IFNが効きやすくなる原理も明らかにされていない点が多い。開発段階ではDFPP療法はIFN投与開始時に施行されたが，IFN投与中にウイルス陰性化が得られない症例に途中で追加すると効果が得られるかなどさまざまな使用方法も考えられる。さらに肝移植症例のHCV再感染を防ぐ可能性，移植後fibrosing cholestatic hepatitisに対する劇的な効果の興味深い報告[12]もあり，現在の主流であるPEG-IFNα/ribavirin併用療法にてSVRが得られなかった症例にDFPP併用による再治療でSVRが得られるかは今後症例の集積が必要である。

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≪発生抑止≫ 石川県の肝癌撲滅戦略 ——モデルケースとして

酒井明人　金子周一*

要旨

- 肝炎ウイルス検診受診率，要精検者受診率は，性・年齢のほかに，地域でも差があった．
- 保健師・行政による要精検者のフォローアップおよび未受診者への受診勧奨は有効であり，適切な医療に結びついていた．
- 地域で多くの症例はかかりつけ医が存在するため，かかりつけ医への肝炎診療に対する考え方，最新情報の普及により，かかりつけ医のボトムアップを行い，最適な医療につなげることが重要である．
- 専門医受診は最適な医療に直接結びつきやすく，厚生労働省ガイドラインに準じた専門医受診を勧奨する施策が必要である．

はじめに

本特集の他稿に述べられているように，肝癌および最大の発生母地であるウイルス性肝疾患の診断，治療に関する進歩は目覚ましい．慢性肝炎，肝硬変，そして肝癌に関してのガイドラインも整備されつつあり，患者が専門医にたどり着けば，全国どこにおいても適切な医療が受けられる状況にある．

肝癌発生抑止には，C型肝炎であればinterferon(IFN)療法によるウイルスの排除の達成，B型肝炎であれば適切に治療対象を選んでの抗ウイルス療法によるコントロールが第一である．しかしながら多くのウイルス性慢性肝炎患者は自覚症状に乏しく，検査を受けなければ自分がウイルスキャリアであることがわからない．

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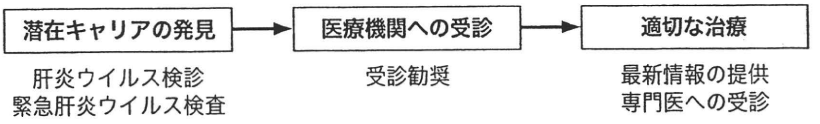
平成14(2002)年から，ウイルスキャリアを発掘し，適切な医療が行われることを目的に肝炎ウイルス検診が行われたが，その目的が達成されたとはいいがたい．本稿では，石川県において肝炎ウイルス検診当初から取り組んできた肝炎診療体制について述べる．

石川県肝炎ウイルス検診の状況

本邦における悪性新生物死亡数の男性第4位，女性第5位である肝癌を撲滅するには，潜在肝炎ウイルスキャリアの発見，キャリアの医療機関への受診，適切な医療(治療)という，三つのステップが必要である(Fig. 1)．キャリアの発見には肝炎ウイルス検診受診率の向上が重要であったし，未受診者には現在行われている緊急肝炎ウイルス検査の周知が必要である．

石川県では，5年間の肝炎ウイルス検診受診率



		節目検診受診率	精密検査受診率	初年度IFN療法施行率	
				精検受診者中	慢性肝炎中
全　国	平成14年	30.0%	65.4%	13.8%	
	平成15年	29.4%	85.6%	13.3%	
	平成16年	25.1%	81.8%	18.2%	
	平成17年	24.7%			
	平成18年	23.2%			
石川県	平成14年	41.5%	66.9%	3.0%	3.8%
	平成15年	41.5%	73.1%	5.7%	8.5%
	平成16年	37.8%	68.3%	14.7%	23.5%
	平成17年	36.8%	65.8%	24.5%	35.3%
	平成18年	36.6%	57.4%	23.7%	31.0%

Fig. 1.　肝癌撲滅に対する施策の全国および石川県の状況
［全国の精密検査受診率，IFN 療法施行率は日野のデータ[1]より引用］

が 36.6～41.5% と，全国平均[1]と比べると 10% ほど受診率がよかったが，満足できるものではない．Fig. 2 に，市町村ごとの平成 14 年肝炎ウイルス検診受診率，および性・年齢・医療圏別での精密検査（精検）受診状況を示す．検診自体の受診率は，能登地方および南加賀で低い傾向にある．しかしながら，能登地方ではウイルスキャリアと判明すると医療機関にはきちんと受診する傾向にある．一方，南加賀ではウイルスキャリアと判明しても医療機関への受診率がわるい．能登地方ではキャリアの発掘が重要であり，南加賀ではキャリアの発掘と受診勧奨の両面が必要なことがうかがえる．

また医療機関受診の時間がとりにくい若年男性の受診率がわるいのは共通しており，受診動機を促す啓蒙活動が必要である．

フォローアップ事業の重要性

石川県では初年度（平成 14 年度）から肝炎ウイルス検診受診時に，検診後も保健師・行政が関わってフォローすることの同意を得ている．保健師・行政は少なくとも年 1 回は，本人あるいは医療機関に問い合わせて受診状況の把握に努め，医療機関を受診していなければ，ときにはパンフレットを用いながら直接受診勧奨している．このようなフォローを毎年続けており，たとえば平成 14 年度 C 型肝炎ウイルス（HCV）精検者は 1, 2, 3, 4, 5 年後にそれぞれ 55，53，57，58，63% が医療機関受診していること，およびその診療内容を把握している．一方，状況不明な症例も 31, 39, 32, 28，17% 存在している．

精検受診率は全国データ（Fig. 1）より低い傾向にあるが，全国報告は精検受診有無が確認できたのが平均 57% ほどのデータから得られたものであり[1]，そのデータの正確性に疑問が残る．本県では受診勧奨に努めた結果，検診初年度には未受診でも翌年以降に 46.3% が医療機関を受診し，そのうち 14.5% で IFN 療法が行われる（Table 1）など，保健師・行政によるフォローアップ事業が医療機関への受診勧奨・適切な医療へ結びついたことがうかがえる．

C 型肝炎に対する IFN 療法普及のための対策

ウイルス性慢性肝炎への適切な医療は，C 型肝炎であれば IFN 療法によるウイルスの排除が目標である．HCV が排除されれば肝疾患の進行は止まり，肝発癌率の低下が得られることは，もはや周知の事実である．肝炎ウイルス検診の最終目標

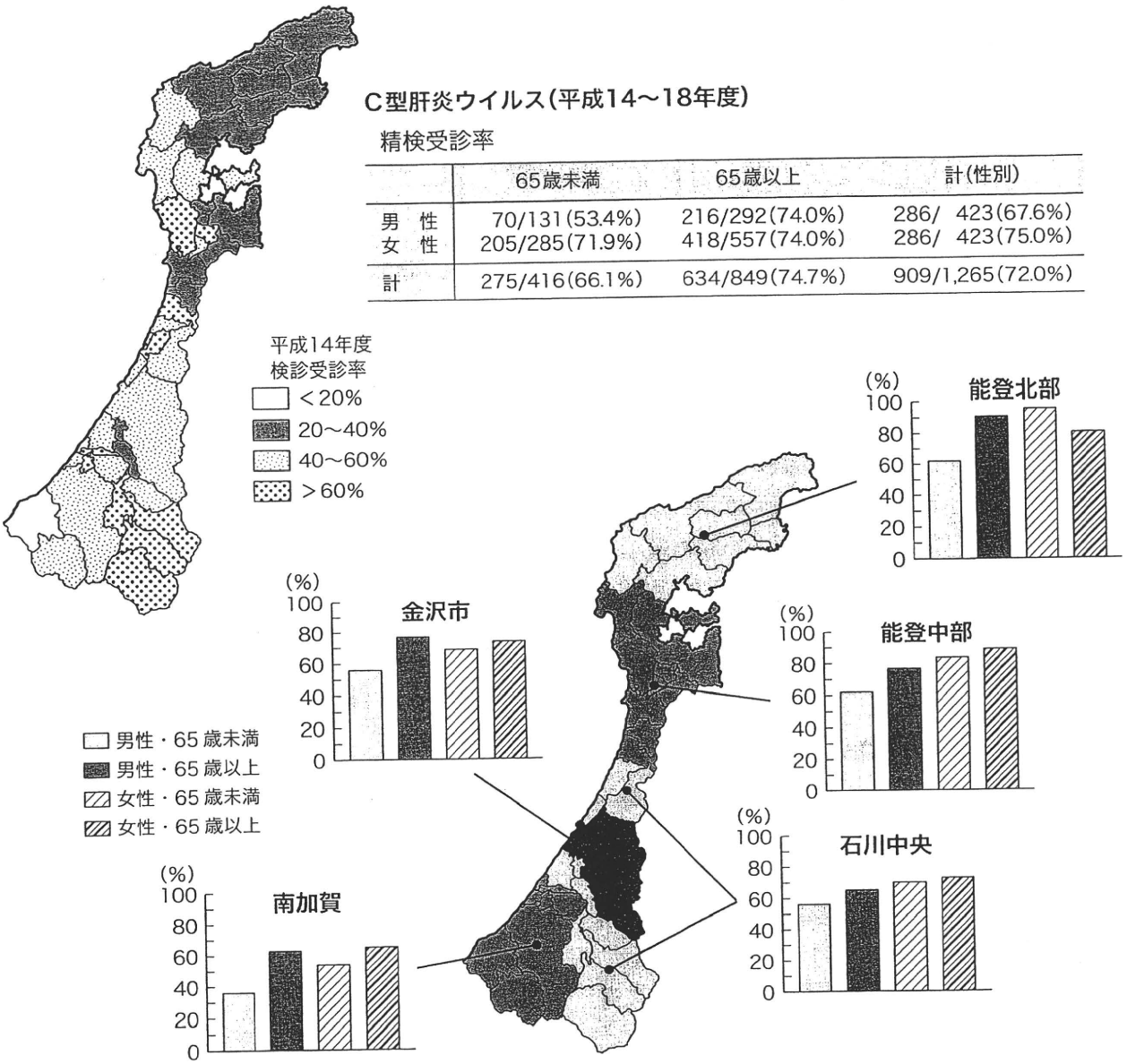


Fig. 2. 石川県肝炎ウイルス検診状況

Table 1. 石川県精検未受診者のその後の状況

	検診初年度精検未受診	翌年以降医療機関受診	IFN 療法/受診者
能登北部	18(14.8%)	12(66.7%)	3(25.0%)
能登中部	32(17.5%)	17(53.1%)	2(11.8%)
石川中央	71(31.8%)	45(63.4%)	7(15.6%)
南加賀	88(40.6%)	52(59.1%)	10(19.2%)
金沢市	147(28.1%)	39(26.5%)	2(5.1%)
計	356(28.1%)	165(46.3%)	24(14.5%)

Table 2. 初年度受診医療機関別の C 型肝炎治療法

治療方針	診療所 (n= 41)	総合病院・専門医 (n= 144)
IFN 療法	4(9.8%)	53(36.8%)
他の注射薬	4(9.8%)	3(2.1%)
→IFN(移行率)	2(50.0%)	3(100.0%)
内服薬	33(80.5%)	88(61.1%)
→IFN(移行率)	2(6.1%)	15(17.0%)
内服薬，65 歳未満	7(17.1%)	28(19.4%)
→IFN(移行率)	0(0.0%)	8(28.6%)
のべ IFN 療法	8(19.5%)	79(54.9%)

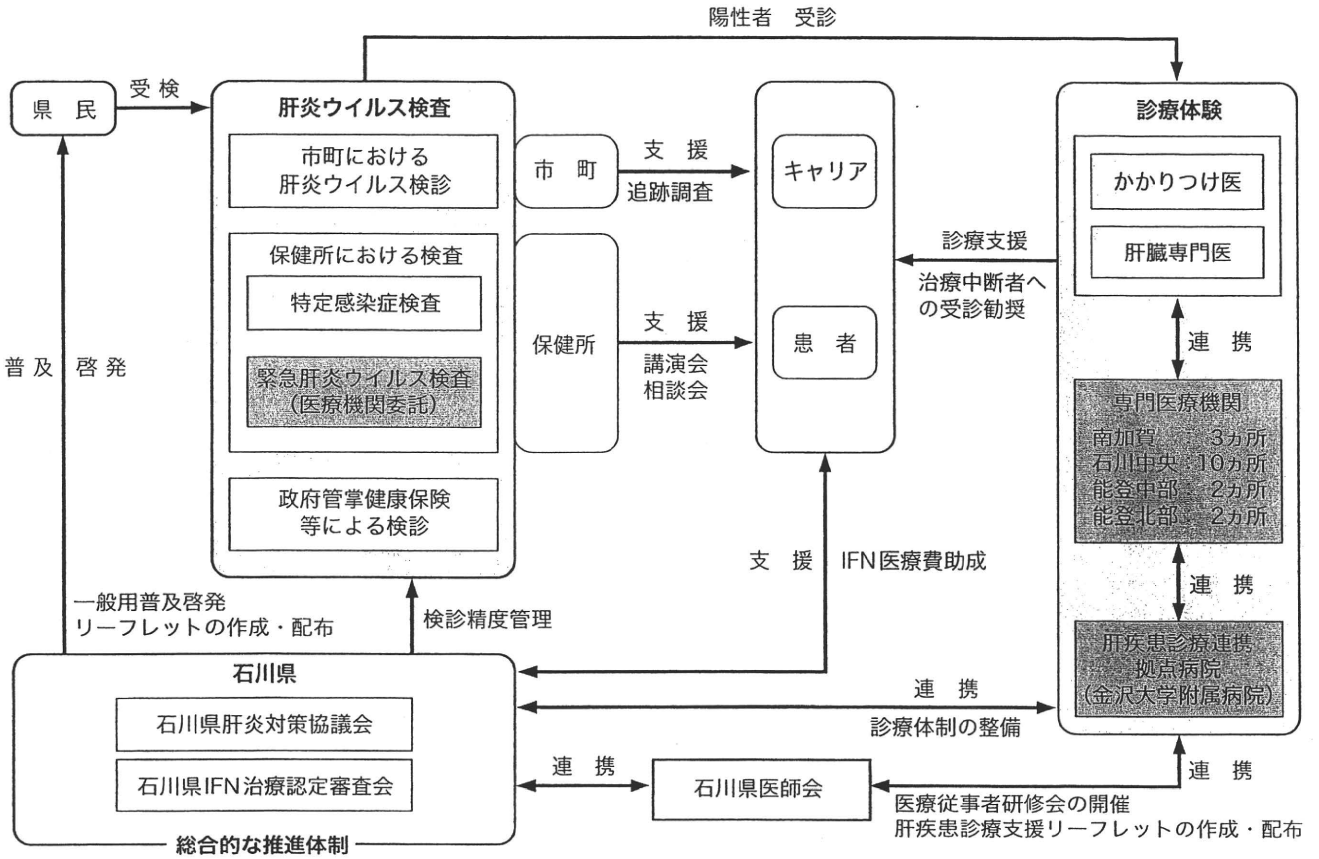


Fig. 3.　石川県における肝炎総合対策体制

は，見出した症例を IFN 療法に結びつけることであるが，平成 14〜16 年全国集計では[1]主に経口薬が用いられ(平成 14 年：38%，平成 15 年：43%，平成 16 年：34%)，IFN 療法施行率は 20% に満たない(Fig. 1)．

石川県においても要精検者全体で平成 14 年 3.0%，15 年 5.7%，また精検診断名として無症候性キャリアでなく慢性肝炎とした症例に絞っても，平成 14 年 3.8%，15 年 8.5% と低率であった(Fig. 1)．とくに 65 歳以上の高齢者では IFN 施行率が 2.6% と，65 歳未満の 9.6% に対して有意に低いことが問題であった[2]．

石川県ではとくに精検実施医療機関を指定せずに，非専門のかかりつけ医であっても精検を担当できることとしている．このため精検の精度を保つために画像診断を義務づけし，診断のフローチャートにも最上位に画像診断を置いている[3]．また検診開始 3 年間は 1 例ごとの事例検討会にて専門医より指導を行い，診断の精度を向上させてきた．しかしながら IFN 施行率の向上には事例検討のみでは不十分であり，まず IFN 療法に対するアンケート調査を行った．その結果 IFN 療法を説明する割合，適応と考える年齢，適応と考える ALT 値などに専門・非専門医で違いがあることが明らかとなった[2]．これを踏まえて IFN 療法に絞った研修会を行い，年ごとにテーマを変え，最新療法，高齢者に対する考え方，ALT 正常例に対する考え方などの知識啓蒙に努めた．この結果，石川県では平成 16，17，18 年での IFN 療法施行率は要精検者全体で 14.7%，24.5%，23.7%，慢性肝炎患者で 23.5%，35.3%，31.0% と，向上している(Fig. 1)．

IFN 療法の施行率を上げるもう一つの方法は，専門医が診ることである．石川県肝炎ウイルス検診において精検を行った年より経過観察のみでなく何らかの治療を行った症例，これはおそらく

ALT 上昇が認められた症例と考えられるが，185 人であった．41 人が非専門医の一般診療所，144 人が総合病院あるいは専門医が精検を担当しているが，初年度からの IFN 療法施行率は診療所で 9.8%，総合病院・専門医では 36.8% と総合病院・専門医で高い IFN 導入率が示された．診療所では実に 80.5% が経口薬にて治療されており，その後の IFN 導入率も低い傾向であった．一方，総合病院・専門医では経口薬あるいは IFN 以外の注射にて当初治療されても，その後に IFN 導入が行われ，のべ IFN 導入率は 54.9% と，診療所の 19.5% と比べ明らかに高かった(Table 2)．

現在，厚生労働省の肝炎検診後の診療ガイドラインでも少なくとも年 1 回の専門医受診が推奨されており，IFN 施行率を上げるうえで，ガイドラインに即した地域診療体制の確立が重要である．石川県ではガイドラインを受けて肝疾患診療連携拠点病院，専門医療機関を整備し，Fig. 3 に示すような県全体での総合対策体制を打ち出している．

おわりに

肝癌撲滅のためには，肝炎ウイルスキャリアの発見から適切な医療まで，一医師，一医療機関だけではできない．どの段階においても行政，かかりつけ医，専門医，拠点病院が関わる必要があり，また一つの施策・方法だけで解決はできない．とくに肝炎ウイルス検診のデータは個人情報保護の問題から，今一つ有効に生かされていないのが現状である．また IFN 療法の補助が行われるのはあと 6 年間であり，対策は急務であるといえる．

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臨床的観点から 3
C型肝炎治療と遺伝子情報

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● はじめに

C型肝炎ウイルス（HCV）キャリアは日本の全人口の約1%に及び，第2の国民病と言われる。慢性肝炎から肝硬変，肝癌へと至り，近年では毎年約3万人がC型肝炎関連肝疾患で死亡しており，対策が急がれる。C型肝炎ウイルスが発見されるまでは対症療法のみであったが，ウイルス発見後，1990年代に入り原因治療としてインターフェロン（IFN）療法が登場し，ウイルスを完全に排除できる症例が得られるようになった。この著効症例は，肝病態は進行せず，肝癌発症率も非常に低く，予後が改善されることが明らかとなった。C型慢性肝炎に対するIFN療法は，遺伝子型1b型で高ウイルス量の症例に対しては著効率は10%前後と低く，費用，副作用の面から問題であった。しかし近年，ペグ化IFNおよびリバビリン併用療法の登場により，1b型高ウイルス量でも50%前後，2型では80%以上の症例で，著効が得られるようになってきている。

IFN療法の治療効果に影響する因子としては，上記のウイルス遺伝子型，ウイルス量，特定領域のウイルス遺伝子配列（NS5A領域遺伝子配列，コア領域アミノ酸変異など）が重要である。しかしながら，1b型高ウイルス量症例でも著効になる，あるいは2型，低ウイルス量症例でも無効となる症例が存在する。このような予測からはずれる症例は未知のウイルス側因子の影響もありうる一方，宿主側因子による可能性も存在する。報告されている宿主側因子の検討は多くはないが，HLA，人種，年齢，性，肥満度，アルコール，肝線維化進展度などがあげられている[1)]。しかしながら，これらの因子のみでIFN療法の効果予測が可能なわけではない。分子生物学的手法，機器の進歩に伴いヒトゲノムが解析され，さらに短時間で個々の症例よりばく大な遺伝子情報が得られるようになり，C型肝炎に対するIFN療法に影響する新たな宿主側因子として，遺伝子情報が重要となってきている。

● IFN関連遺伝子と治療効果

IFNは細胞膜表面の受容体に結合すると，細胞内にその受容体の下流に存在するシグナル伝達を起こし，核内に存在するIFN誘導遺伝子を発現させて各種抗ウイルス蛋白を誘導すると考えられている（図1）。受容体から転写活性因子複合体であるIFN-stimulating genes factor 3（ISGF3）を構成するまではTyk2，JAK1，STATなどが関与し，このISGF3が結合するプロモーターであるISRE（IFN-stimulating response element）は，IFN遺伝子発現にアクセルをかけるinterferon reguratory factor-1（IRF-1），ブレーキをかけるIRF-2の両転写因子と結合す

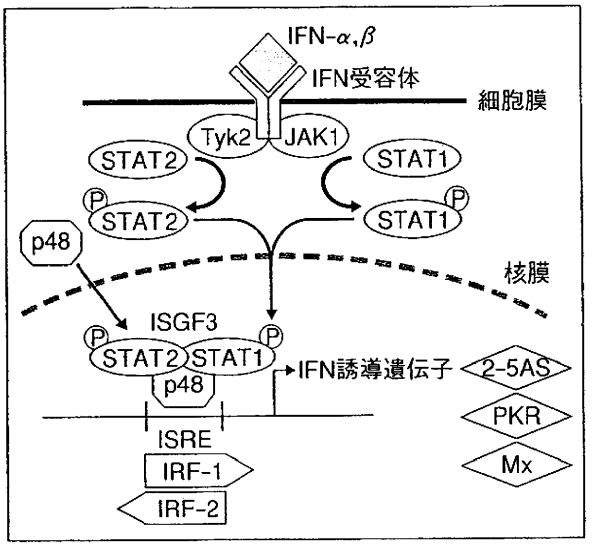

図 1　インターフェロン（IFN）シグナルの伝達経路

ることが可能である。

抗ウイルス蛋白である 2-5AS や PKR の発現は上流に位置する ISRE によって制御されており，このような転写調節因子の発現には個体差が存在することが予想される。また，IFN シグナル伝達経路である JAK-STAT 経路のネガティブ・フィードバック機構として STAT の活性を抑制する cytokine-inducible SH2 protein (CIS) や JAK に結合してそのシグナルの発現を遮断する JAK-binding protein の存在も報告され，これらの IFN 発現遺伝子を制御する因子が IFN 治療効果に関連する可能性がある。

報告されている IFN 関連遺伝子と IFN 治療効果についてはまず，IFN 受容体に関するものがあげられる。IFN が結合する受容体としては IFNAR1 と IFNAR2 があり，前者は IFNα のサブタイプのひとつである IFNα8 にのみ結合し，後者はすべての IFNα と IFNβ に結合することが知られている。この IFN 受容体 mRNA の肝での発現量が高い症例は IFN 療法の効果が高いことが報告されており[2,3]，これは肝での IFNAR2 の蛋白レベルでの発現においても確認されている[4]。また逆に，可溶性 IFNAR2 の発現量が多い症例は IFN の効果が低く[5]，これは IFNα がレセプターに結合するのと拮抗するためと考えられている[6]。また，肝細胞 IFN シグナル関連 mRNA では，JAK-binding protein の発現が高い症例で IFN 著効例が多いと報告されている[7]。

表 1　末梢血リンパ球中の遺伝子発現の組み合わせによるインターフェロン（IFN）治療効果の予測（文献 19 より引用改変）

IFN 投与前 末梢血リンパ球中の遺伝子の組み合わせ	精度，% training	test
1 topoisomerase (DNA) I	53.9	46.2
2 catenin (cadherin-associated protein) β1 (88 kDa)	66.0	57.1
3 Ras-related C3 botulinum toxin substrate 2	91.0	89.1
IFN 投与開始 2 週間後 末梢血リンパ球中の遺伝子の組み合わせ	**精度，% training**	**test**
1 differentiation 6 (septin 2)	28.8	25.8
2 cyclin G1	75.0	64.4
3 cell division cycle 20 homolog (*Saccharomyces cerevisiae*)	90.2	87.9
1 MIHC	28.8	25.0
2 cyclin G1	75.0	64.4
3 cell division cycle 20 homolog (*S. cerevisiae*)	90.2	87.9
1 apoptosis inhibitor 1 (baculoviral IAP repeat-containing 3)	28.8	25.0
2 cyclin G1	75.0	64.4
3 cell division cycle 20 homolog (*S. cerevisiae*)	90.2	87.9

また，IFN はサイトカインの一種であり，免疫系に関与するサイトカインは IFN の作用と密接に関連している。インターロイキン 10(IL-10) は suppressor of cytokine signaling (SOCS) ファミリーとよばれる蛋白を発現して IFNα のシグナルを阻害することが知られており，血清中 IL-10 が高い症例は IFN 療法の効果が低いと報告されてる[8]。同様に IL-6, IL-1, TNF-α なども SOCS や，STAT の活性化を抑制する CIS を誘導することが知られており，これらのサイトカインも血清中濃度が高い症例は IFN の効果が低いと報告されている[9~11]。

● IFN 療法における単一塩基多型（SNP）

遺伝子がコードする open reading frame の中にアミノ酸変異が存在すれば，蛋白の機能に個

体差が生じることになる。また非翻訳領域においても，イントロンやプロモーターの領域に点変異，欠失や挿入などが起これば，mRNA の発現量に大きな影響を与える可能性がある。この特定の単一塩基の違いにより蛋白の質あるいは発現量に個体差が生じる現象を単一塩基多型(single nucleotide polymorphism：SNP)という。今まで述べてきた各種 IFN 関連遺伝子の発現，サイトカイン濃度などは，この SNP 解析にて遺伝子と関連付けられているものがある。

抗インフルエンザウイルス活性を示す Mx1 蛋白が高発現している症例は IFN の効果が高いとされているが，Mx 蛋白のプロモーター領域の-88 の部位に G/T の SNP が存在していることと関連している[12]。このようなプロモーター領域の SNP は IL-10[13]，細胞障害性 T 細胞の活性を抑制する CTLA4[14]，Th1 反応開始に関連する osteopontin[15]などでも認められている。SNP 解析により，IFN 療法に関連した遺伝子の発現量に差がある原因が求められ，同じウイルス量，同じ HCV ジェノタイプでも IFN 療法に差が出る背景であると考えられる。

● 包括的遺伝子情報解析

上述のように IFN 関連遺伝子，サイトカインなどのそれぞれの単一の遺伝子の発現量，あるいは蛋白の発現量により IFN 療法の効果が規定されている可能性が示唆されている。しかしながら，このような単一の遺伝子発現量のみを調べることでは，ウイルス側因子を超える IFN 療法効果予測はできない。これは，それぞれ報告されている遺伝子のみならず，未知の遺伝子を含め IFN 療法に一見関連しないと思われる多数の遺伝子が，実際には複雑に関係し合っているためと考えられる。

われわれは serial analysis of gene expression 法を用いて，正常肝を含むさまざまな肝病態における遺伝子発現プロファイルを検討し，正常肝，慢性 C 型肝炎，肝細胞癌において，それぞれ異なった遺伝子プロファイルを示すことを報告している[16]。また，cDNA マイクロアッセイ法を用いて，同じ慢性肝炎でも，C 型肝炎感染と B 型肝炎感染では異なった遺伝子発現を示すことも明らかにしてきた[17]。

このように包括的に肝内で発現している遺伝子を解析したデータをもとに，われわれは新たに IFN 効果を予測するために，IFN 療法と関連が報告されているものを中心に 295 遺伝子を載せた cDNA chip を作成し，IFN 療法を行った症例について IFN 投与前の肝生検サンプルで検討した。IFN 単独療法を行った 15 症例において発現遺伝子量を適当な解析アルゴリズムを設定することにより，IFN 投与中に血中 HCV RNA の消失が得られなかった症例と，IFN 投与中に HCV RNA 消失が得られた症例(著効例と再燃例をまとめた群)を，ウイルス量，HCV ジェノタイプと無関係に分けることが可能であった[18]。

さらに肝生検よりサンプル取得が容易な末梢血リンパ球中の遺伝子発現をみることで，IFN 治療効果予測が可能であるか検討を行った[19]。遺伝子発現の高低にかかわらず単一の遺伝子では治療効果予測の精度は良くても 50%であったが，**表 1** に示すように適切な遺伝子の組み合わせを SWEEP operator method で選んでいくと，投与前では topoisomerase (DNA) I, catenin, Ras-related C3 botulinum toxin substrate 2 の組み合わせで 91.0%の精度で治療効果予測が可能であった。同様に IFN 投与開始 2 週間後の末梢血リンパ球でも，投与前とは異なった 3 種類の遺伝子発現の組み合わせで精度 90.2%で効果予測が可能であった。

● おわりに

本稿では述べなかったが，IFN 療法のみならず C 型肝炎の病態，進行度に関しても SNP をはじめとするさまざまな遺伝子情報が蓄積されてきている。現在は研究の域を出ていないが，大量，迅速かつ安価に遺伝子情報が解析されるようになれば，個々の患者に合わせたテーラーメイド治療の時代がくるものと期待される。

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Genome-wide association of *IL28B* with response to pegylated interferon-α and ribavirin therapy for chronic hepatitis C

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The recommended treatment for patients with chronic hepatitis C, pegylated interferon-α (PEG-IFN-α) plus ribavirin (RBV), does not provide sustained virologic response (SVR) in all patients. We report a genome-wide association study (GWAS) to null virological response (NVR) in the treatment of patients with hepatitis C virus (HCV) genotype 1 within a Japanese population. We found two SNPs near the gene *IL28B* on chromosome 19 to be strongly associated with NVR (rs12980275, $P = 1.93 \times 10^{-13}$, and rs8099917, 3.11×10^{-15}). We replicated these associations in an independent cohort (combined *P* values, 2.84×10^{-27} (OR = 17.7; 95% CI = 10.0–31.3) and 2.68×10^{-32} (OR = 27.1; 95% CI = 14.6–50.3), respectively). Compared to NVR, these SNPs were also associated with SVR (rs12980275, $P = 3.99 \times 10^{-24}$, and rs8099917, $P = 1.11 \times 10^{-27}$). In further fine mapping of the region, seven SNPs (rs8105790, rs11881222, rs8103142, rs28416813, rs4803219, rs8099917 and rs7248668) located in the *IL28B* region showed the most significant associations ($P = 5.52 \times 10^{-28}$–2.68×10^{-32}; OR = 22.3–27.1). Real-time quantitative PCR assays in peripheral blood mononuclear cells showed lower *IL28B* expression levels in individuals carrying the minor alleles ($P = 0.015$).

Hepatitis C is a global health problem that affects a significant proportion of the world's population. The World Health Organization estimated that in 1999, there were 170 million HCV carriers worldwide, with 3–4 million new cases appearing each year. HCV infection affects more than 4 million people in the United States, where it represents the leading cause of cirrhosis and hepatocellular carcinoma as well as the leading cause of liver transplantation[1].The American Gastroenterological Association estimated that drugs are the largest direct costs of hepatitis C[1].

The most effective current standard of care in patients with chronic hepatitis C, a combination of PEG-IFN-α with ribavirin, does not produce SVR in all patients treated. Large-scale studies on 48-week-long PEG-IFN-α/RBV treatment in the United States and Europe showed that 42–52% of patients with HCV genotype 1 achieved SVR[2–4], and similar results were found in Japan. However, older patients (greater than 50 years of age) had a significantly lower rate of SVR due to poor adherence resulting from adverse events and laboratory-detectable abnormalities such as neutropenia and thrombocytopenia[5,6]. Specifically, various well-described side effects (such as a flu-like syndrome, hematologic abnormalities and adverse neuropsychiatric events) often necessitate dose reduction, and 10–14% of patients require premature withdrawal from interferon-based therapy[7]. To avoid these side effects in patients who will not be helped by the treatment, as well as to reduce the substantial cost of PEG-IFN-α/RBV treatment, it would be useful to be able to predict an individual's response before or early in treatment. Several viral factors, such as genotype 1, high baseline viral load, viral

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Received 29 June; accepted 21 August; published online 13 September 2009; doi:10.1038/ng.449

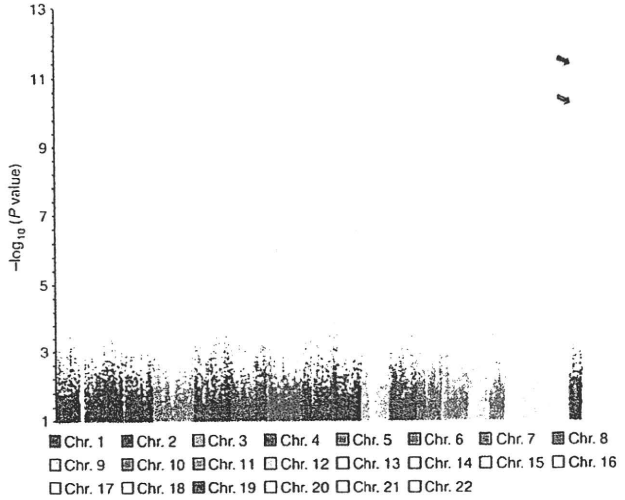

Figure 1 Genome-wide association results with PEG-IFN-α/RBV treatment in 142 Japanese patients with HCV (78 NVR and 64 VR samples). *P* values were calculated by using a χ^2 test for allele frequencies. The dots with arrows for chromosome 19 denote SNPs that showed significant genome-wide associations ($P < 8.05 \times 10^{-8}$) with response to PEG-IFN-α/RBV treatment.

kinetics during treatment, and amino acid pattern in the interferon sensitivity–determining region, have been reported to be significantly associated with the treatment outcome in a number of independent studies[8–10]. Studies have also provided strong evidence that ~20% of patients with HCV genotype 1 and 5% of patients with genotype 2 or 3 have a null response to PEG-IFN-α/RBV. No definite predictor of this resistance is currently available that make it possible to bypass the initial 12–24 weeks' treatment before deciding whether treatment should be continued. If a reliable predictor of non-response were identified for use in patients before treatment initiation, then an estimated 20%, including those who have little or no chance to achieve SVR, could be spared the side effects and cost of treatment.

Host factors, including age, sex, race, liver fibrosis and obesity, have also been reported to be associated with PEG-IFN-α/RBV therapy outcome[11,12]. However, little is known about the host genetic factors that might be associated with the response to therapy: thus far only a few candidate genes, including those encoding type I interferon receptor-1 (*IFNAR1*) and mitogen-activated protein kinase–activated protein kinase 3 (*MAPKAPK3*), have been reported to be associated with treatment response[13,14]. We describe here a GWAS for response to PEG-IFN-α/RBV treatment.

We conducted this GWAS to identify host genes associated with response to PEG-IFN-α/RBV treatment in 154 Japanese patients with HCV genotype 1 (82 with NVR and 72 with virologic response (VR), based on the selection criteria as described in Online Methods). We used the Affymetrix SNP 6.0 genome-wide SNP typing array for 900,000 SNPs. A total of 621,220 SNPs met the following criteria: (i) SNP call rate ≥95%, (ii) minor allele frequency (MAF) ≥1% and (iii) deviation from Hardy-Weinberg equilibrium (HWE) $P \geq 0.001$ in VR samples. After excluding 4 NVR and 8 VR samples that showed quality control (QC) call rates of <95%, 78 NVR and 64 VR samples were included in the association analysis. **Figure 1** shows a genome-wide view of the single-point association data based on allele frequencies. Two SNPs located close to *IL28B* on chromosome 19 showed strong associations, with a minor allele dominant model (rs12980275, $P = 1.93 \times 10^{-13}$, and rs8099917, $P = 3.11 \times 10^{-15}$, respectively), with NVR to PEG-IFN-α/RBV treatment (**Table 1**). The rs8099917 lies between *IL28B* and *IL28A*, ~8 kb downstream from *IL28B* and ~16 kb upstream from *IL28A*. These associations reached genome-wide levels of significance for both SNPs in this initial GWAS cohort (Bonferroni criterion $P < 8.05 \times 10^{-8}$ (0.05/621,220)). The frequencies of minor allele–positive patients were much higher in the NVR group than in the VR group for both SNPs (74.3% in NVR, 12.5% in VR for rs12980275; 75.6% in NVR, 9.4% in VR for rs8099917). Notably, individuals homozygous for the minor allele were observed only in the NVR group. The VR group, as compared to the NVR group, showed genotype frequencies closer to those in the healthy Japanese population[15], yet the minor allele frequencies were slightly higher in the transient virologic response (TVR) group (23.1%, 15.4%) than in the SVR group (9.8%, 7.8%) (**Table 1**). We applied the Cochrane-Armitage test on all the SNPs and found a genetic inflation factor, λ, of 1.029 for the GWAS stage (**Supplementary Fig. 1**). We also carried out principal component analysis in 142 samples for the GWAS stage together with the HapMap samples (CEU, YRI, CHB and JPT) (**Supplementary Fig. 2**); this suggested that the effect of population stratification was negligible.

Table 1 Significant association of two SNPs (rs12980275 and rs8099917) with response to PEG-IFN-α/RBV treatment

					Null responder (NVR[a], *n* = 128)			Responder (VR[a], *n* = 186)			Responder (SVR[a], *n* = 140)			NVR vs. VR		NVR vs. SVR	
dbSNP rsID	Nearest gene	MAF[b] (allele)	Allele (1/2)	Stage	11	12	22	11	12	22	11	12	22	OR (95% CI)[c]	*P* value[d]	OR (95% CI)[c]	*P* value[d]
rs12980275	*IL28B*	0.15 (G)	A/G	GWAS	20 (25.6)	54 (69.2)	4 (5.1)	56 (87.5)	8 (12.5)	0 (0.0)	46 (90.2)	5 (9.8)	0 (0.0)	20.3 (8.3-49.9)	1.93×10^{-13}	26.7 (9.3-76.5)	7.41×10^{-13}
				Replication	10 (20.0)	37 (74.0)	3 (6.0)	101 (82.8)	21 (17.2)	0 (0.0)	73 (82.0)	16 (18.0)	0 (0.0)	19.2 (8.3-44.4)	5.46×10^{-15}	18.3 (7.6-44.0)	8.37×10^{-13}
				Combined	30 (23.4)	91 (71.1)	7 (5.5)	157 (84.4)	29 (15.6)	0 (0.0)	119 (85.0)	21 (15.0)	0 (0.0)	17.7 (10.0-31.3)	2.84×10^{-27}	18.5 (10.0-34.4)	3.99×10^{-24}
rs8099917	*IL28B*	0.12 (G)	T/G	GWAS	19 (24.4)	56 (71.8)	3 (3.8)	58 (90.6)	6 (9.4)	0 (0.0)	47 (92.2)	4 (7.8)	0 (0.0)	30.0 (11.2-80.5)	3.11×10^{-15}	36.5 (11.6-114.6)	5.00×10^{-14}
				Replication	11 (22.0)	37 (74.0)	2 (4.0)	108 (88.5)	14 (11.5)	0 (0.0)	78 (87.6)	11 (12.4)	0 (0.0)	27.4 (11.5-65.3)	9.47×10^{-18}	25.1 (10.0-63.1)	1.00×10^{-14}
				Combined	30 (23.4)	93 (72.7)	5 (3.9)	166 (89.2)	20 (10.8)	0 (0.0)	125 (89.3)	15 (10.7)	0 (0.0)	27.1 (14.6-50.3)	2.68×10^{-32}	27.2 (13.9-53.4)	1.11×10^{-27}

[a]NVR, null virologic response; VR, virologic response; SVR, sustained virologic response. The 186 VRs consisted of 46 transient virologic response (TVRs) and 140 SVRs. [b]Minor allele frequency and minor allele in 184 healthy Japanese individuals[15]. The MAF of the SNPs in SVR is similar to that of TVR group, whereas that of NVR is much higher (76.6%). [c]Odds ratio for the minor allele in a dominant model. [d]*P* value by χ^2 test for the minor allele dominant model.

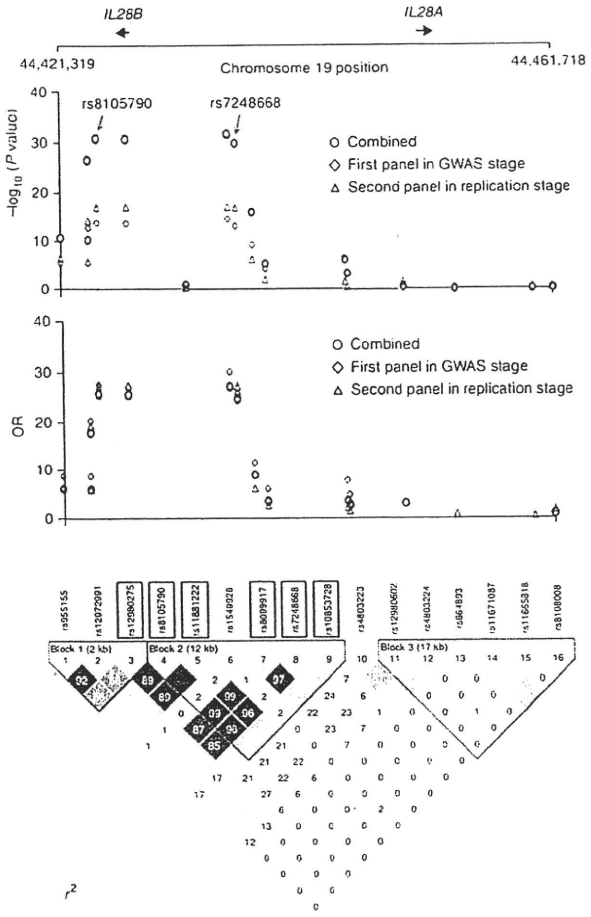

Figure 2 Genomic structure, *P* value and OR plots in association analysis and LD map around *IL28B* and *IL28A* (chr.19, nucleotide positions 44421319–44461718; build 35). *P* values by the χ^2 test for minor allele dominant effect model are shown for the first panel of 142 samples in the GWAS stage, the second panel of 172 samples in the replication stage, and the combined analysis. Below are estimates of pairwise r^2 for 16 SNPs selected in the replication study using a total of 314 Japanese patients with HCV treated with PEG-IFN-α/RBV. Boxes indicate the significantly associated SNPs with response to PEG-IFN-α/RBV treatment both in the GWAS stage and in the replication stage. Dotted lines indicate the region with the strongest associations from the positions of rs8105790 to rs7248668.

We analyzed the region of ~40 kb (chr. 19, nucleotide positions 44421319–44461718; build 35) containing the significantly associated SNPs (rs12980275 and rs8099917) using Haploview software for linkage disequilibrium (LD) and haplotype structure based on the HapMap data for individuals of Japanese ancestry. The LD blocks were analyzed using the four-gamete rule, and four blocks were observed (**Supplementary Fig. 3**). We selected 16 SNPs for both replication study and high-density association mapping, including tagging SNPs estimated on the basis of the haplotype blocks, one SNP located within *IL28B* (rs11881222) and the significantly associated SNPs from the GWAS stage (rs12980275 and rs8099917) (**Supplementary Table 1**).

To validate the results of the GWAS stage, 16 SNPs selected for the replication stage, including the original SNPs, were genotyped using the DigiTag2 assay in an independent set of 172 Japanese patients with HCV treated with PEG-IFN-α/RBV treatment (50 NVR and 122 VR samples), together with the first panel of 142 samples analyzed in the GWAS stage (**Supplementary Table 1**). The associations of the original SNPs were replicated in the replication cohort of 172 patients ($P = 5.46 \times 10^{-15}$, OR = 19.2 for rs12980275; $P = 9.47 \times 10^{-18}$, OR = 27.4 for rs8099917; **Table 1**). The combined *P* values for both stages reached 2.84×10^{-27} (OR = 17.7; 95% CI = 10.0–31.3) and 2.68×10^{-32} (OR = 27.1; 95% CI = 14.6–50.3), respectively (**Table 1**). Notably, when we compared the SVR ($n = 140$) with the NVR group ($n = 128$), the original two SNPs (rs12980275 and rs8099917) again showed strong associations: both *P* values and ORs were similar to those observed in the comparison between VR and NVR, and the combined *P* values for both stages reached 3.99×10^{-24} (OR = 18.5; 95% CI = 10.0–34.4) and 1.11×10^{-27} (OR = 27.2; 95% CI = 13.9–53.4), respectively (**Table 1**). Comparing SVR ($n = 140$) versus NVR plus TVR ($n = 174$), we again found that these SNPs were significantly associated ($P = 1.71 \times 10^{-16}$, OR = 8.8; 95% CI 5.1–15.4 for rs12980275; $P = 1.18 \times 10^{-18}$, OR = 12.1; 95% CI 6.5–22.4 for rs8099917, **Supplementary Table 2**), suggesting that these SNPs would predict NVR as well as SVR before PEG-IFN-α/RBV therapy.

Among the newly analyzed SNPs in the replication study, six (rs12980275, rs8105790, rs11881222, rs8099917, rs7248668 and rs10853728) showed significant associations both in the GWAS stage ($P < 8.05 \times 10^{-8}$) and in the replication stage ($P < 0.0031$ (0.05/16)) after Bonferroni correction. These SNPs are located within a 15.7-kb region that includes *IL28B* (Fig. 2 and **Supplementary Table 1**). In particular, the strongest associations with NVR were observed for four SNPs, rs8105790, rs11881222, rs8099917 and rs7248668, that are located in the downstream flanking region, the third intron and the upstream flanking region of *IL28B*. The combined *P* values for these polymorphisms were 1.98×10^{-31} (OR = 25.7; 95% CI = 13.9–47.6), 2.84×10^{-31} (OR = 25.6; 95% CI = 13.8–47.3), 2.68×10^{-32} (OR = 27.1; 95% CI = 14.6–50.3) and 1.84×10^{-30} (OR = 24.7; 95% CI = 13.3–45.8), respectively (**Supplementary Table 1**). We then sequenced this region to identify further variants and found three SNPs (rs8103142, rs28416813 and rs4803219) located in the third exon, the first intron and the upstream flanking region of *IL28B*, and a few infrequent variations. These SNPs also showed strong associations in the combined dataset of 128 NVR and 186 VR samples ($P = 1.40 \times 10^{-29}$, OR = 26.6 for rs8103142; $P = 5.52 \times 10^{-28}$, OR = 22.3 for rs28416813; $P = 2.45 \times 10^{-29}$, OR = 23.3 for rs4803219; **Supplementary Table 3**). We also performed LD and haplotype analyses with seven SNPs. These SNPs were in strong LD, and the risk haplotype showed a level of association similar to those of individual SNPs ($P = 1.35 \times 10^{-25}$, OR = 11.1; 95% CI = 6.6–18.6) (**Table 2**). These results suggest that the association with NVR was primarily driven by one of these SNPs.

Table 2 Association analysis of response to treatment by *IL28B* haplotype

SNP							Frequencies			
rs8105790	rs11881222	rs8103142	rs28416813	rs4803219	rs8099917	rs7248668	NVR group	VR group	*P* value	OR (95% CI)
T	A	T	C	C	T	G	0.543	0.942	1.81×10^{-32}	0.1 (0.04–0.12)
C	G	C	G	T	G	A	0.387	0.054	1.35×10^{-25}	11.1 (6.6–18.6)

Association analysis of haplotypes consisting of seven SNPs with response to PEG-IFN-α/RBV treatment in 314 Japanese patients with HCV. Boldface letters: rs11881222 (third intron); rs8103142 (third exon).

Table 3 Factors associated with NVR by logistic regression model

Factors	Odds ratio	95% CI	*P* value
rs8099917 (G allele)	37.68	16.71–83.85	<0.0001
Age	1.02	0.98–1.07	0.292
Gender (Female)	3.32	1.49–7.39	0.003
Re-treatment[a]	1.12	0.55–2.33	0.750
Platelet count	0.93	0.87–1.01	0.080
Aminotransferase level	1.00	0.99–1.00	0.735
Fibrosis stage[2,6]	1.10	0.73–1.66	0.658
HCV-RNA level	1.01	0.99–1.02	0.139

[a]Re-treatment, non-response to previous treatment with interferon-α (plus RBV).

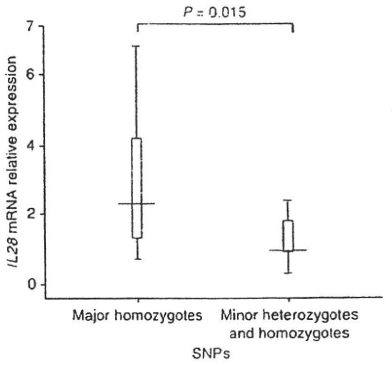

Figure 3 Quantification of *IL28* mRNA expression. The expression level of *IL28* genes was determined by real-time quantitative RT-PCR using RNA purified from peripheral blood mononuclear cells. Distribution of relative gene expression levels was compared between the individuals homozygous for major alleles ($n = 10$) and the heterozygous or homozygous individuals carrying minor alleles ($n = 10$) of rs8099917 by using the Mann-Whitney *U*-test. The bars indicate the median. All samples were obtained from HCV-infected patients before PEG-IFN-α/RBV therapy.

To examine the relative contribution of factors associated with NVR, we used a logistic regression model. One tagging SNP located within *IL28B* (minor allele of rs8099917) was the most significant factor for predicting NVR, followed by gender (**Table 3**). Clinically, viral factors such as HCV genotype and HCV RNA level are important for the outcome of PEG-IFN-α/RBV therapy. Indeed, mean HCV-RNA level was significantly lower in SVR (SVR versus TVR, $P = 0.002$; SVR versus NVR, $P = 0.016$; **Supplementary Table 4**). Mean platelet count and the proportion of mild fibrosis (F1–F2) were significantly higher in SVR than in NVR.

Real-time quantitative PCR assays in peripheral blood mononuclear cells revealed a significantly lower level of *IL28* mRNA expression in individuals with the minor alleles (**Fig. 3**), suggesting that variant(s) regulating *IL28* expression is associated with a response to PEG-IFN-α/RBV treatment. *IL28B* encodes a cytokine distantly related to type I (α and β) interferons and the interleukin (IL)-10 family. This gene and *IL28A* and *IL29* (encoding IL-28A and IL-29, respectively) are three closely related cytokine genes that encode proteins known as type III IFNs (IFN-λs) and that form a cytokine gene cluster at chromosomal region 19q13 (ref. 16). The three cytokines are induced by viral infection and have antiviral activity[16,17]. All three interact with a heterodimeric class II cytokine receptor that consists of IL-10 receptor beta (IL10Rβ) and IL-28 receptor alpha (IL28Rα, encoded by *IL28RA*)[16,17], and they may serve as an alternative to type I IFNs in providing immunity to viral infection.

Notably, a recent report showed that the strong antiviral activity evoked by treating mice with TLR3 or TLR9 agonists was significantly reduced in both $IL28RA^{-/-}$ and $IFNAR^{-/-}$ mice, indicating that IFN-λ is important in mediating antiviral protection by ligands for TLR3 and TRL9 (ref. 18). IFN-λ induced a steady increase in the expression of a subset of IFN-stimulated genes, whereas IFN-α induced the same genes with more rapid and transient kinetics[19]. Therefore, it is possible that IFN-λ induces a slower but more sustained response that is important for TLR-mediated antiviral protection. This might be one of the ways that a genetic variant regulating *IL28* expression influences the response to PEG-IFN-α/RBV treatment. Further research will be required to fully understand the specific mechanism by which a genotype might affect the response to treatment.

In conclusion, the strongest associations with NVR were observed for seven SNPs, rs8105790, rs11881222, rs8103142, rs28416813, rs4803219, rs8099917 and rs7248668, that are located in the downstream flanking region, the third intron, the third exon, the first intron and the upstream flanking region of *IL28B*. Further studies following our report of this robust genetic association to NVR may make it possible to develop a pre-treatment predictor of which individuals are likely to respond to PEG-IFN-α/RBV treatment. This would remove the need for the initial 12–24 weeks of treatment that is currently used as a basis for a clinical decision about whether treatment should be continued. That would allow better targeting of PEG-IFN-α/RBV treatment, avoiding the unpleasant side effects that commonly accompany the treatment where it is unlikely to be beneficial, and reduce overall treatment costs. Because of the small number of samples in this study, we plan to conduct a further prospective multicenter study to establish these SNPs as a clinically useful marker.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

This study was supported by a grant-in-aid from the Ministry of Health, Labour, and Welfare of Japan (H19-kannen-013). This study is based on 15 multicenter hospitals throughout Japan, in the Hokkaido area (Hokkaido University Hospital), Kanto area (Saitama University Hospital; Konodai Hospital; Musashino Red Cross Hospital; Tokyo Medical and Dental University Hospital), Koshin area (Shinshu University Hospital; Kanazawa University Hospital), Tokai area (Nagoya City University Hospital), Kinki area (Kyoto Prefectural University of Medicine Hospital; National Hospital Organization Osaka National Hospital; Hyogo College of Medicine Hospital) and Chugoku/Shikoku area (Tottori University Hospital; Ehime University Hospital; Yamaguchi University Hospital; Kawasaki Medical College Hospital). We thank Y. Uehara-Shibata, Y. Ogasawara, Y. Ishibashi and M. Yamaoka-Sageshima (Tokyo University) for technical assistance; A. Matsumoto (Shinshu), K. Naiki (Saitama), K. Nishimura (Kyoto), H. Enomoto (Hyogo), K. Oyama (Tottori) and the Ochanomizu Liver Conference Study Group for collecting samples; M. Watanabe (Tokyo Medical and Dental University), S. Kaneko (Kanazawa University) and M. Onji (Ehime University) for their advice throughout the study; and H. Ito (Aichi Cancer Center) for conducting statistical analyses.

AUTHOR CONTRIBUTIONS

Study design and discussion: Y.T., N.N., N.M., K.T., M.M.; sample collection: Y.T., M.K., K.M., N.S., M.N., M.K., K.H., S.H., Y.I., E.M., E.T., S.M., Y.M., M.H., A.S., Y.H., S.N., I.S., M.I., K.I., K.Y., F.S., N.I.; genotyping: N.N.; statistical analysis: N.N., A.K., K.I.; quantitative RT-PCR: M.S.; manuscript writing: Y.T., N.N., K.T., M.M.

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ONLINE METHODS

Study cohorts. From April 2007 to April 2009, samples were obtained from 314 patients with chronic HCV (genotype 1) infection who were treated at 15 multicenter hospitals (liver units with hepatologists) throughout Japan. Each patient was treated with PEG-IFN-α2b (1.5 μg per kg body weight (μg/kg) subcutaneously once a week) or PEG-IFN-α2a (180 μg/kg once a week) plus RBV (600–1,000 mg daily depending on body weight). As a reduction in the dose of PEG-IFN-α and RBV can contribute to a less sustained virological response[21], only patients with an adherence of >80% dose for both drugs during the first 12 weeks were included in this study. HBsAg-positive and/or anti-HIV–positive individuals were excluded from this study.

NVR (seen in ~20% of total treated patients) was defined as less than a 2-log-unit decline in the serum level of HCV RNA from the pre-treatment baseline value within the first 12 weeks and detectable viremia 24 weeks after treatment. VR was defined as the achievement of SVR or transient TVR in this study; SVR was defined as undetectable HCV RNA in serum 6 months after the end of treatment, whereas TVR was defined as a reappearance of HCV RNA in serum after treatment was discontinued in a patient who had undetectable HCV RNA during the therapy or on completion of the therapy. Of 878 patients with HCV genotype 1 treated by PEG-IFN-α/RBV at 14 hospitals, only 114 (13.0%) met the criteria for NVR in this study. For the GWAS stage of the study, a case-control study was conducted comparing individuals with NVR (82 individuals) and VR (72 individuals). For the replication stage, an independent cohort of samples from 172 Japanese patients with HCV genotype 1, including 50 with NVR and 122 with VR, was obtained from an independent cohort study at Tokyo Medical and Dental University Hospital (Ochanomizu Liver Conference Study Group) and Musashino Red Cross Hospital. Clinical data from the combined cohorts, with a total of 140 SVR, 46 TVR and 128 NVR patients, are shown in **Supplementary Table 4**.

Informed consent was obtained from each patient who participated in the study. The study protocol conforms to the relevant ethical guidelines as reflected in *a priori* approval by the ethics committees of all the participating universities and hospitals.

SNP genotyping and data cleaning. In the GWAS stage, we genotyped 154 Japanese patients with HCV receiving PEG-IFN-α/RBV treatment using the Affymetrix Genome-Wide Human SNP Array 6.0 according to the manufacturer's instructions. After exclusion of 4 NVR samples and 8 SVR samples with QC call rates <95%, the remaining 142 samples were recalled using the Birdseed version 3 software (Affymetrix). The average overall call rate of 78 NVR and 64 VR samples reached 99.46% and 99.46%, respectively. We then applied the following thresholds for QC in data cleaning: SNP call rate ≥95% for all samples, MAF ≥1% for all samples and HWE *P* value ≥0.001 for VR group[22,23]. A total of 621,220 SNPs on autosomal chromosomes passed the QC filters and were used for association analysis. All cluster plots for the SNPs showing $P < 0.001$ in association analyses by comparing allele frequencies in NVR and VR groups were checked by visual inspection. SNPs with ambiguous genotype calls were excluded. **Supplementary Table 5** shows SNPs that might be weakly associated with NVR ($P < 10^{-4}$).

Although the 12 samples noted above were excluded from the GWAS stage by data cleaning, their quality was good enough for the SNP typing in the replication study, and thus they were included in the replication stage. In the subsequent replication stage with high-density association mapping, SNP genotyping in the independent set of 172 patients was completed using the DigiTag2 assay[24] and direct sequencing using the Applied Biosystems 3730 DNA Analyzer (Applied Biosystems). In addition, strongly associated SNPs identified in the GWAS stage were also genotyped for the GWAS samples using the DigiTag2 assay, and the results were 100% concordant to those from the GWAS platform.

Screening for new polymorphisms. To determine possible genomic variants in the region of *IL28B* and its promoter, we sequenced the 3.3-kb region in a total of 48 Japanese patients with HCV (28 NVR and 20 VR). We selected 7 samples from NVR patients who were minor allele homozygotes for 2 SNPs (rs12980275 and rs8099917), 11 samples from NVR and 10 samples from VR heterozygotes, and 10 samples from NVR and 10 samples from VR major allele homozygotes. The sequencing primers were designed using the Visual OMP Nucleic Acid software (**Supplementary Table 6**). PCR was carried using TaKaRa LA *Taq* polymerase (Takara Biochemicals) under the following thermal cycler conditions: stage 1, 94 °C for 1 min; stage 2, 98 °C for 10 s, 68 °C for 15 min, for a total of 30 cycles; stage 3, 72 °C for 10 min. A 50-μl PCR analysis was performed using 2.5 U TaKaRa LA *Taq* with 1× LA PCR buffer II, 0.4 mM dNTP, 10 pmol of each primer and 10 ng of genomic DNA. For sequencing, 7.0 μl of the PCR products were incubated with 3 μl of Exonuclease I/Shrimp Alkali Phosphatase (Takara Biochemicals) first for 90 min at 37 °C and then for another 10 min at 80 °C. Sequencing reactions were performed with the use of a BigDye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems). After purification with MultiScreen-HV (Millipore) and Sephadex G-50 Fine (GE Healthcare UK Ltd.), the reaction products were applied to the Applied Biosystems 3730 DNA Analyzer.

In the variation screening, three SNPs (rs8103142, rs28416813 and rs4803219) and a few infrequent variations were detected. We then typed these SNPs in all of the 314 patients.

Statistical analysis. The observed association between a SNP and response to PEG-IFN-α/RBV treatment was assessed by χ^2 test with a two-by-two contingency table in three genetic models: allele frequency model, dominant-effect model and recessive-effect model. SNPs on the X chromosome were removed because gender was not matched between the NVR group and the VR group. A total of 621,220 SNPs passed the QC filters in the GWAS stage; therefore, significance levels after the Bonferroni correction for multiple testing were $P = 8.05 \times 10^{-8}$ (0.05/621,220) in the GWAS stage and $P = 0.0031$(0.05/16) in the replication stage. None of the 16 markers genotyped in the replication stage showed deviations from Hardy-Weinberg equilibrium in the VR group ($P > 0.05$).

The inflation factor λ was estimated based on the median χ^2 and revealed to be 1.029 (median) and 1.011 (mean), suggesting that the population substructure should not have any substantial effect on the statistical analysis (**Supplementary Fig. 1**). In addition, the principal component analysis on the 142 patients (78 NVR samples and 64 VR samples) analyzed in the GWAS stage together with the HapMap samples also revealed that the effect of population stratification was negligible (**Supplementary Fig. 2**).

For the replication study and the high-density association mapping, 16 SNPs were selected from the region of ~40 kb (chr. 9, nucleotide positions 44421319–44461718; build 35) containing the significantly associated SNPs (rs12980275 and rs8099917) in the GWAS stage by analyzing, using Haploview software, LD and haplotype structure based on the HapMap data for individuals of Japanese descent. These SNPs included tagging SNPs estimated on the basis of haplotype blocks, SNPs located within the *IL28B* and *IL28A* genes (rs11881222 and rs576832, respectively) and the significantly associated SNPs identified in the GWAS stage (**Supplementary Table 1**). On the basis of the genotype data from the total of 314 patients in the GWAS stage and replication stages, haplotype blocks were estimated using the four-gamete rule, and three blocks were observed (**Fig. 2**). Association of haplotype with response to PEG-IFN-α/RBV treatment was analyzed using Haploview software.

The logistic regression model was used to assess the factors associated with NVR. STATA 10 (Statacorp LP) was used for all analysis. Age, platelet count, and aminotransferase (ALT) and HCV-RNA levels were applied as continuous variables.

Real-time quantitative RT-PCR for *IL28* gene. A layer of mononuclear cells was collected via Ficoll from peripheral blood. Total RNA was isolated using the RNeasy Mini Kit and the RNase-Free DNase Set (Qiagen) according to the manufacturer's protocol. First-strand cDNA was synthesized using SuperScript II reverse transcriptase with Oligo $(dT)_{12-18}$ primer (Invitrogen). The relative quantification of the target gene was determined using Custom TaqMan Gene Expression Assays, and the expression of glyceraldehyde-3-phosphate dehydrogenase was used to normalize the gene expression level (Applied Biosystems) according to the manufacturer's protocol. The data were analyzed by the $2\{-\Delta\Delta C_t\}$ method using Sequence Detector version 1.7 software (Applied Biosystems). A standard curve was prepared by serial tenfold dilutions of

doi:10.1038/ng.449

human cDNA. The curve was linear over 7 logs with a correlation coefficient of 0.998. The specific detection of *IL28B* in real-time PCR is hard to establish, because the nucleotide differences between *IL28A* and *IL28B* consist of only 9 nucleotides scattered throughout the gene. Primers and probes are designed for the *IL28* gene (**Supplementary Table 6**).

URLs. The results of the present GWAS have been registered at a public database: https://gwas.lifesciencedb.jp/cgi-bin/gwasdb/gwas_top.cgi.

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Transfusion Medicine, 2009, **19**, 172–179 doi: 10.1111/j.1365-3148.2009.00930.x

ORIGINAL ARTICLE

Effect of selective vaccination on a decrease in the rate of hepatitis B virus-positive Japanese first-time blood donors

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Received 6 November 2008; accepted for publication 4 February 2009

SUMMARY. The government of Japan started a selective vaccination programme to prevent mother-to-infant infection by hepatitis B virus (HBV) since January 1986. The effect of the programme on first-time blood donors has not been examined in detail. Data of first-time blood donors aged 16–25 years from 1996 to 2007 were extracted from the Japanese Red Cross (JRC) donors' database. Principal component analysis (PCA) was used to visualize the birth-year-dependent group of rate of HBV-positive donors. According to the birth of year, donors were divided into four groups by PCA. After the start of the programme, donors born in 1986–1989 comprised a single group. Before the start of the programme, three groups (1980, 1981–1984 and 1985) were identified. Although a significant time-dependent decrease in the rate of HBV-positive donors was observed before the start of the programme, a significant difference in the rate of HBV-positive donors was observed around the start of the programme by regression analysis for 16–19-year-old first-time blood donors. The selective vaccination programme has been effective to prevent the vertical transmission of HBV from the analysis of first-time blood donors. On the other hand, vaccination of blood donors should be considered to reduce the risk of post-transfusion HBV infection, because the horizontal transmission increases in HBV-positive blood donors.

Key words: first-time blood donors, HBV selective vaccination, principal component analysis, regression analysis.

South and East Asia including Japan was an epidemic area of hepatitis B virus (HBV). From the report of the Japanese Ministry of Health, Labour and Welfare in 2002, the number of HBV-infected patients was 97 000, and asymptomatic carriers were estimated to be 1·1–1·4 million. It is reported that the estimated number of HBV carriers was 0·63% and that of hepatitis C virus (HCV) carriers was 0·49% among Japanese first-time blood donors in 1995–2000 (Tanaka *et al.*, 2004). However, recently, HBV infection rate among Japanese first-time blood donors has been decreasing markedly. The recent rate of positive first-time blood donors for the HBV surface antigen (HBsAg) was < 0·22%.

Infection routes of HBV were divided into two main routes, the vertical (mother-to-infant) and horizontal routes. Most of the vertical infections become chronic and most of the horizontal infections end transiently.

Since January 1986, the government of Japan started a nationwide programme to prevent mother-to-infant infection by HBV (Shiraki, 1994; Shiraki *et al.*, 1996; Inui *et al.*, 2007). Every pregnant woman has been screened for serum HBsAg and the HBV e antigen

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(HBeAg). Newborn infants whose mothers were positive for HBeAg were received an immunoprophylaxis treatment by administrating a hepatitis B vaccine and hepatitis B immunoglobulin (HBIG)

The Ministry of Health and Welfare issued a notification on the use of disposable syringes in addition to disposable needles in 1988. This notification might contribute to reducing the risk of iatrogenic HBV infections when babies were administered several mandatory vaccines. With the implementation of this prevention programme, transmission of HBV decreased markedly yearly. On the other hand, horizontal infection with HBV genotype A, which seldom appeared in Japan several years ago, has increased recently in both patients and donors (Orito *et al.*, 2001; Murokawa *et al.*, 2005; Sugauchi *et al.*, 2006; Takeda *et al.*, 2006; Hayashi *et al.*, 2007).

To investigate the recent epidemiology of HBV infection and the effectiveness of the Japanese vaccination programme for the prevention of mother-to-infant transmission of HBV, the data of HBsAg-positive Japanese donors aged 16–25 years were used for principal component analysis (PCA) and regression analysis.

MATERIALS AND METHODS

The Japanese government started a nationwide hepatitis B vaccination programme in January 1986 for infants born to HBV-carrier mothers to prevent perinatal infection of HBV (Shiraki, 1994; Shiraki *et al.*, 1996; Inui *et al.*, 2007). Initially, the Japanese vaccination programme covered only neonates born to mothers who were positive for both HBsAg and the HBeAg. In 1995, the vaccination programme was extended to all neonates born to mothers who were HBsAg carriers regardless of the mother's HBeAg/antibody status. More than 92% of all the pregnant women in Japan were enrolled in the programme (Inui *et al.*, 2007).

The number of first-time blood donors and HBsAg-positive donors aged 16–25 years was extracted from JRC database from 1996 to 2007. To investigate the present state of HBV infection, the presence of the immunoglobulin-M antibody against the HBV core antigen (IgM-HBcAb) was determined among all HBsAg-positive donors from October 2006 to September 2007. The Japanese screening system was reported previously (Iizuka *et al.*, 1992; Yugi *et al.*, 2006). The nucleic acid amplification technology (NAT) system has been reported elsewhere (Mine *et al.*, 2003). IgM-HBcAb was tested by enzyme immunoassay (Abbott Laboratories, IL, USA).

Statistical analysis

The effect of the Japanese vaccination programme on the rate of HBsAg-positive donors was examined by principal component analysis (PCA) and regression analysis.

The rate of HBsAg-positive 16–18-year-old donors for every birth of year from 1980 to 1989 was visually grouped by PCA (Appendix) using the free software R (http://www.r-project.org/).

The difference in the rate of HBsAg-positive donors between before and after the implementation of the vaccination programme was analysed. We assumed the different slope around 1986 and intended to verify the assumption by regression analysis using the following equation

$$y_n = \alpha_n + \beta_n x_1 + \gamma_n x_2 + \delta_n D + \varepsilon_n, \qquad (1)$$

where α is a constant, β the coefficient of slope after 1980, γ the additional coefficient of slope after 1986, δ the coefficient (D) that shows the gap of HBsAg-positive rate around 1986, ε the error term that meets the standard assumption, n the age from 16 to 25 years old and x_1, x_2 the rate of positive donors of years born from 1980 to 1991. Regression analysis was carried out using the Microsoft Office Excel software.

RESULTS

Time-dependent changes in the rate of HBsAg-positive first-time blood donors of each generation from 1996 to 2007 are shown in Fig. 1. The rate of HBsAg-positive younger generations was lower than those of older generations. The rate of HBsAg-positive donors of all generations decreased yearly from 0·83% in 1996 to 0·22% in 2007 (data not shown).

Present state of numbers of blood donors and numbers of HBV-infected blood donors in Japan is shown in Table 1. From October 2006 to September 2007, the total number of donors was 4 974 911: the number of HBsAg-positive donors was 2043 (0·041%), the number of first-time blood donors was 594 096 and the number of HBsAg-positive first-time blood donors was 1362 (0·229%). Among 61 IgM-HBcAb-positive donors, 35 were repeat donors who have been infected after the last donation. Among 90 HBsAg-negative and NAT-positive donors, 22 were considered to have occult HBV infection on the basis of their being HBsAg-negative, HBV-DNA-positive and IgG-HBcAb-positive. Then serological window period donors were 68 among NAT-positive donors.

The rate of HBsAg-positive Japanese donors from 16 to 25 years old was extracted from JRC database from

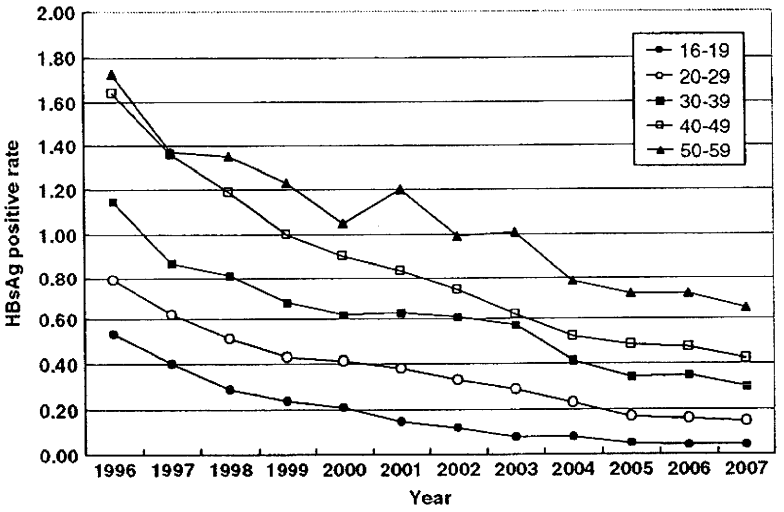

Fig. 1. Age–time-dependent rate of HBsAg-positive first-time blood donors from 1996 to 2007. Data of donors in their 60s are omitted because the number of first-time blood donors in their 60s is so small that the amplitude of the rate of HBsAg-positive donors becomes large unexpectedly.

Table 1. Present state of numbers of blood donors and numbers of HBV-infected blood donors from October 2006 to September 2007 in Japan

	Total blood donors			First-time blood donors			Horizontal infection	
Age	Number of donors	Number of HBsAg-positive donors	Rate (%) of HBsAg-positive donors	Number of donors	Number of HBsAg-positive donors	Rate (%) of HBsAg-positive donors	IgM-HBcAb-positive donors	NAT-positive donors
16	37 717	3	0·008	30 436	3	0·010	0	0
17	53 388	10	0·019	29 277	10	0·034	0	0
18	118 711	31	0·026	66 617	29	0·044	1	0
19	130 391	25	0·019	51 080	20	0·039	2(1)*	2
20	124 224	31	0·025	33 847	27	0·080	4(3)	2
21	120 609	28	0·023	25 583	22	0·086	3(2)	4
22	118 215	38	0·032	22 806	32	0·140	2(1)	1
23	118 974	38	0·032	20 640	30	0·145	3(2)	4
24	115 434	37	0·032	17 873	32	0·179	2(1)	3
25	110 247	38	0·034	15 574	30	0·193	2	4
26–29	452 645	172	0·038	50 433	130	0·258	6(3)	9
30–39	1 375 372	499	0·036	112 620	333	0·296	24(19)	25
40–49	1 077 348	487	0·045	64 232	286	0·445	9(2)	10
50–59	773 571	484	0·063	44 004	296	0·673	3(1)	15(12)†
60–69	248 065	122	0·049	9 074	82	0·904	0	11(10)
Total	4 974 911	2043	0·041	594 096	1362	0·229	61(35)	90(22)

*Number of repeated donors are shown in parenthesis.
†Number of IgG-HBcAb-positive donors (occult donors) are shown in parenthesis.

1996 to 2007 to investigate the effectiveness of the Japanese vaccination programme. The rates of HBsAg-positive first-time blood donors from 16 to 25 years old who are born from 1980 to 1991 are shown in Table 2.

The bold line between data in 1985 and those in 1986 shows the boundary before and after the implementation of the Japanese vaccination programme. The lowest column in Table 2 shows that the rate of HBsAg-positive 16-year-old donors who were born in 1991, and became acceptable as blood donors for the first time in 2007, was 0·018%.

To visualize the difference in the rate of HBsAg-positive donors around the start of the vaccination programme, PCA was carried out using the data within the frame of the dotted line in Table 2. From the result of the PCA of HBsAg-positive 16- to 18-year-old donors born from 1980 to 1989, the donors can be divided into four groups (Fig. 2).

