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Review Article

λ-Interferons and the single nucleotide polymorphisms: A milestone to tailor-made therapy for chronic hepatitis C

Yasuhito Tanaka,[1] Nao Nishida,[2] Masaya Sugiyama,[1] Katsushi Tokunaga[2] and Masashi Mizokami[1,3]

[1]Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, [2]Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, and [3]Research Center for Hepatitis and Immunology, Kohnodai Hospital, National Center for Global Health and Medicine, Ichikawa, Japan

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Type III interferons (IFN) (IFN-λ1, -λ2, -λ3/interleukin [IL]-29, -28A, -28B) are cytokines with type I IFN-like antiviral activities. Most cells have expressed both type I and III IFN following Toll-like receptor (TLR) stimulation or viral infection, whereas the ability of cells to respond to IFN-λ was restricted to a specific subset of cells. It was reported that signal transduction pathway of IFN-λ was similar to that of IFN-α/β although a receptor adapted by IFN-λ were distinct from that of IFN-α/β. However, the clinical significance and the role of each IFN-λ were unclear. Recent genome-wide association studies (GWAS) of the human whole genome revealed several single nucleotide polymorphism sites (SNP) strongly associated with the response to pegylated IFN-α (PEG-IFN) plus ribavirin (RBV) treatment in chronic hepatitis C patients. The SNP, which are located near the *IL-28B* gene of chromosome 19, were discovered simultaneously by three independent studies opening a new prospective in hepatitis C research. The present review highlights significant insights that can be derived from the GWAS approach, and summarizes current knowledge of *in vitro* and *in vivo* study on the role of IFN-λ in antiviral effect.

Key words: IL28B, Polymorphism, Interferon-lambda, Pegylated-interferon, Ribavirin

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INTRODUCTION

HEPATITIS C IS a global health problem which affects a significant portion of the world's population. The World Health Organization estimated that, in 1999, 170 million hepatitis C virus (HCV) carriers were present worldwide, with 3–4 million new cases appearing per year.[1] The most effective current standard of care in patients with chronic hepatitis C is a combination of pegylated α-interferon (PEG-IFN) with ribavirin (RBV) treatment. However, in the USA and Europe, only 42–52% of patients with HCV genotype 1 achieve sustained virological response (SVR),[2–4] and similar results have been reported in the relatively older Japanese population. Furthermore, various well-described side-effects often necessitate dose reduction, and 10–14% of patients require premature withdrawal from IFN-based therapy.[5] To avoid these side-effects in patients who are unlikely to benefit from the treatment, as well as to reduce the treatment cost, it is important to predict an individual's response before treatment with PEG-IFN/RBV. Several viral factors such as genotype 1, high baseline viral load, viral kinetics during treatment and amino acid pattern in the IFN sensitivity-determining region have been found to be significantly associated with the outcome by a number of independent studies.[6–8] Accumulated data have provided strong evidence that approximately 20% of patients with HCV genotype 1 and 5% of patients with genotype 2 or 3 have a null virological response (NVR) to PEG-IFN/RBV. Reliable NVR prediction would allow avoidance of side-effects and reduce the cost of treatment in the 20% of patients before the treatment initiation.

Host factors were shown to be associated with the outcome of the therapy, including age, sex, race, liver fibrosis and obesity.[9,10] Little had been known about the host genetic factors that might be associated with the

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Correspondence: Dr Yasuhito Tanaka, Department of Virology, Liver Unit, Nagoya City University Graduate School of Medical Sciences, Kawasumi, Mizuho, Nagoya 467-8601, Japan. Email: ytanaka@med.nagoya-cu.ac.jp

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response to the therapy: thus far, only a few candidate genes including type I IFN receptor-1 (*IFNAR1*) and mitogen-activated protein kinase-activated protein kinase 3 (*MAPKAPK3*) have been reported;[11,12] however, a recent genome-wide association study (GWAS) approach using high-throughput genotyping technology to relate hundreds of thousands of genetic markers (genotypes) to clinical conditions and measurable traits (phenotypes), was able to confirm that numerous polymorphisms affect disease susceptibility[13] and drug response.[14] On the basis of the GWAS, three independent groups have showed several significant single nucleotide polymorphisms (SNP) associated with the response to PEG-IFN plus RBV treatment and opened a door to a new era in hepatitis C research.[13,15,16]

In the present article, we first describe a susceptible gene, *IL-28B*, which can predict NVR to the combination therapy for chronic hepatitis C, and then we summarize current knowledge of basic research and the putative mechanisms to be studied further.

GENETIC ASSOCIATIONS WITH RESPONSE TO PEG-IFN PLUS RBV TREATMENT

GE *ET AL.* analyzed 1137 US patients with HCV genotype 1 and identified a genetic polymorphism (rs12979860) near the *IL-28B* gene on chromosome 19, encoding IFN-λ3 (IFN-λ3), is associated with an approximately twofold change in response to PEG-IFN plus RBV treatment, both among patients of European ancestry ($P = 1.06 \times 10^{-25}$) and African-Americans ($P = 2.06 \times 10^{-3}$).[15] An important finding from this study is the population distribution of the advantageous SNP, which is significantly more frequent in European-Americans and Asian populations than in African-Americans. Approximately 23–55% of Africans (~40% of African-Americans) carry advantageous C-allele frequency of rs12979860, compared with approximately 53–85% of Europeans (~70% of European-Americans) and approximately 90% of Chinese and Japanese. Ge *et al.* showed that the SVR rates across different population groups displayed a striking concordance with the C-allele frequency. Because the genotype leading to better response is in substantially greater frequency in European than African populations, this genetic polymorphism also explains the twofold difference in response rates between African-Americans and patients of European ancestry.

Another GWAS conducted to identify host genes associated with response to PEG-IFN/RBV treatment in 154 Japanese HCV patients with PEG-IFN plus RBV treatment, including 82 NVR and 72 virological responders (VR).[16] As the dose reduction of PEG-IFN and RBV can confound statistical estimation,[15] only patients with an adherence of more than 80% dose of both drugs during the first 12 weeks were included in this study. Two SNP (rs12980275 and rs8099917) located close to the *IL-28B* gene on chromosome 19 showed strong associations in the minor allele dominant model ($P = 1.93 \times 10^{-13}$ and 3.11×10^{-15}; odds ratio [OR] = 20.3, 95% confidence interval [CI] = 8.3–49.9 and OR = 30.0, 95% CI = 11.2–80.5, respectively) with NVR to PEG-IFN plus RBV treatment. The result was validated in an independent replication cohort consisting of 172 Japanese patients (combined $P = 2.84 \times 10^{-27}$ and 2.68×10^{-32}; OR = 17.7, 95% CI = 10.0-31.3 and OR = 27.1, 95% CI = 14.6–50.3, respectively).[16] The rs8099917 lies in between *IL-28B* and *IL-28A*, approximately 8 kb downstream from *IL-28B* and approximately 16 kb upstream from *IL-28A*. Interestingly, as the minor allele frequency (MAF) of these two SNP in the SVR group is similar to that of a transient virological response (TVR) group of East Asian patients (Fig. 1), the prediction of SVR (SVR vs non-SVR) using the SNP had lower statistical power (OR = 8.8 and 12.1), compared to that of NVR prediction (17.7 and 27.1, respectively), indicating that these SNP are strongly associated with the outcome of NVR. The TVR patients with the major allele, however, would achieve SVR by prolonged therapy or PEG-IFN/RBV plus protease inhibitor.

The association of this SNP (rs8099917) was also supported by an independent study which conducted a GWAS of SVR to PEG-IFN/RBV combination therapy in 293 Australian individuals (Northern European ancestry) with HCV genotype 1, with validation in an independent replication cohort consisting of 555 Europeans from the UK, Germany, Italy and Australia (combined $P = 9.25 \times 10^{-9}$, OR = 1.98, 95% CI = 1.57–2.52).[13] Note that the OR for predicting SVR was much higher in the Japanese population than that in the European and African populations (Fig. 2). One of the explanations could be the different allele frequency between the Japanese and European populations. Another is the difference in the case–control study design; Tanaka *et al.* examined effects between VR (SVR + TVR) versus NVR, whereas Ge *et al.* or Suppiah *et al.* compared SVR versus non-SVR (TVR + NVR).

To evaluate the significance of these SNP including rs12979860,[15] we reanalyzed 11 SNP (rs12980275, rs8105790, rs11881222, rs8103142, rs28416813, rs4803219, rs12979860, rs1549928, rs8099917, rs7248668 and rs10853728) around the *IL-28B* gene

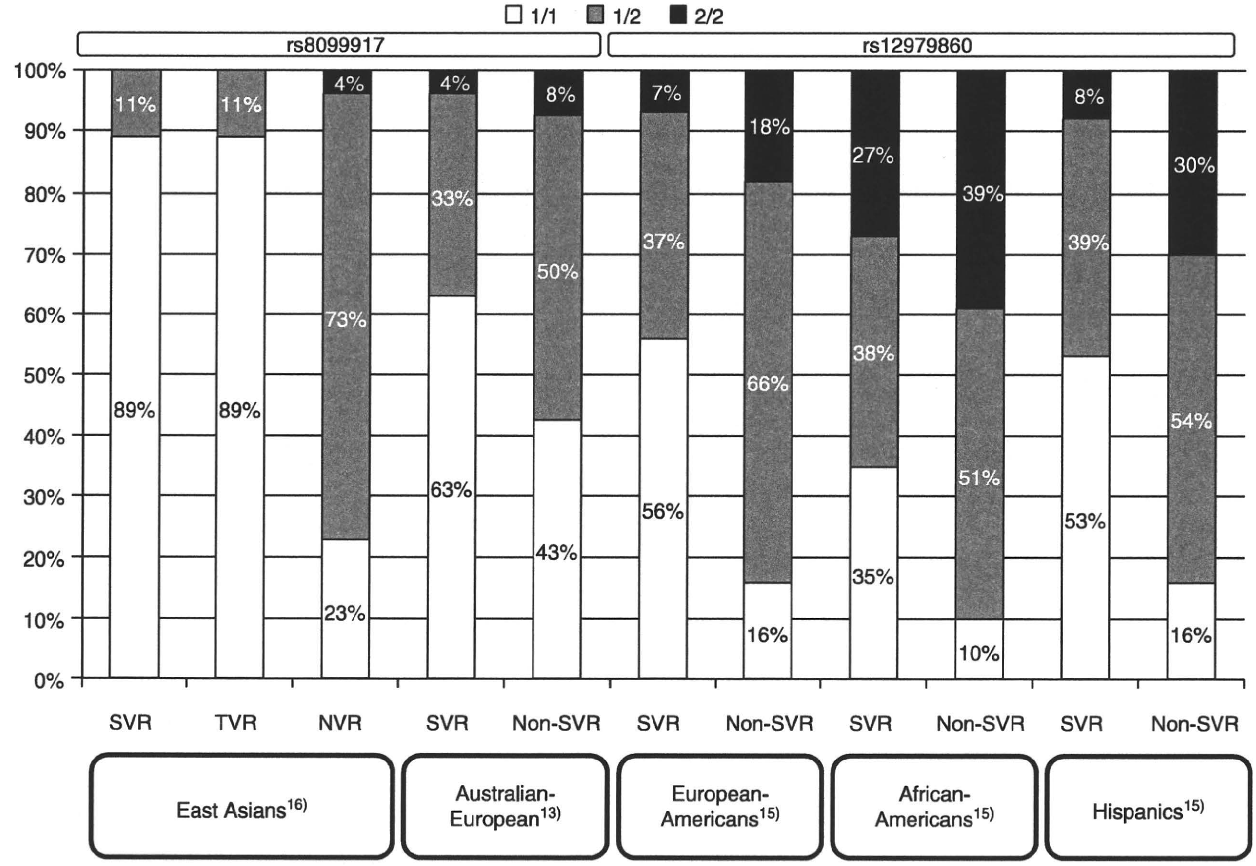


Figure 1 Comparison of genotypes proportion on representative single nucleotide polymorphisms (SNP) between ethnic population groups. Three original papers reporting significant SNP on hepatitis C virus (HCV) therapy cover several population groups. This graph shows the proportion of genotypes related to HCV therapy between the population groups. NVR, null virological response; SVR, sustained virological response.

using a total of 255 Japanese chronic hepatitis C patients receiving PEG-IFN-α/RBV therapy (97 with NVR and 158 with VR).[16] The most significant associations with NVR were observed for seven SNP (rs8105790, rs11881222, rs8103142, rs28416813, rs4803219, rs8099917 and rs7248668). The rs11881222, rs8103142 and rs28416813 are located in the third intron, the third exon and the first intron within the *IL-28B* genome, respectively. The *P*-values for these polymorphisms reached 5.44×10^{-24} (OR = 21.0; 95% CI = 10.9–40.4) in the minor allele dominant model (Table 1). In addition, we newly analyzed the region of approximately 13.4 kb containing 11 SNP using Haploview software for linkage disequilibrium (LD) and haplotype structure (Fig. 3). These SNP were in strong LD ($r^2 > 0.91$) except for rs1549928 and rs10853728, and the risk haplotype showed a level of association similar to those of individual SNP ($P = 7.3 \times 10^{-19}$, OR = 8.7; 95% CI = 5.10–14.70) (Table 2), suggesting that the observed strong association with NVR was primarily driven by one of these SNP. Which SNP is the primary one for the prediction? In this study, we compared several SNP to determine it in a Japanese population. The association study between 97 NVR and 158 VR showed that OR of the above seven SNP reached 21.0, as well as that of rs12979860 carried out on an Illumina Human610-quad BeadChip which was 19.9. As these SNP were in strong LD ($r^2 > 0.96$, Fig. 3), however, it might be difficult to determine the primary SNP using a Japanese population. In European and African populations, based on seven SNP within a 17-kb region around the *IL-28B* gene showing genome-wide association with SVR, including the top hit, rs12979860, Ge *et al.* showed that the other six SNP displayed different degrees of LD with rs12979860, and the most strongly associated SNP in this region after accounting for rs12979860 was rs8099917.

Table 1 Significant association of single nucleotide polymorphisms with response to pegylated α-interferon plus ribavirin treatment

		rs12980275	rs8105790	rs11881222	rs8103142	rs28416813	rs4803219	rs12979860	rs1549928	rs8099917	rs7248668	rs10853728
NVR	AA	23	24	24	24	25	25	23	85	23	24	17
	AB	69	69	69	62	62	61	70	12	70	70	66
	BB	5	4	4	11	10	11	4	0	4	3	14
	mAF	0.407	0.397	0.397	0.433	0.423	0.428	0.402	0.062	0.402	0.392	0.485
VR	AA	130	137	137	137	137	137	136	131	137	137	102
	AB	28	21	21	19	19	19	22	24	21	21	51
	BB	0	0	0	2	2	2	0	3	0	0	5
	mAF	0.089	0.066	0.066	0.073	0.073	0.073	0.070	0.095	0.066	0.066	0.193
Allele (A/B)		A/G	T/C	A/G	T/C	C/G	C/T	C/T	A/G	T/G	G/A	C/G
P-value		1.89E-20	2.33E-23	2.33E-23	2.33E-23	9.78E-23	9.78E-23	1.88E-23	3.10E-01	5.44E-24	2.33E-23	2.70E-13
OR		14.9	19.8	19.8	19.8	18.8	18.8	19.9	0.7	21.0	19.8	8.6
(95% CI)		(8.0–27.8)	(10.3–38.0)	(10.3–38.0)	(10.3–38.0)	(9.8–35.9)	(9.8–35.9)	(10.4–38.1)	(0.3–1.4)	(10.9–40.4)	(10.3–38.0)	(4.6–15.9)

CI, confidence interval; NVR, null virological response; OR, odds ratio; VR, virological response.

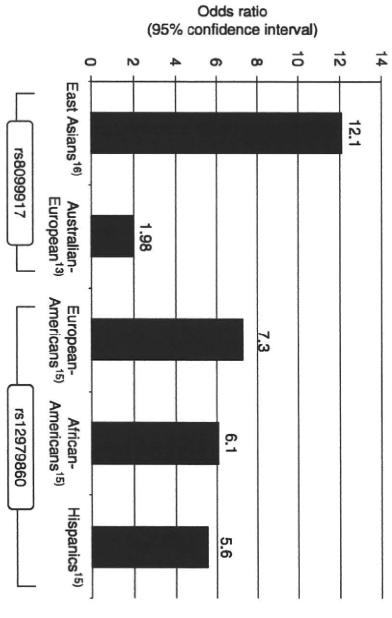
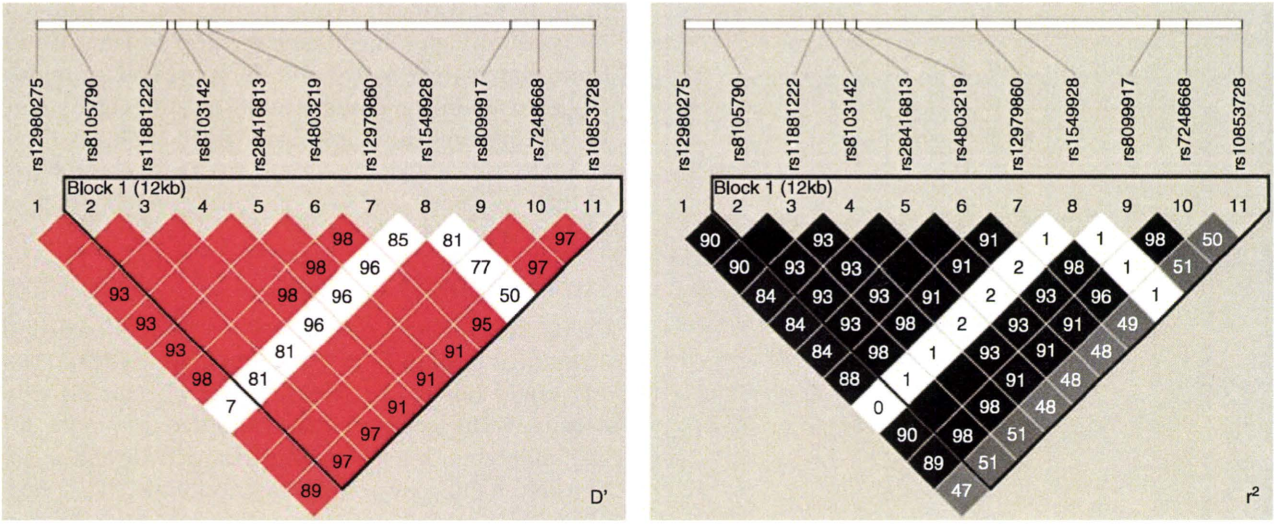


Figure 2 Odds ratios of representative single nucleotide polymorphisms (SNP) with response to PEG-IFN/RBV treatment in comparison between non-SVR (null virological response + transient virological response) and sustained virological response. The odds ratios were compared with those between ethnic population groups. Two representative SNP (rs8099917, or rs12979860) indicating most significant difference in each paper were shown as being lower.

To analyze the difference of LD pattern between races, we performed LD mapping with these SNP toward JPT (Japanese in Tokyo), CEU (Utah residents with ancestry from Northern and Western Europe) or YRI (Yoruba in Ibada, Nigeria) populations. These SNP were in strong LD in JPT and CEU populations though low LD was predicted in the YRI population (Fig. 4). These results would indicate that any of the SNP contained in this region could be the responsible one. Owing to the high degree of correlation between rs12979860 and additional SNP (rs28416813 and rs8103142) obtained by sequencing the *IL-28B* gene, tests for independence among these variants were not able to reveal which of these SNP is uniquely responsible for the association with SVR or NVR. Additional HCV-infected cohorts may help to determine whether one of these SNP, or any other SNP in the region, is causal for the association, as the pattern of association suggests the possibility of more than one functional variant in the region. Ultimately, identification and elucidation of the primary SNP will depend on high-density association mapping and analyses for LD and haplotype structure based on the HapMap data for individuals of different ancestry, as well as functional studies.

GENETIC ASSOCIATIONS WITH SPONTANEOUS CLEARANCE OF HCV

APPROXIMATELY 30% OF individuals spontaneously clear acute HCV infection. Epidemiological, viral and host factors have been associated with the



#	Name	Position	ObsHET	PredHET	HWpval	%Geno	FamTrio	Mend Err	MAF	Alleles
1	rs12980275	44423623	0.35	0.307	0.0736	100	0	0	0.19	A:G
2	rs8105790	44424341	0.33	0.289	0.0588	100	0	0	0.175	T:C
3	rs1881222	44426763	0.33	0.289	0.0588	100	0	0	0.175	A:G
4	rs8103142	44426946	0.31	0.301	0.8942	100	0	0	0.185	T:C
5	rs28416813	44427484	0.31	0.301	0.8942	100	0	0	0.185	C:G
6	rs4803219	44427759	0.31	0.301	0.8942	100	0	0	0.185	C:T
7	rs12979860	44430627	0.335	0.292	0.0495	100	0	0	0.177	C:T
8	rs1549928	44431549	0.143	0.158	0.34	100	0	0	0.086	A:G
9	rs8099917	44435005	0.33	0.289	0.0588	100	0	0	0.175	T:G
10	rs7248668	44435661	0.335	0.285	0.0145	100	0	0	0.172	G:A
11	rs10853728	44436989	0.458	0.406	0.0997	100	0	0	0.283	C:G

Figure 3 Pairwise linkage disequilibrium (D' and r^2) diagrams for the 13.4-kb region (chromosome 19: 44423623–44436986) including *IL-28B* using a total of 255 Japanese patients with hepatitis C virus treated with pegylated α-interferon plus ribavirin. The linkage disequilibrium block described in the upper panel was delineated using the Four Gamete rule as implemented in Haploview software (MAF ≥ 5% and HWE $P \geq 0.001$). Lower panel shows profiles of 11 SNP in the 13.4-kb region.

differences in HCV clearance or persistence, and variation in genes involved in the immune response has already been linked to outcome of acute HCV infection[8,9] presumably owing to alteration in the strength and quality of the immune response. However, most variability in spontaneous HCV clearance remains unclear.

In a recent GWAS, the above SNP (rs12979860) 3 kb upstream of the *IL-28B* gene was shown to associate strongly with response to PEG-IFN plus RBV treatment. To determine the potential effect of rs12979860 variation on outcome to HCV infection in a natural history setting, Thomas *et al.* genotyped this variant in HCV cohorts comprised of individuals who spontaneously cleared the virus ($n = 388$) or had persistent infection ($n = 620$).[17] They showed that the C/C (major allele) genotype strongly enhances resolution of HCV infection among individuals of both European and African ancestry, implicating a primary role for *IL-28B* in resolution of HCV infection. Although this might be the strongest and most significant genetic effect associated with natural clearance of HCV, GWAS should be conducted to find additional genetic factors.

FUNDAMENTAL FEATURE OF IFN-λ

THE FOCUSED SNP of IL-28B and the fringe region revealed significant differences between VR and NVR. The *IL-28B* gene has been recently discovered and classified into type III IFN that is a member of the class II cytokine family. IL-28B, known as IFN-λ3, is part of the IFN-λ family, which also consists of IL-29/IFN-λ1 and IL-28A/IFN-λ2. This class II family includes type I, II and III IFN and the IL-10 family (IL-10, IL-19, IL-20,

Table 2 Association analysis of response to treatment by haplotype using nine single nucleotide polymorphisms

SNP									Frequency		*P*-value	OR (95% CI)
rs8105790	rs11881222	rs8103142	rs28416813	rs4803219	rs12979860	rs1549928	rs8099917	rs7248668	NVR group	VR group		
T	A	T	C	C	C	A	T	G	0.504	0.829	5.76E-15	0.2 (0.13–0.32)
C	G	C	G	T	T	A	G	A	0.381	0.066	7.30E-19	8.7 (5.10–14.70)

CI, confidence interval; NVR, null virological response; OR, odds ratio; SNP, single nucleotide polymorphisms; VR, virological response.

IL-22, IL-24, IL-26, IL-28 and IL-29). IFN-λ signaling is initiated through a membrane receptor system distinct from that of type I IFN and composed of a unique IL-28RA subunit and the IL-10R2 chain (Fig. 5). The IL-10R2 receptor subunit is shared by IL-10, IL-22, IL-26 and IFN-λ. Although these cytokines share one subunit of their receptor complexes, they have widely different biological activities.

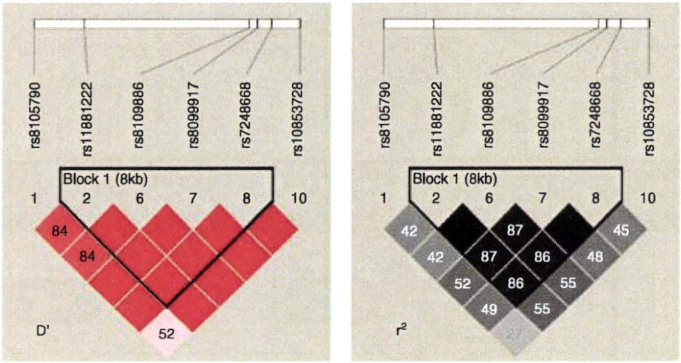
Structural basis of IFN-λ

Crystal structure of a type III IFN has been diffracted to a maximal resolution of 2.8 Å although residues 118–127 could not be modeled because of poor electron density.[18] The structure has suggested a model for ligand–receptor interaction. Antiviral activity and signal transducer and activator of transcription (STAT)2 phosphorylation was assayed by structure-based site-directed mutagenesis of human IL-28B. A comparison between the structure of IL-28B and structures of other class II cytokines (IFN-α2, IFN-β, IL-10, IL-19 and IL-22) shows that IL-28B is structurally more similar to members of the IL-10 family, especially IL-22, than to type I IFN. However, sequence alignment of the amino acid sequences of these cytokines did not show the same pattern of similarities. The sequence similarity between IL-28B and IFN-α2 or IFN-β is 33% and 31%, respectively, whereas the similarity between the IL-28B and IL-10 family is approximately 23%. Interestingly, the pattern of disulfide bond in the structure of IL-28B is similar to type I IFN but is not observed in the IL-10 family. They determined a cluster of six residues at the center of the A and F helices in IL-28B. The most effective residue for antiviral effect was Phe-158 of helix F.

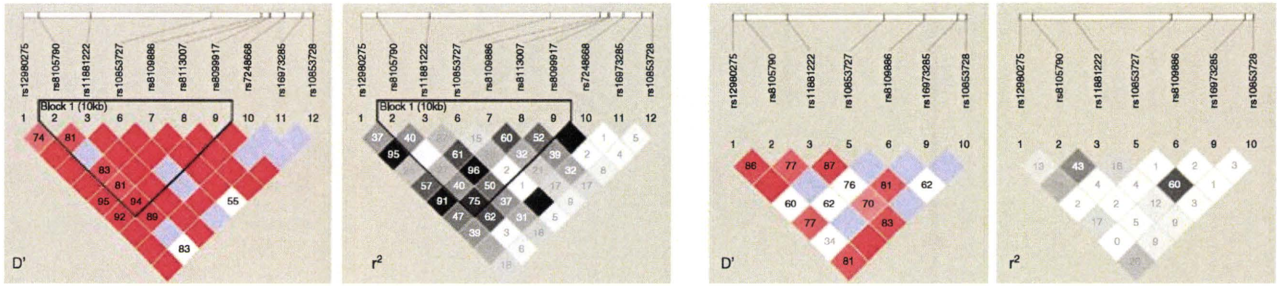
Expression profile of IFN-λ

The IFN-λ gene is expressed by several stimulations. The stimulation of encephalomyocarditis virus (EMCV), poly (I:C), LPS, Sendai virus or influenza A, but not R848 or Pam_3Cysz,[19,20] induced IFN-λ expression. These results suggest IFN-λ expression is dependent on a pattern recognition receptor.

A search for the *cis*-regulatory element of the human IL-29 revealed a cluster of IFN regulatory factor (IRF)-binding sites and an NF-κB-binding site.[21] Functional analyses demonstrated that all of these sites are essential for gene activation by the virus. Another group investigated the promoter function of IFN-λ compared with type I IFN.[22] Structural and functional characterization of the IL-29 and IL-28B gene promoters revealed them to be similar to IFN-β and IFN-α genes, respectively. Both of these promoters had functional IFN-stimulated



HapMap-JPT



HapMap-CEU HapMap-YRI

Figure 4 Comparison of pairwise linkage disequilibrium (D′ and r^2) diagrams between three populations (JPT, CEU and YRI) for the 13.4-kb region (chromosome 19: 44423623–44436986) using the HapMap release 23a. The linkage disequilibrium block was delineated using the Four Gamete rule as implemented in Haploview software (MAF ≥ 5% and HWE $P \geq 0.001$) for the 13.4-kb region.

response element and NF-κB binding sites. The *IL-29* gene is regulated by virus-activated IRF-3 and IRF-7, thus resembling that of the IFN-β gene, whereas IL-28A/B gene expression is mainly controlled by IRF-7, thus resembling those of IFN-α genes. These results strongly suggest that types I and III IFN genes are regulated by a common mechanism.

A certain degree of tissue specificity in the production of IFN-λ was observed. In the brain, IFN-α/β was readily produced after infection with various RNA viruses, whereas expression of IFN-λ was low in this organ.[23] Another paper reported that endogenous IL-29 but not IL-28A/B was expressed in human neuronal cells.[24] Virus infections in the liver induced the expression of both IFN-α/β and IFN-λ genes. The IFN-λ expression was significant in the stomach, intestine and lungs, but very low in the central nervous system and spleen. The IFN-λ signaling probably expanded to specifically protect epithelial tissue. IFN-λ might contribute to the prevention of viral invasion through skin and mucosal surfaces.

Cells of hematopoietic and non-hematopoietic compartments were isolated to determine the cellular source of IFN-λ. IFN-λ was induced to varying degrees in most cell types, with pDC and cDC being the two most prominent sources of both types of cytokines.[14]

Characteristics of receptors used by IFN-λs

The receptor complex for type I IFN consists of two subunits, IFNRA1 and IFNRA2. IFN-α/β subtypes differ in their affinity for IFNAR1 and that this receptor subunit is the limiting factor for ternary complex formation.[25] Binding to the IFNAR1 subunit would indicate signal pathways leading to antiproliferative activity, whereas binding to the IFNAR2 subunit would direct signal pathways leading to antiviral responses.[26] These fine differences of cytokine bindings could interpret the distinct quality observed in the activity of different IFN subtypes.

The IFN-λ receptor is composed of two chains, IL-28R1 and IL-10R2, and mediates the tyrosine phos-

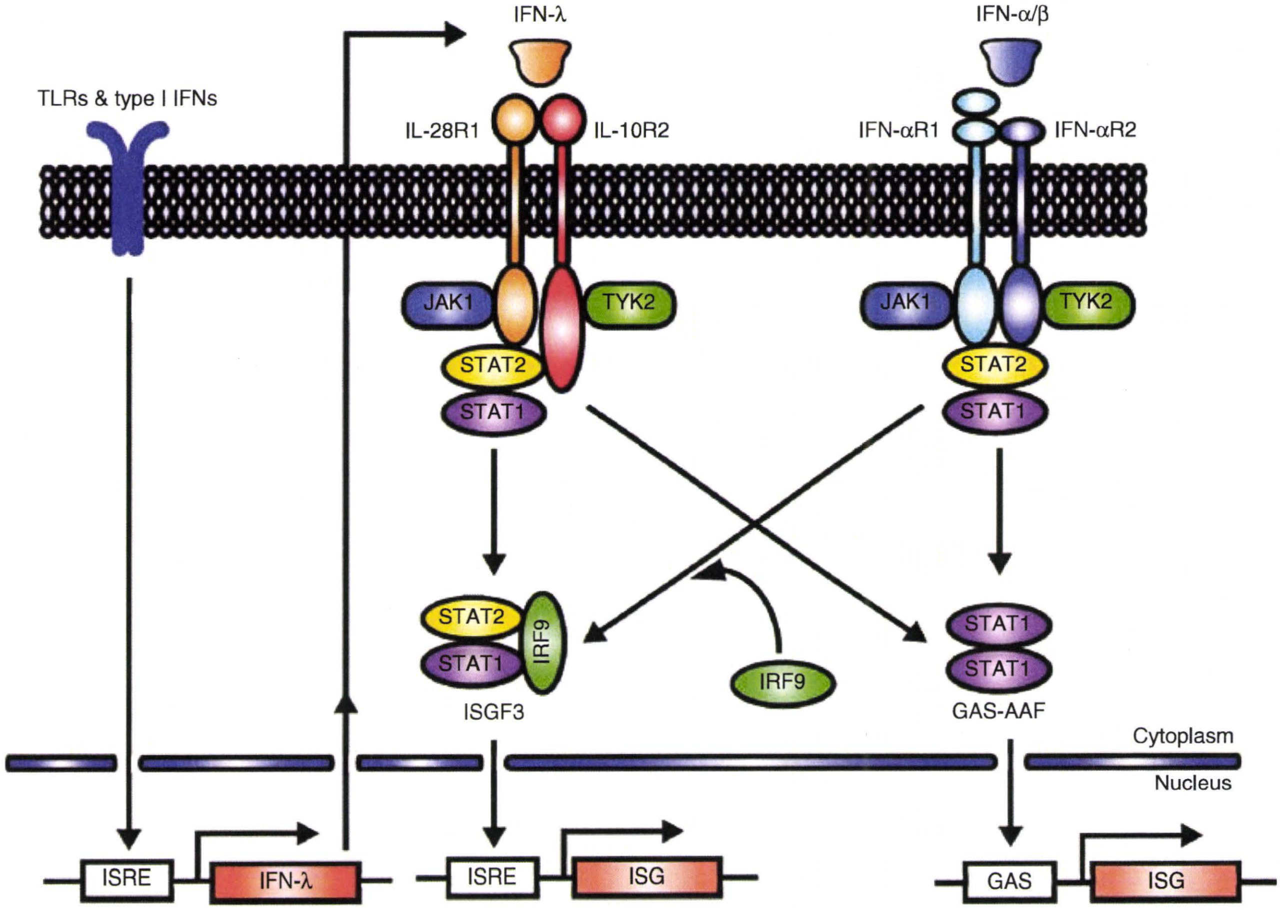


Figure 5 Schematics of expression pathway of λ-interferons (IFN-λ). IL, interleukin; IRF, interferon regulatory factor; STAT, signal transducer and activator of transcription; TLR, Toll-like receptor.

phorylation of STAT1, STAT2, STAT3 and STAT5.[19,27] A distinct characteristic between the type I and III IFN systems is the expression pattern of their receptors. The expression of the IL-28R1 is highly restricted,[23,28] whereas several types of cells express the type I IFN receptor complex and the IL-10R2. The response to type III IFN is also restricted in each organ because both receptor subunits are crucial for type III IFN signaling.

The signaling pathway of IFN-λ was influenced by splicing variants of IL-28R1 as well as the levels of IL-28R1 expression influence. Immune cells expressed high levels of a short IL-28R1 splice variant although immune cells were the main resource of IFN-λ. The truncated receptor binds IFN-λ and prevented the IFN-λ to bind the wild-type IL-28R1.[29] Skin cell population, karatinocytes and melanocytes, but not fibroblasts, endothelial cells or subcutaneous adipocytes, showed lower expression of the truncated IL-28R1. These results might suggest that the skin cell is important target of IFN-λ for homeostasis.

The second receptor of IFN-λ, IL-10R2, has unique characteristics. Several novel IL-10-related cytokines have recently been discovered. These include IL-22, IL-26 and IFN-λ. The main chains for IL-22, IL-26 and IFN-λ are distinct from that of IL-10; however, all of these cytokines use a common second chain, IL-10R2, to activate their receptor signaling. Although IL-10R2 is broadly expressed on a wide variety of tissues, only a subset of tissues expresses the ligand-specific R1 receptor. IL-10 binds to IL-10R1; IL-22 binds to IL-22R1; IL-26 binds to IL-20R1; and IFN-λ binds to IL-28R1. These ligand bindings to their specific R1 chains develop a conformational change, and its reconstitution of the main receptor enables IL-10R2 to interact with the complexes. This interaction activates a signal-transduction cascade that results in rapid activation of several tran-