curtailed further evaluations. Also in AIDS, an RCT of rIFN-γ at 100 and 200 μg three times weekly added to antifungal therapy showed important trends in improvement in cryptococcal meningitis, with more rapid cerebrospinal fluid sterilization (32–36% rIFN-γ recipients versus 13% recipients ($P = 0{\cdot}072$) and reduction in cryptococcal antigen (12- to 24-fold versus eightfold decrease, respectively) at 2 weeks.[111] At 10 weeks, improved combined mycological and clinical success was seen in the rIFN-γ recipients (26% versus 8%; $P = 0{\cdot}078$). Unfortunately the study was under-powered. A follow-up study comparing two and six doses of 100 μg of rIFN-γ in cryptococcal meningitis all treated with amphotericin B and flucytosine, showed faster organism clearance in both rIFN-γ groups compared with those not receiving rIFN-γ.[112] No differences in mortality were seen. In pulmonary TB, an RCT comparing the addition of rIFN-γ given by nebulizer to anti-tuberculous therapy showed a significant difference in the rate of clearance of *M. tuberculosis* from the sputum smear at 4 weeks ($P = 0{\cdot}03$) anti-tuberculous therapy alone.[113] Both nebulized and subcutaneous rIFN-γ significantly reduced fever, wheeze, and night sweats at 4 weeks. Some open studies of rIFN-γ are suggestive of benefit in invasive aspergillosis,[108,114–116] but no RCTs have been published. In contrast to these encouraging data, other RCTs have been negative. A large RCT in pulmonary fibrosis was stopped early with lack of benefit.[117] In Chinese patients with chronic hepatitis B, rIFN-γ showed no benefit.[118] Aerosolized rIFN-γ was ineffective in mild to moderate cystic fibrosis.[119]

IFN-γ genetic and epigenetic variation and fungal disease

As discussed, IFN-γ appears to be important in the immune response to fungi; in aspergillosis in particular, Th1 (IFN-γ-producing) responses appear beneficial in CPA, while uncontrolled Th2 responses are detrimental,[102,103] and impaired IFN-γ responses are associated with aspergillosis, including CPA.[102,103,105,107] Immune cells from aspergillosis patients have deficient IFN-γ production, and patients benefit from treatment with recombinant IFN-γ. The production of IFN-γ that is measured by this assay can be affected by genetic and epigenetic variations within the IFN-γ gene. It is likely that the deficient responses observed in cells from CPA patients are a result of genetic or epigenetic factors within the DNA encoding the IFN-γ gene.

Identification of IFN-γ SNPs affecting expression, either constitutively or in response to stimuli, may be useful as

indicators for IFN-γ treatment in aspergillosis patients or those with other fungal disease. In addition, identification of variations that are associated with aspergillosis or other fungal diseases may be useful as genetic markers of susceptibility to these diseases and could help to identify at risk individuals. Therefore, genetic studies of IFN-γ and its role in fungal diseases such as aspergillosis would be invaluable.

In addition, although there is no evidence yet as to whether the IFN-γ is epigenetically altered in patients with aspergillosis or other fungal diseases, it is likely that epigenetic changes that reduce expression of IFN-γ, such as hypermethylation of the gene or promoter, may increase susceptibility to this disease. Investigation and identification of these would also be invaluable.

Conclusions

The IFN-γ gene is subject to both genetic and epigenetic variations, some of which have been associated with gene expression and with disease. IFN-γ therapy is given to patients with profound defects in IFN-γ and IL-12 production. A high proportion of patients with CPA are poor producers of IFN-γ in response to multiple stimuli and IFN-γ therapy appears to be beneficial for patients with IA and CPA. The investigation and management of patients with possible or demonstrated IFN-γ deficiency in adulthood are poorly studied and could be greatly enhanced with the integration of genetic data. Variation in the IFN-γ gene may be important in fungal disease, including aspergillosis, particularly in CPA and IA, and genetic and epigenetic studies investigating IFN-γ in aspergillosis would be useful tools to elucidate a possible role for this variation in both susceptibility to aspergillosis and in identification and stratification of patients who would benefit from IFN-γ therapy.

Disclosures

The authors have no competing interests.

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