Res 2010；40：851-852.
18) Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008；359：378-390.
19) Villanueva A, Hernandez-Gea V, Llovet JM. Medical therapies for hepatocellular carcinoma: a critical view of the evidence. Nat Rev Gastroenterol Hepatol 2013；10：34-42.
20) Wei Z, Doria C, Liu Y. Targeted therapies in the treatment of advanced hepatocellular carcinoma. Clin Med Insights Oncol 2013；7：87-102.
21) Finn RS. Emerging targeted strategies in advanced hepatocellular carcinoma. Semin Liver Dis 33 Suppl 2013；1：S11-19.
22) Yeo W, Chung HC, Chan SL, et al. Epigenetic therapy using belinostat for patients with unresectable hepatocellular carcinoma: a multicenter phase I/II study with biomarker and pharmacokinetic analysis of tumors from patients in the Mayo Phase II Consortium and the Cancer Therapeutics Research Group. J Clin Oncol 2012；30：3361-3367.
23) Fujimoto A, Totoki Y, Abe T, et al. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. Nat Genet 2012；44：760-764.
24) Li X, Feng GS, Zheng CS, et al. Expression of plasma vascular endothelial growth factor in patients with hepatocellular carcinoma and effect of transcatheter arterial chemoembolization therapy on plasma vascular endothelial growth factor level. World J Gastroenterol 2004；10：2878-2882.
25) Pawlik TM, Reyes DK, Cosgrove D, et al. Phase II trial of sorafenib combined with concurrent transarterial chemoembolization with drug-eluting beads for hepatocellular carcinoma. J Clin Oncol 2011；29：3960-3967.
26) Scheel C, Weinberg RA. Cancer stem cells and epithelial-mesenchymal transition: concepts and molecular links. Semin Cancer Biol 2012；22：396-403.
27) Fortune BE, Umman V, Gilliland T, et al. Liver transplantation for hepatocellular carcinoma: a surgical perspective. J Clin Gastroenterol 47 2013；Suppl：S37-42.
28) Todo S, Furukawa H, Tada M. Extending indication: role of living donor liver transplantation for hepatocellular carcinoma. Liver Transpl 2007；13：S48-54.
29) 嶋村 剛，藤堂 省．脈管浸潤からみた肝移植成績．肝胆膵 2013；66：967-979.
30) Cheng JW, Lv Y. New progress of non-surgical treatments for hepatocellular carcinoma. Med Oncol 2013；30：381.
31) Yamashita YI, Shirabe K, Tsuijita E, et al. Third or more repeat hepatectomy for recurrent hepatocellular carcinoma. Surgery 2013（in press）

Summary

Current perspectives for treatment of hepatocellular carcinoma.

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Begining in the 1980s, multidisciplinary therapies for hepatocellular carcinoma, including liver resection, radiofrequency ablation, ethanol injection, and/or transarterial chemoembolization were developed and the prognosis of patients with hepatocellular carcinoma (HCC) markedly improved. Recently, in addition to those conventional therapies, new strategies were introduced, including laparoscopic and robotic (Da Vinci) liver resection, liver transplantation, molecular targeted therapy, and proton therapy. In this review, we present perspectives on recent treatments for the hepatocellular carcinoma. We think that regular follow-up of the patients and the construction of a multidisciplinary medical treatment system as well as the development of new treatments and strategies through accumulation of new knowledge will lead to improvement of the prognosis of hepatocellular carcinoma.

一般演題

高齢のC型肝炎症例に対するインターフェロン治療成績

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索引用語　C型慢性肝炎、インターフェロン、高齢者

要　旨　高齢者のC型慢性肝炎症例における治療効果と副作用に関し、非高齢者と比較検討した。高齢者群ではジェノタイプ1b、高ウイルス量の難治例の割合が有意に高かった。全体のSVR率は、高齢者群で31%、非高齢者群で48%であり、ジェノタイプ1bで高ウイルス量の症例におけるSVR率は高齢者群で19%、非高齢者群で36%であった。薬剤のadherenceをみると、高齢者でリバビリン投与量が減量となる症例が多かった。インターフェロン・リバビリン併用療法における治療中止理由の検討では、高齢者で細菌感染症に伴う中止例が多かった。単独治療では高齢者群でも副作用による中止はなく、安全に施行しうると考えられた。

はじめに

C型慢性肝炎は肝硬変・肝細胞癌の最も大きな原因であり、特に高齢者においては発癌リスクが非高齢者よりも有意に高いことが指摘されており[1,2]、治療ガイドラインにおいて65歳以上の高齢者群に対して積極的な抗ウイルス治療が勧められるようになった。特に本邦においては、欧米と比較すると高齢者の占める割合が高く[3]、高齢者の治療においては副作用が強くSVR率が低いことも指摘されているため[4]、個々の症例に応じた適切な治療方針を考えていく必要がある。そこで、高齢のC型慢性肝炎症例に対する治療方針決定における注意点を検討する目的で、当院における治療症例の治療効果と副作用に関し、非高齢者と比較検討した。

対象と方法

当院でインターフェロン治療を施行したC型慢性肝炎75例（2010年10月以降）および、慶友会吉田病院肝臓病センターでインターフェロン治療を施行したC型慢性肝炎160例（1999～2010年9月）の計235例を対象とした。

インターフェロン・リバビリン併用治療症例（220例）における治療効果および副作用に関して、65歳以上の高齢者と65歳未満の非高齢者を比較検討した。治療内容の内訳は、PegIFNα2a＋リバビリンが95症例、PegIFNα2b＋リバビリンが119症例、βIFN＋リバビリンが6症例であった。これらのうち195症例が初回治療、25症例が2回目以上の治療であった。また、インターフェロン単独長期投与症例（15例）にお

ける治療継続状態および副作用に関して検討した。単独治療症例では、IFNα自己注射（週2～3回）が5症例、PegIFNα2a（週1回～2週に1回）が10症例であり、7症例が初回治療、8症例が2回目以上の治療であった。

統計学的解析は、Mann-Whitney testおよびChi-square testを用いた。

成　績

近年の治療対象症例の動向を確認する目的で、1999～2010年9月に治療された160例と2010年10月以降に治療された75例の年齢分布を比較した。2010年以前の治療症例の中では、65歳以上の高齢者は15%にすぎなかったのに対し、2010年10月以降の治療症例の中では38%が65歳以上で、そのうち18%は70歳以上となっており、近年の治療症例において高齢者の割合が高くなっていることが確認された（**図1**）。

IFN・リバビリン併用治療を行った症例の中で、高齢者群と非高齢者群の臨床的背景を比較すると、過去の治療症例・最近2年間の治療症例ともに、高齢者群でジェノタイプ1Bの症例の割合が有意に高く、しかもジェノタイプ1Bでかつ高ウイルス量のいわゆる難治性の症例の割

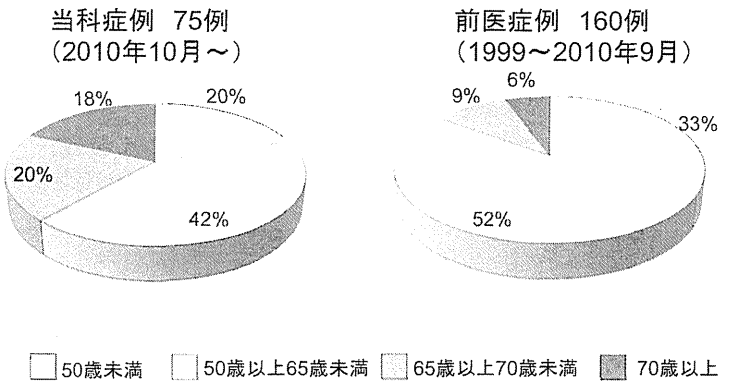

図1　対象症例の年齢別割合
2010年9月以前の症例の中では、高齢者の割合は15%のみであったが、2010年10月以降の症例では38%が65歳以上、うち18%が70歳以上と、高齢者の割合が著明に増加している。

表1　高齢者群と非高齢者群の臨床的背景
ペグインターフェロン・リバビリン併用治療症例

		高齢者群（65歳以上）	非高齢者群（65歳未満）	P値
当院症例（2010年10月以降）60例	年　齢	69（65～77）	54（24～64）	–
	性別（M：F）	10：13	18：19	ns
	ジェノタイプ（1b：2a/b）	16：7	14：23	0.031
	RNA量（5以上：5未満）	18：5	32：5	ns
	1b-high：others	15：8	13：24	0.024
前医症例（1999～2010年9月）160例	年　齢	68（65～76）	53（22～64）	–
	性別（M：F）	12：12	81：55	ns
	ジェノタイプ（1b：2a/b：判定不能）	23：1	85：50：1	0.003
	RNA量（5以上：5未満）	21：3	128：8	ns
	1b-high：others：判定不能	21：3	84：51：1	0.032
全　体220例	年　齢	68（65～77）	54（22～64）	–
	性別（M：F）	22：25	99：74	ns
	ジェノタイプ（1b：2a/b：判定不能）	39：8	99：73：1	0.012
	RNA量（5以上：5未満）	39：8	160：13	ns
	1b-high：others：判定不能	36：11	97：75：1	0.035

合が高かった（**表1**）。SVR率（ITT解析）は、最近の症例では高齢者群で40％（6/15）、非高齢者群で74％（17/23）と高齢者群で有意に低率であり、過去の治療症例では高齢者群で25％（5/20）、非高齢者群で43％（53/122）と高齢者群で低い傾向であった。これらの症例全体におけるSVR率（ITT解析）は、高齢者群では31％（11/35）、非高齢者群で48％（70/145）であり、ジェノタイプ1b・高ウイルス量の症例におけるSVR率は高齢者群で19％（5/27）、非高齢者群で36％（32/89）であった（**表2**）。

以下の検討は、治療中の詳細な情報が確認されている2010年10月以降の症例に関して行った。インターフェロン製剤とリバビリンそれぞれについての総投与量を算出し、身長・体重から決定される理想投与量に対する比を、高齢者群と非高齢者群で比較した（**図2**）。インターフェロンに関しては理想量の80％以上を投与しえた症例の割合は高齢者群で65％、非高齢者群で69％と、ほとんど差はなかった。一方、リバビリン投与総量をみると、高齢者群のうち46％と半数近い症例で、理想量の60％以下の投与し

表2　高齢者群と非高齢者群の治療効果の比較
ペグインターフェロン・リバビリン併用治療症例

		高齢者群（65歳以上）	非高齢者群（65歳未満）	P値
当院症例（2010年10月以降）60例	SVR：nonSVR：未判定例	6：9：8	17：6：14	0.004
	1b-high症例の SVR：nonSVR：未判定例	2：8：5	7：5：1	ns
前医症例（1999～2010年9月）160例	SVR：nonSVR：未判定例	5：15：4	53：69：14	ns
	1b-high症例の SVR：nonSVR：未判定例	3：14：4	25：52：7	ns
全　体 220例	SVR：nonSVR：未判定例	11：24：12	70：75：28	ns
	1b-high症例の SVR：nonSVR：未判定例	5：22：9	32：57：8	ns

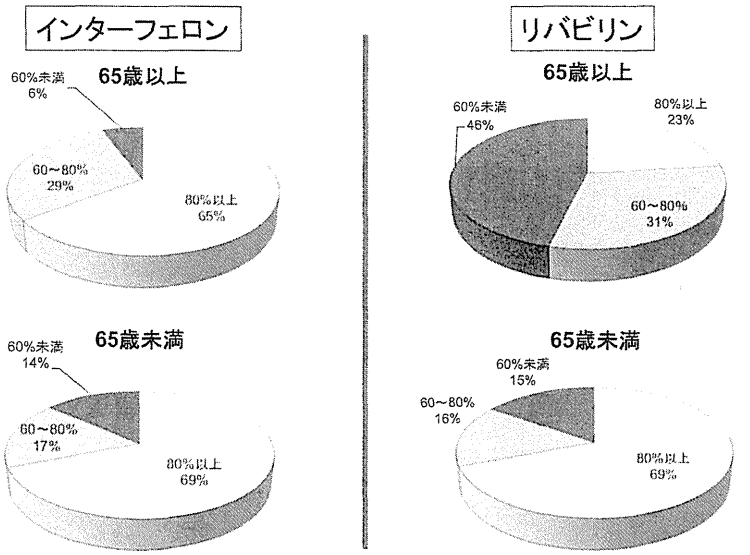

図2　高齢者群と非高齢者群におけるAdherenceの比較
インターフェロンは高齢者でもadherenceの低下はみられないが、リバビリンのadherenceは高齢者群で60％以下であった症例の割合が高い。

かできず、80%以上を内服できた症例はわずかに23%のみであった。非高齢者群ではリバビリン総投与量が60%未満であったのはわずか15%で、69%の症例が80%以上内服できており、このリバビリン内服量に関して、高齢者群と非高齢者群との間で顕著な差がみられた。

治療中止となった症例が高齢者群で4例、非高齢者群で5例みられたが、それらの症例の中止に至った理由を比較検討した(**表3**)。高齢者群では4例中3例が肺炎・腎盂腎炎・蜂窩織炎といった種々の細菌感染症により、残りの1例は出血性胃潰瘍により中止となっていた。感染症の治療に難渋する症例や、発熱によって脱水・急性腎障害を併発した症例もみられた。一方、非高齢者群の中止理由では1例で急性腸炎をみるのみで、細菌感染症による中止例はなく、副作用や自身の都合による中止が多かった。このことより、高齢者に対する併用療法では特に細菌感染症に注意する必要があると考えられた。

インターフェロン少量長期投与に関しては、高齢者7例・非高齢者8例で導入されていたが、高齢者群において副作用による中止症例はなく、中止に至った理由で最も多いのは効果がみられないということであった(**表4**)。

考　察

我が国のC型慢性肝炎症例は、欧米と比較して平均年齢が高く、65歳以上の高齢者の割合が高くなっている[3]。今回の検討症例の年齢分布でも、2010年以前の約10年間に比べ、2010年10月以降の群では、明らかに高齢者の割合が高かった。これは単純に対象症例の加齢のためばかりではなく、近年において高齢者に対する治療の必要性がより一層強調されるようになった結果であろうと考えられる。

一方、高齢者群の治療成績に関してみると、SVR率は非高齢者群に対し有意に低いといわれており[4]、SVRが期待しにくい症例に対してはインターフェロン少量長期投与やβ製剤導入などの治療法も積極的にすすめていく必要性が示されている[1,5,6]。今回我々の報告した症例

表3　ペグインターフェロン・リバビリン併用治療における中止症例

	年　齢	性　別	Geno type	治療前RNA量	治療内容	中止理由
高齢者群	74	F	1b	5.5	α 2a + R	胃潰瘍出血
	74	M	1b	6.5	α 2a + R	急性肺炎
	73	M	1b	6.3	α 2a + R	急性腎盂腎炎
	68	F	1b	7.1	α 2a + R	右腕蜂窩織炎
非高齢者群	63	F	2b	6.6	α 2b + R	食事摂取不良・脱水
	58	F	1b	6.1	α 2a + R	血小板低下
	57	F	2a	6.7	α 2b + R	急性腸炎
	53	M	1b	3.2	α 2a	仕事の都合
	47	M	2b	4.7	α 2a	来院せず

表4　インターフェロン単独療法における中止症例

	年　齢	性　別	治療期間(年)	中止理由	治療後病態
高齢者群	79	M	0.5	無効	LC、HCC発生、脳梗塞発症
	73	M	0.5	無効	LC、SNMC治療継続
	70	F	3	転移性肺がん	CH、ALT安定で無症状
非高齢者群	62	F	1	副作用(倦怠感)	LC、SNMC治療継続
	64	F	4	効果減弱	AIH合併でステロイド治療
	50	F	2	個人的理由	フォローから脱落
	58	F	0.7	無効	LC、SNMC治療継続

では、高齢者群における全体のSVR率は31%、ジェノタイプ1b・高ウイルス量の難治性症例でのSVR率は19%と、非高齢者群における48%、36%に比べ低率であった。

このように、高齢者群においてSVRが得られにくい原因のひとつとして、副作用に伴うadherenceの低下があると指摘されている[1,7]。今回検討した併用療法の症例でみると、インターフェロン投与量の分布は高齢者と非高齢者の間に差はみられなかったが、リバビリン投与量は高齢者群で減量を必要とした割合が高く、80%以上の十分量が投与できた症例はわずか23%にすぎなかった。このように、高齢者群においては特にリバビリンによる貧血の進行が治療の支障となることが多い。その一因として、加齢に伴う腎機能低下によるリバビリン排泄低下というメカニズムが指摘されており、安全な投与量設定のための工夫も提唱されている[8]。欧米ではエリスロポエチン（EPO）製剤の併用投与が行われている[9]が、本邦ではまだ認可されていない現状であり、今後はこのような副作用に対する対策の強化が望まれる。

さらに、治療の中断もまた最終的治療効果に影響する大きな要因となる。今回の検討では、高齢者群において中止に至った症例の中止原因の多くが細菌感染症によるものであったのが特徴的である。治療中はインターフェロンの作用で好中球数の低下が高頻度にみられるため、好中球数のレベルによるインターフェロン減量基準が定められている。高齢者でも非高齢者でも同等の減量基準に従って投与量の調節を行っているものの、高齢者においては加齢に伴う何らかの免疫能低下も加わって、細菌感染に対する防御能が低下している可能性が示唆される。

併用治療が困難な症例や過去の治療で無効であった症例に対し、インターフェロン単独少量長期投与を行うことも多くなっており[6]、高齢者7例、非高齢者8例に対してこの投与が行われている。少量長期投与で中止に至った症例の中止理由をみると、高齢者群の中止例3例中2例は無効のため、1例は他臓器悪性腫瘍のためという結果であり、高齢者群において副作用で中止となった症例はみられなかった。このように、肝炎の進展予防・発癌予防を目的としたインターフェロン単独治療は、高齢の症例に対しても比較的安全に施行しうると思われ、適応症例に対しての積極的導入が望ましいと考えられた。

文　献

1) 池田房雄、岩崎良章、山本和秀：高齢者のC型慢性肝炎に対する治療．日本臨床　2011; 69: 221-226
2) 熊田　卓、豊田秀徳、多田俊史：高齢者C型肝炎ではALT低値、血小板高値でも発癌する．The Proceeding of the 28th Inuyama Symposium 2010 肝炎・肝癌の新しい診断と治療，犬山シンポジウム記録刊行会編，メディカルトリビューン，東京，2011, 125-130
3) 泉　並木：インターフェロン治療，リバビリン併用療法の歴史的変遷．モダンフィジシャン 2008; 28: 5-7
4) 井出達也、宮島一郎、佐田通夫：高齢者のC型慢性肝疾患の治療．肝胆膵2008; 57: 983-987
5) 荒瀬康司：うつ状態あるいは70歳以上の高齢C型慢性肝炎症例に対するIFN-β/RBV併用療法の効果と安全性．医学のあゆみ2011; 239: 1171-1174
6) 高谷広章、名越澄子、近山　琢、ほか：高齢C型慢性肝炎患者に対するリバビリン併用ペグインターフェロン2b療法補強治療としてのペグインターフェロンα2a単独療法の有用性．日本高齢消化器病学会誌2011; 13: 157-161
7) 中本晋吾、今関文夫、神田達郎、ほか：高齢者C型慢性肝炎に対するペグインターフェロン、リバビリン併用治療効果．日本高齢消化器病学会誌2011; 13: 162-169
8) 豊田成司、狩野吉康、赤池　淳、ほか：リバビリンの全身クリアランス（CL/F）は貧血に伴う減量・中止に対する最も感度の高い因子である．肝臓2005; 46: 107-118
9) Younossi ZM, Nader FH, Bai C, et al: A Phase II dose finding study of darbepoetin alpha and filgrastim for the management of anaemia and neutropenia in chronic hepatitis C treatment. J Viral Hepat 2008; 15: 370-378

Efficacy of interferon treatment in elderly patients with hepatitis C

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Abstract We evaluated the efficacy and side effect of interferon therapy in chronic hepatitis C patients comparing elderly patients aged 65 years and more (elderly group) and younger patients (younger group). In elderly group, percentage of genotype 1b and high viral RNA was higher than that in younger group. The overall SVR rate was 31% in elderly group and 48% in younger group, and SVR rate among genotype 1b with high RNA level cases was 19% in elderly group and 36% in younger group. With regard to adherence of each medicine, dose reduction of ribavirin was required more frequently in elderly group. Main reason of discontinuance of the therapy in elderly group was various kinds of bacterial infection. Among the interferon nomotherapy cases, severe adverse effect was not observed even in the elderly group.

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ORIGINAL RESEARCH

Clinical factors related to long-term administration of sorafenib in patients with hepatocellular carcinoma

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Background: Sorafenib has been approved in the indication of unresectable hepatocellular carcinoma, but there are many cases in which administration of the drug is discontinued due to severe side effects. In this study, we compared the characteristics of patients who continued and discontinued sorafenib.

Methods: Ninety-six patients (75 men and 21 women) were initiated on sorafenib from July 2009 through September 2011. The patient characteristics of interest included gender, age, etiology, Child-Pugh classification, treatment history and frequency, and levels of α-fetoprotein, des-gamma-carboxy prothrombin, aspartate amino acid transferase, and alanine aminotransferase. Duration of administration of sorafenib and reasons for its discontinuation were compared.

Results: Median overall survival was 11.8 months. Discontinuation of sorafenib within 90 days was identified as an independent prognostic factor for overall survival on multivariate analysis ($P < 0.0001$). Transarterial chemoembolization performed six times or more ($P = 0.013$) was also identified as an independent factor contributing to discontinuation of sorafenib within 90 days in multivariate analysis. Patients who received sorafenib for ≥90 days had significantly longer overall survival than those who discontinued it ($P < 0.0001$).

Conclusion: Prolonged treatment with sorafenib is an important factor in achieving extended overall survival. We recommend starting sorafenib before latent liver damage has occurred as a result of too many transarterial chemoembolization procedures.

Keywords: sorafenib, hepatocellular continuation, discontinuation, efficacy

Introduction

In general, hepatocellular carcinoma in its early stages can be treated by surgical resection, radiofrequency ablation,[1,2] or liver transplantation when there is a single nodule ≤ 5 cm or three nodules ≤ 3 cm (Milan criteria).[3] However, many people are diagnosed in the advanced stages, when only transcatheter arterial infusion chemotherapy and transarterial chemoembolization are performed,[4,5] but despite recently improved embolization devices,[6] even these therapies have limited success in cases of vascular invasion or extrahepatic spread.[7] Because hepatocellular carcinoma has a high recurrence rate, it is important to prevent secondary disease, and several therapies have been reported to prevent recurrence, including interferon,[8] retinoids,[9] and branched-chain amino acids.[10]

Recently, a number of molecularly targeted agents have been investigated throughout the world,[11–13] and some agents are entering Phase II or III trials.[14,15] Sorafenib inhibits the serine/threonine kinases, RAF-1and B-Raf,[16,17] inhibits the tyrosine kinase activity of vascular endothelial growth factor receptors 1, 2, and 3 and

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platelet-derived growth factor receptor β,[16,17] which has been shown to trigger production of reactive oxygen species and death of hepatocellular carcinoma cells.[18]

Sorafenib was approved to treat hepatocellular carcinoma in 2007,[19] and is used in patients with advanced stage disease. In two well known studies, sorafenib significantly increased survival time in patients with hepatocellular carcinoma, although there were absolute differences in survival time between the two patient populations because the definition of advanced disease differed between the two studies.[20,21]

Although administration of 800 mg of sorafenib is recommended, there are many cases in which administration is discontinued or the dosage is reduced because of severe side effects.[15,20,22] In this study, we demonstrated the therapeutic effects of sorafenib retrospectively and compared the characteristics of patients who continued and discontinued treatment with this agent.

Materials and methods

Patients

We enrolled 96 patients who had started to receive the drug in our hospital or at one of six affiliated hospitals from July 2009 through September 2011 and were able to be observed for more than 90 days. The patient characteristics investigated included gender, age, etiology, Child-Pugh classification, treatment history and frequency, levels of α-fetoprotein, des-gamma-carboxy prothrombin, aspartate amino acid transferase, and alanine aminotransferase, as well as treatment received prior to administration of sorafenib, eg, surgery, percutaneous ethanol injection therapy, percutaneous microwave coagulation therapy, radiofrequency ablation, transarterial chemoembolization, transcatheter arterial embolization, and/or transcatheter arterial infusion. The effectiveness of the treatments was evaluated according to modified Response Evaluation Criteria In Solid Tumors (RECIST)[23] using an enhanced computed tomography scan every 3 months. Duration of administration of sorafenib was noted and the reasons for its discontinuation were identified.

Treatment plan and toxicity evaluation

Sorafenib was initiated at 800 mg/day in two divided doses,[24] and dose reduction was allowed for unacceptable adverse effects, ie, grade 3/4 toxicities, and treatment was continued until disease progression, development of intolerable drug toxicity, or patient refusal to continue taking the drug. Patients were followed up on an outpatient basis every 2–4 weeks. Adverse events were assessed according to the National Cancer Institute Common Toxicity Criteria for Adverse Events, version 4.0.

Statistical analysis

Overall survival was estimated using the Kaplan-Meier method, and differences in survival between the groups were compared using the log-rank test. Cox's proportional hazard model and logistic regression were used to examine likely prognostic factors in each group. The results were reported as hazard ratios with 95% confidence intervals. $P < 0.05$ was considered to be statistically significant for all analyses. All statistical analyses were performed using JMP version 7.2 software (SAS Institute, Cary, NC).

Results

Patient characteristics

The 96 patients comprised 75 men and 21 women of mean age of 71.2 ± 16.8 (median 71) years (see Table 1). The etiology was hepatitis C virus in 56, hepatitis B virus in 15, and others in 25. Median (range) levels of

Table 1 Baseline patient characteristics and previous therapy before administration of sorafenib

Variable	Median (range)
Age	71 (52–87)
Gender (M/F)	75/21
HCV/HBV/others	56/15/25
Aspartate transaminase (U/L)	54 (19–165)
Alanine aminotransferase (U/L)	33 (12–150)
Platelets (per μL)	12 (5–32)
α-fetoprotein (ng/mL)	88.7 (3.2–245,500)
des-gamma-carboxy prothrombin (mAU/mL)	559 (5–75,000)
Child-Pugh classification (5/6/7)	62/30/4
BCLC stage B/C	37/59
Extrahepatic spread (±)	27/69
Macroscopic vascular invasion (±)	30/66
ECOG performance status (0/1/2)	50/44/2
Previous therapy	**n (%)**
Operation	32 (33)
Percutaneous ethanol injection therapy	11 (11)
Percutaneous microwave coagulation therapy	1 (1)
Radiofrequency ablation	42 (44)
TA(C)E	72 (75)
Transcatheter arterial infusion	19 (20)
Radiation	5 (5)
Efficacy in all patients	
Level of response	**n (%)**
CR	0 (0)
PR	13 (14)
SD	31 (32)
PD	30 (31)
Not evaluable	22 (23)
Response rate	14%
Disease-control rate	46%

Abbreviations: BCLC, Barcelona Clinic Liver Cancer; ECOG, Eastern Cooperative Oncology Group; HBV, hepatitis B virus; HCV, hepatitis C virus; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; TACE, transarterial chemoembolization.

α-fetoprotein, des-gamma-carboxy prothrombin, aspartate (U/L), and alanine aminotransferase (U/L) were 88.7 (3.2–245,500) ng/mL, 559 (5–75,000) mAU/mL, 54 (19–165) IU/L, and 33 (12–150) IU/L, respectively. Fifty, 44, and two patients had an Eastern Cooperative Oncology Group performance status of 0, 1, and 2, respectively. Child-Pugh classification was 5 in 62, 6 in 30, and 7 in four. Barcelona Clinic Liver Cancer (BCLC) staging was B in 37 and C in 59. Twenty-seven patients had metastatic disease in organs other than the liver. Prior medical history at the time of initiation of sorafenib therapy was surgery in 32, percutaneous ethanol injection therapy in 11, percutaneous microwave coagulation therapy in one, radiofrequency ablation in 42, transarterial chemoembolization and/or transcatheter arterial embolization in 72, transcatheter arterial infusion in 19, and radiation in five cases.

Efficacy, response, and disease control rates

Median overall survival was 11.8 months. No patients had a complete response, 13 patients (14%) had a partial response, and 31 (32%) had stable disease (according to modified RECIST criteria), whereas 30 patients (31%) had progressive disease and 22 patients were not evaluable. The disease control rate was 46% and the response rate was 14% (see Figure 1 and Table 1).

Compliance with treatment

The mean sorafenib dose was 800 mg in 26 patients (27%), 600 mg in nine (9%), 400 mg in 46 (48%), and ≤200 mg in 15 (16%). Because the median duration of treatment was 87 (range 2–737) days, we divided the patients into two groups, ie, one with ≥90 days of treatment with sorafenib (n = 45) and another within 90 days of treatment with sorafenib (n = 51), and examined factors influencing the patient's ability to take the drug on a longer-term basis (Table 2). Common reasons for discontinuation within 90 days were adverse events (40 patients) and radiologic progression (n = 11). The adverse events were hand-foot skin reactions (n = 3), diarrhea (n = 3), general fatigue (n = 2), rash (n = 3), fever (n = 3), renal failure (n = 2), pancreatitis (n = 1), liver dysfunction (n = 15), and others (n = 8), whereas severe liver dysfunction included liver failure (n = 7), hepatic encephalopathy (n = 3), ascites (n = 3), elevation of aspartate or alanine aminotransferase (n = 1), and jaundice (n = 1).

Prognostic factors for overall survival by univariate and multivariate analysis

On univariate analysis, BCLC (C) staging ($P = 0.04$), tumor volume ≥ 50% of the liver ($P < 0.0001$), macroscopic vascular invasion ($P = 0.006$), and discontinuation of sorafenib administration within 90 days ($P < 0.0001$) were significant prognostic factors, but only discontinuation of sorafenib within 90 days was identified as an independent prognostic factor contributing to overall survival on multivariate analysis ($P < 0.0001$, Table 3).

Relationship between administration for ≥90 days and overall survival

In the group that continued on sorafenib for ≥90 days, overall survival was significantly longer than in the group that discontinued sorafenib within 90 days ($P < 0.0001$), and the same relationship was found in the 61 patients who had their dose reduced to 400 mg and 200 mg ($P = 0.0026$,

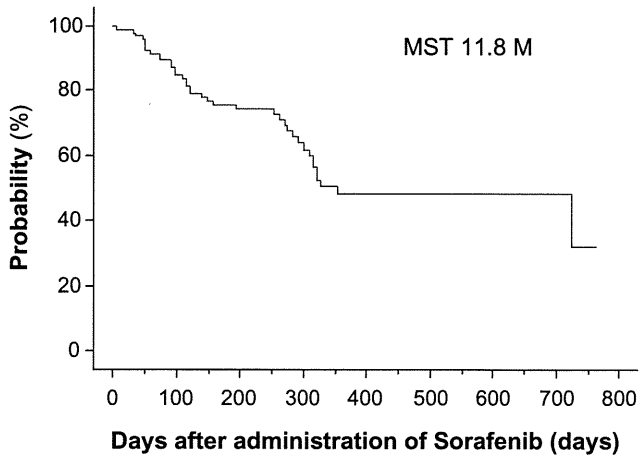

Figure 1 Overall survival for all patients.
Note: Median overall survival was 11.8 (range 7–763) days.

Table 2 Reasons for discontinuation of sorafenib within 90 days

Adverse events without liver dysfunction	**36**
Progressive disease	11
Hand-foot skin reaction	3
Diarrhea	3
General fatigue	2
Rash	3
Fever	3
Renal failure	2
Pancreatitis	1
Others	8
Liver dysfunction	**15**
Liver failure	7
Hepatic encephalopathy	3
Ascites	3
Elevation of AST or ALT	1
Jaundice	1

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase.

Table 3 Risk factors contributing to overall survival (n = 96)

	Subgroup	Univariate analysis			Multivariate analysis		
		Risk ratio	95% CI	*P* value	Risk ratio	95% CI	*P* value
Age	≥71 or <71	0.74	0.39–1.41	0.36			
Gender	Female	1.53	0.94–2.51	0.08			
Child-Pugh classification	≥6 or 5	0.54	0.28–1.04	0.06			
α-fetoprotein (ng/mL)	<50 or ≥50	1.95	0.94–4.0	0.71			
Des-gamma-carboxy prothrombin (mAU/mL)	<400 or ≥400	1.69	0.85–3.3	0.13			
Etiology	HCV, others	0.74	0.39–1.41	0.37			
PS	≥1 or 0	0.66	0.35–1.27	0.22			
BCLC	C or B	0.46	0.22–0.97	0.04	0.96	0.38–2.45	0.94
RFA	0 or ≥1	1.25	0.65–2.40	0.51			
TACE	<6 or ≥6	1.5	0.74–3.03	0.26			
Tumor volume of liver	<50% or ≥50%	4.41	2.24–8.69	<0.0001	1.70	0.77–3.74	0.19
Macroscopic vascular invasion	None or +	3.06	1.61–5.82	0.006	1.76	0.75–4.09	0.19
Discontinuation	<90 or ≥90	0.1	0.04–0.26	<0.0001	0.13	0.05–0.34	<0.0001
Extrahepatic spread	None or +	1.61	0.83–3.13	0.16			

Abbreviations: BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; CR, complete response; RFA, radiofrequency ablation; TACE, transarterial chemoembolization.

Figures 2 and 3). Therefore, we concluded that treatment with sorafenib for ≥90 days achieves better overall survival, even at a reduced dose.

Risk factors for discontinuation of sorafenib within 90 days by univariate and multivariate analysis

To identify risk factors for discontinuation of sorafenib within 90 days, patients whose observed periods were less than 90 days were eliminated. The total number was 82, with 45 being ≥90 days and 37 being <90 days (Table 4). On univariate analysis, des-gamma-carboxy prothrombin (≤400 mAU/mL, $P = 0.04$), tumor volume ≥ 50% of the liver ($P = 0.02$), macroscopic vascular invasion ($P = 0.03$), and six or more transarterial chemoembolizations ($P = 0.02$) were significant factors, and tumor volume ≥ 50% of the liver ($P = 0.04$) and six or more transarterial chemoembolizations ($P = 0.013$) were identified as independent risk factors on multivariate analysis.

Discussion

Sorafenib is considered a drug that should be used for advanced hepatocellular carcinoma, but there are no suitable criteria for treatment of hepatocellular carcinoma at this stage, because the treatment outcomes are affected by multiple variables, including liver function, patient performance status, and tumor stage.[25,26]

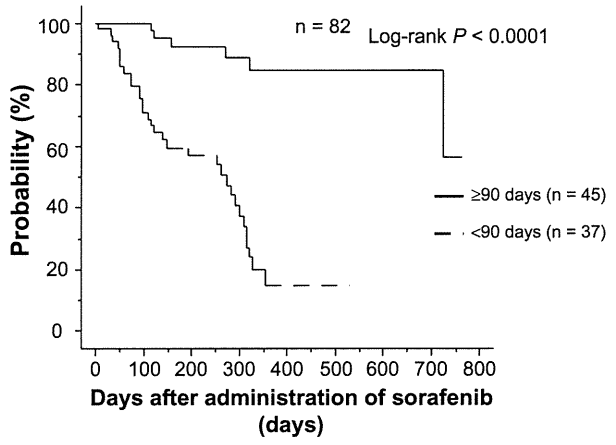

Figure 2 Relationship between continuation of administration and overall survival.
Note: In the group that continued administration for ≥90 days, overall survival was significantly higher than in the group that discontinued administration within 90 days.

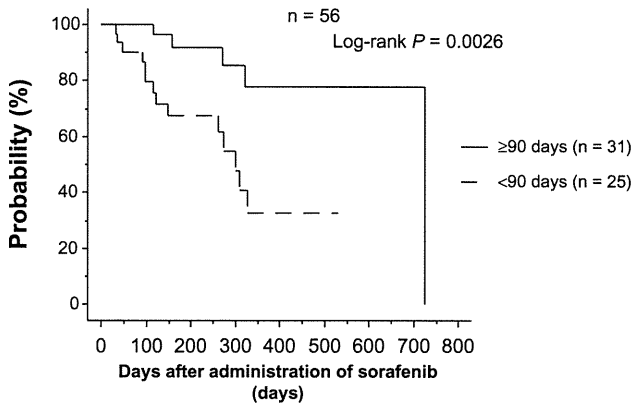

Figure 3 Relationship between continuation of administration and overall survival in patients receiving 200 mg or 400 mg.
Note: In the group that continued administration for ≥90 days, overall survival was significantly higher than in the group who discontinued administration within 90 days.

Table 4 Risk factors contributing to discontinuation of sorafenib administration within 90 days (n = 82)

	Subgroup	Univariate analysis			Multivariate analysis		
		Risk ratio	95% CI	*P* value	Risk ratio	95% CI	*P* value
Child-Pugh classification	≥6 or 5	2.10	0.83–5.30	0.11			
α-fetoprotein (ng/mL)	<50 or ≥50	0.55	0.22–1.35	0.19			
DCP (mAU/mL)	<400 or ≥400	0.39	0.16–0.97	0.04	0.59	0.22–1.60	0.30
Radiofrequency ablation	0 or ≥1	0.78	0.32–1.88	0.58			
Tumor volume of liver	<50% or ≥50%	0.08	0.01–0.69	0.02	0.99	0.01–0.91	0.04
Macroscopic vascular invasion	None or +	0.33	0.12–0.87	0.03	0.46	0.15–1.39	0.17
TACE	<6 or ≥6	0.26	0.08–0.83	0.02	0.21	0.06–0.72	0.013
Extrahepatic spread	None or +	1.81	0.67–4.92	0.24			

Abbreviations: CI, confidence interval; DCP, des-gamma-carboxy prothrombin; TACE, transarterial chemoembolization.

Many trials have reported on the use of sorafenib to prevent hepatocellular carcinoma recurrence after treatment.[27] Sorafenib is used with sirolimus or with inhibitors of mammalian target of rapamycin[29] to reduce the risk of recurrence of hepatocellular carcinoma after liver transplantation,[28] and it has been reported that such combination therapy can be effective. Some adjuvant therapy studies after curative treatment for hepatocellular carcinoma, such as the STORM (Sorafenib as adjuvant Treatment in the prevention Of Recurrence of hepatocellular carcinoMa) trial, are ongoing,[27] and concurrent treatment of hepatocellular carcinoma with conventional transarterial chemoembolization and sorafenib has demonstrated a longer time to progression and possible efficacy.[30,31] The overall median survival of our subjects was 11.8 months. Our data are slightly more robust than those of the Phase III SHARP (Sorafenib in Advanced Hepatocellular Carcinoma Assessment Randomized Protocol) trial (10.7 months)[20] and the tandem study in the Asia-Pacific region (6.5 months), probably because our study included a higher number of BCLC stage B patients than were included in the registration trials.[21]

Sorafenib has several side effects and is often discontinued when their grade becomes severe. Multikinase inhibitors such as sorafenib have unique clinicopathologic consequences, including hand-foot skin reactions and severe side effects.[32–34] The severity of hand-foot skin reactions is dose-related and depends on the duration, dosage, and accumulation of the drug.[35] Our data show that almost all patients had side effects, with about 40% of patients discontinuing sorafenib due to adverse events. However, some side effects may predict a response to sorafenib, such as early skin toxicity and diarrhea,[22,36–38] and methods have been reported for evaluating efficacy and overall survival.[39,40] No studies have reported the efficacy of treatment dose or duration because the Phase III trial for sorafenib used only 800 mg.[20] Okuwaki et al reported late-onset progressive disease, indicating that prolonged treatment with sorafenib may be beneficial.[41] In our data, several factors in univariate analysis, such as BCLC stage C, tumor volume ≥ 50% of the liver, macroscopic vascular invasion, and discontinuation of sorafenib within 90 days reduced overall survival, but discontinuation of sorafenib within 90 days was the only factor found to reduce overall survival in multivariate analysis. Patients able to take sorafenib for longer than 90 days had better overall survival than those who discontinued within 90 days, even if they could take only 400 mg or less than 400 mg because of side effects. Our data demonstrate the benefit of a long duration of treatment, even in cases of reduced dosage. Interestingly, upon further investigation of patients who could be observed for 90 days or more, multivariate analysis showed that a tumor volume occupying ≥50% of the liver and six or more transarterial chemoembolization procedures prior to initiation of sorafenib were significant prognostic indicators. Our data indicate that prolonged administration is an important factor in obtaining good overall survival, and is better started when the number of transarterial chemoembolizations is less than six. Many transarterial chemoembolization procedures can worsen liver function and, although the Child-Pugh score does not change, there might be latent liver damage.

Sorafenib is indicated for patients with BCLC stage C and transarterial chemoembolization is recommended for patients with BCLC stage B,[7,42] so sorafenib is usually used in patients whose tumors are progressing despite locoregional therapy. We recommend starting sorafenib before latent liver damage has occurred as a result of too many transarterial chemoembolization procedures, and prolonged administration of sorafenib is important for long overall survival, even if the dose of sorafenib needs to be reduced because of side effects.

Disclosure

The authors declare that they do not have anything to disclose regarding funding or conflicts of interest with respect to this manuscript.

References

1. Bruix J, Llovet JM. Prognostic prediction and treatment strategy in hepatocellular carcinoma. *Hepatology*. 2002;35:519–524.
2. Park KW, Park JW, Choi JI, et al. Survival analysis of 904 patients with hepatocellular carcinoma in a hepatitis B virus-endemic area. *J Gastroenterol Hepatol*. 2008;23:467–473.
3. Mazzaferro V, Regalia E, Doci R, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med*. 1996;334:693–699.
4. Llovet JM, Real MI, Montana X, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet*. 2002;359:1734–1739.
5. Lo CM, Ngan H, Tso WK, et al. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology*. 2002;35:1164–1171.
6. Varela M, Real MI, Burrel M, et al. Chemoembolization of hepatocellular carcinoma with drug eluting beads: efficacy and doxorubicin pharmacokinetics. *J Hepatol*. 2007;46:474–481.
7. Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology*. 2011;53:1020–1022.
8. Shiratori Y, Shiina S, Teratani T, et al. Interferon therapy after tumor ablation improves prognosis in patients with hepatocellular carcinoma associated with hepatitis C virus. *Ann Intern Med*. 2003;138:299–306.
9. Muto Y, Moriwaki H, Ninomiya M, et al. Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma. Hepatoma Prevention Study Group. *N Engl J Med*. 1996;334:1561–1567.
10. Muto Y, Sato S, Watanabe A, et al. Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis. *Hepatol Res*. 2006;35:204–214.
11. Semela D, Piguet AC, Kolev M, et al. Vascular remodeling and antitumoral effects of mTOR inhibition in a rat model of hepatocellular carcinoma. *J Hepatol*. 2007;46:840–848.
12. Dempke WC, Zippel R. Brivanib. A novel dual VEGF-R2/bFGF-R inhibitor. *Anticancer Res*. 2010;30:4477–4483.
13. Huynh H. Molecularly targeted therapy in hepatocellular carcinoma. *Biochem Pharmacol*. 2010;80:550–560.
14. Kudo M. Hepatocellular carcinoma 2009 and beyond: from the surveillance to molecular targeted therapy. *Oncology*. 2008;75 Suppl 1:1–12.
15. Villanueva A, Llovet JM. Targeted therapies for hepatocellular carcinoma. *Gastroenterology*. 2011;140:1410–1426.
16. Wilhelm SM, Carter C, Tang L, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res*. 2004;64:7099–7109.
17. Chang YS, Adnane J, Trail PA, et al. Sorafenib (BAY 43-9006) inhibits tumor growth and vascularization and induces tumor apoptosis and hypoxia in RCC xenograft models. *Cancer Chemother Pharmacol*. 2007;59:561–574.
18. Coriat R, Nicco C, Chereau C, et al. Sorafenib-induced hepatocellular carcinoma cell death depends on reactive oxygen species production in vitro and in vivo. *Mol Cancer Ther*. 2012;11:2284–2293.
19. Wilhelm SM, Adnane L, Newell P, et al. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. *Mol Cancer Ther*. 2008;7:3129–3140.
20. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med*. 2008;359:378–390.
21. Cheng AL, Kang YK, Chen Z, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol*. 2009;10:25–34.
22. Otsuka T, Eguchi Y, Kawazoe S, et al. Skin toxicities and survival in advanced hepatocellular carcinoma patients treated with sorafenib. *Hepatol Res*. 2012;42:879–886.
23. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis*. 2010;30:52–60.
24. Shim JH, Park JW, Choi JI, et al. Practical efficacy of sorafenib monotherapy for advanced hepatocellular carcinoma patients in a Hepatitis B virus-endemic area. *J Cancer Res Clin Oncol*. 2009;135:617–625.
25. Han KH, Kudo M, Ye SL, et al. Asian consensus workshop report: expert consensus guideline for the management of intermediate and advanced hepatocellular carcinoma in Asia. *Oncology*. 2011;81 Suppl 1:158–164.
26. Llovet JM, Di Bisceglie AM, Bruix J, et al. Design and endpoints of clinical trials in hepatocellular carcinoma. *J Natl Cancer Inst*. 2008;100:698–711.
27. Kudo M. Adjuvant therapy after curative treatment for hepatocellular carcinoma. *Oncology*. 2011;81 Suppl 1:50–55.
28. Weinmann A, Niederle IM, Koch S, et al. Sorafenib for recurrence of hepatocellular carcinoma after liver transplantation. *Dig Liver Dis*. 2012;44:432–437.
29. Gomez-Martin C, Bustamante J, Castroagudin JF, et al. Efficacy and safety of sorafenib in combination with mammalian target of rapamycin inhibitors for recurrent hepatocellular carcinoma after liver transplantation. *Liver Transpl*. 2012;18:45–52.
30. Sansonno D, Lauletta G, Russi S, et al. Transarterial chemoembolization plus sorafenib: a sequential therapeutic scheme for HCV-related intermediate-stage hepatocellular carcinoma: a randomized clinical trial. *Oncologist*. 2012;17:359–366.
31. Park JW, Koh YH, Kim HB, et al. Phase II study of concurrent transarterial chemoembolization and sorafenib in patients with unresectable hepatocellular carcinoma. *J Hepatol*. 2012;56:1336–1342.
32. Lacouture ME, Reilly LM, Gerami P, et al. Hand foot skin reaction in cancer patients treated with the multikinase inhibitors sorafenib and sunitinib. *Ann Oncol*. 2008;19:1955–1961.
33. Lacouture ME, Wu S, Robert C, et al. Evolving strategies for the management of hand-foot skin reaction associated with the multitargeted kinase inhibitors sorafenib and sunitinib. *Oncologist*. 2008;13:1001–1011.
34. Rombola F, Caravetta A, Mollo F, et al. Sorafenib, risk of bleeding and spontaneous rupture of hepatocellular carcinoma. A clinical case. *Acta Medica (Hradec Kralove)*. 2011;54:177–179.
35. Boudou-Rouquette P, Ropert S, Mir O, et al. Variability of sorafenib toxicity and exposure over time: a pharmacokinetic/pharmacodynamic analysis. *Oncologist*. 2012;17:1204–1212.
36. Vincenzi B, Santini D, Russo A, et al. Early skin toxicity as a predictive factor for tumor control in hepatocellular carcinoma patients treated with sorafenib. *Oncologist*. 2010;15:85–92.
37. Bettinger D, Schultheiss M, Knuppel E, et al. Diarrhea predicts a positive response to sorafenib in patients with advanced hepatocellular carcinoma. *Hepatology*. 2012;56:789–790.
38. Zhao Y, Yang M, Qi X, et al. Drug-related adverse events may predict efficacy in sorafenib therapy for hepatocellular carcinoma. *Hepatology*. 2012;56:790–791.
39. Horger M, Lauer UM, Schraml C, et al. Early MRI response monitoring of patients with advanced hepatocellular carcinoma under treatment with the multikinase inhibitor sorafenib. *BMC Cancer*. 2009;9:208.
40. Lee JH, Park JY, Kim do Y, et al. Prognostic value of 18F-FDG PET for hepatocellular carcinoma patients treated with sorafenib. *Liver Int*. 2011;31:1144–1149.
41. Okuwaki Y, Nakazawa T, Hidaka H, et al. Late-onset benefit in progressive advanced hepatocellular carcinoma with continued sorafenib therapy: a case report. *J Med Case Rep*. 2012;6:38.
42. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol*. 2012;56:908–943.

Genome-Wide Association Study Confirming Association of HLA-DP with Protection against Chronic Hepatitis B and Viral Clearance in Japanese and Korean

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Abstract

Hepatitis B virus (HBV) infection can lead to serious liver diseases, including liver cirrhosis (LC) and hepatocellular carcinoma (HCC); however, about 85–90% of infected individuals become inactive carriers with sustained biochemical remission and very low risk of LC or HCC. To identify host genetic factors contributing to HBV clearance, we conducted genome-wide association studies (GWAS) and replication analysis using samples from HBV carriers and spontaneously HBV-resolved Japanese and Korean individuals. Association analysis in the Japanese and Korean data identified the *HLA-DPA1* and *HLA-DPB1* genes with $P_{meta}=1.89\times10^{-12}$ for rs3077 and $P_{meta}=9.69\times10^{-10}$ for rs9277542. We also found that the *HLA-DPA1* and *HLA-DPB1* genes were significantly associated with protective effects against chronic hepatitis B (CHB) in Japanese, Korean and other Asian populations, including Chinese and Thai individuals ($P_{meta}=4.40\times10^{-19}$ for rs3077 and $P_{meta}=1.28\times10^{-15}$ for rs9277542). These results suggest that the associations between the *HLA-DP* locus and the protective effects against persistent HBV infection and with clearance of HBV were replicated widely in East Asian populations; however, there are no reports of GWAS in Caucasian or African populations. Based on the GWAS in this study, there were no significant SNPs associated with HCC development. To clarify the pathogenesis of CHB and the mechanisms of HBV clearance, further studies are necessary, including functional analyses of the HLA-DP molecule.

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Introduction

Overall, one-third of the world's population (2.2 billion) is infected with hepatitis B virus (HBV), and about 15% of these are chronic carriers. About 75% of the chronic carriers live in the east-south Asia and east pacific area, and there are 1.3–1.5 million chronic carriers living in Japan [1]. Of chronic carriers, 10–15% develop liver cirrhosis (LC), liver failure and hepatocellular carcinoma (HCC), and the remaining individuals eventually achieve a state of nonreplicative infection, resulting in hepatitis B surface antigen (HBsAg) negative and hepatitis B core antibody (anti-HBc) positive, i.e. HBV-resolved individuals [2–3]. In Japan, although the major route of HBV transmission was perinatal transmission and horizontal transmission in early childhood, infant HBV carriers have successfully been reduced since 1986 through a selective vaccination policy by the Japanese government [4–7]. However, the prevalence of HBV genotype A in acute HBV (AHB) infection has increased markedly since 2000, reaching approximately 52% in 2008 due to the lack of a universal HB vaccination, and around 10% of AHB cases could be persistent infection [8–9]. Viral factors, as well as host factors, are thought to be associated with persistent HB infection.

In 2009, significant associations between chronic hepatitis B (CHB) and a region including *HLA-DPA1* and *HLA-DPB1* were identified using 786 Japanese individuals having CHB and 2,201 control individuals through a two-stage genome-wide association study (GWAS) [10]. The same group was also subjected to a second GWAS using a total of 2,667 Japanese persistent HBV infection cases and 6,496 controls, which confirmed significant associations between the *HLA-DP* locus and CHB, in addition to associations with another two SNPs located in the genetic region including the *HLA-DQ* gene [11]. The associations between *HLA-DP* variants with HBV infection were replicated in other Asian populations, including Thai and Han Chinese individuals [10,12–13]. With regard to HBV clearance, the association between the human leukocyte antigen (HLA) class II allele and clearance of HBV was confirmed by the candidate gene approach in African, Caucasian and Asian populations [14–18]. However, in a previous GWAS using samples of Japanese CHB and control individuals, the clinical data on HBV exposure in the control individuals were unknown, and this may have led to bias. Moreover, there have been no reports of GWAS using samples from HBV carriers and HBV-resolved individuals to identify host genetic factors associated with HBV clearance other than HLA class II molecules.

Here, we performed a GWAS using samples from Japanese HBV carriers, healthy controls and spontaneously HBV-resolved individuals in order to confirm or identify the host genetic factors related to CHB and viral clearance. In the subsequent replication analysis, we validated the associated SNPs in the GWAS using two independent sets of Japanese and Korean individuals. In our study, healthy controls were randomly selected with clinically no evidence of HBV exposure, therefore, HBV-resolved individuals were prepared to clearly identify the host genetic factors related with CHB or HBV clearance.

Results

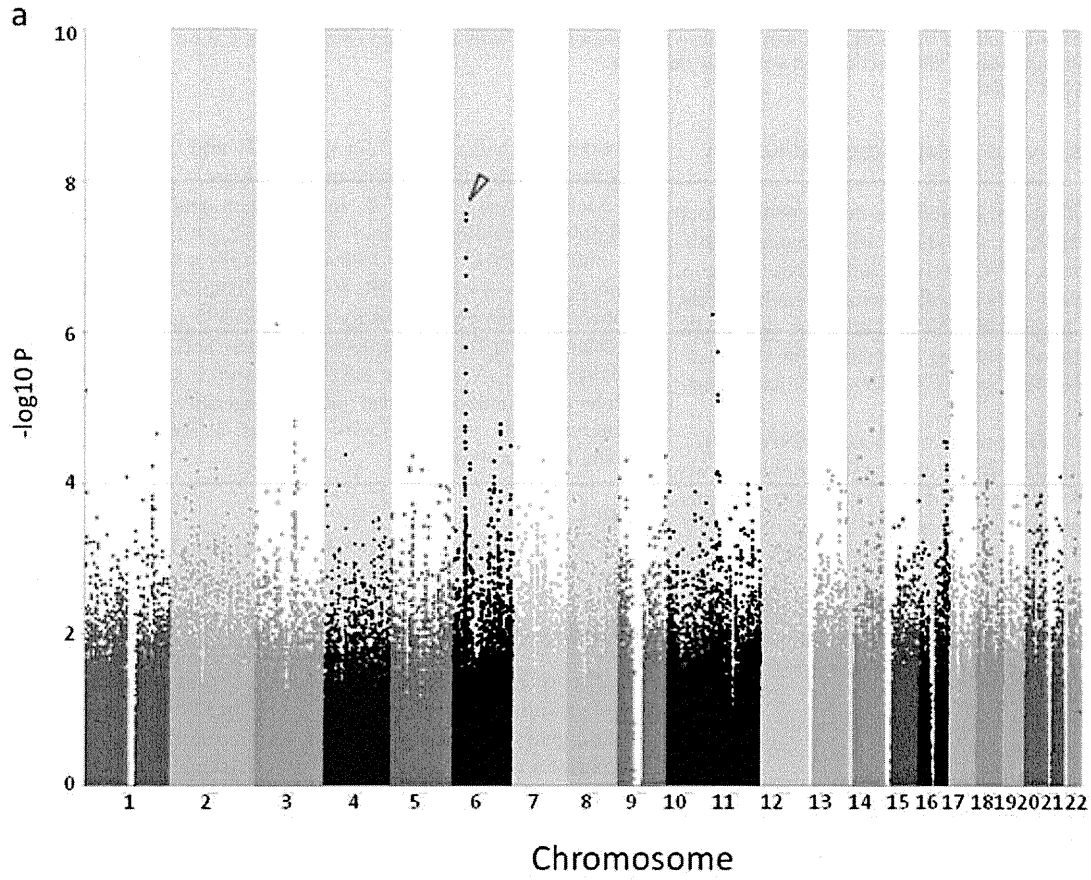
Protective Effects Against Chronic Hepatitis B in Japanese and Korean Individuals

In this study, we conducted a GWAS using samples from 181 Japanese HBV carriers (including asymptomatic carriers (ASC), CHB cases, LC cases and HCC cases, based on the criteria described in Materials and Methods) and 184 healthy controls in order to identify the host genetic factors related to progression of CHB. All samples were genotyped using a genome-wide SNP typing array (Affymetrix Genome-Wide Human SNP Array 6.0 for 900 K SNPs). Figure 1a shows a genome-wide view of the single point association data based on allele frequencies using the SNPs that met the following filtering criteria: (i) SNP call rate ≥95%; (ii) minor allele frequency (MAF) ≥1% for HBV carriers and healthy controls; and (iii) no deviation from Hardy-Weinberg equilibrium (HWE) $P \geq 0.001$ in healthy controls. We identified significant associations of protective effects against CHB with two SNPs (rs3077 and rs9277542) using the allele frequency model, both of which are located in the 3′ UTR of *HLA-DPA1* and in the sixth exon of *HLA-DPB1*, respectively (rs3077, $P = 1.14 \times 10^{-7}$, and rs9277542, $P = 5.32 \times 10^{-8}$, respectively). The association for rs9277542 reached a genome-wide level of significance in the GWAS panel (Bonferroni criterion $P < 8.36 \times 10^{-8}$ (0.05/597,789)).

In order to validate the results of GWAS, a total of 32 SNPs, including the associated two SNPs (rs3077 and rs9277542), were selected for replication in two independent sets of HBV carriers and healthy controls (replication-1:256 Japanese HBV carriers and 236 Japanese healthy controls; and replication-2:344 Korean HBV carriers and 151 Korean healthy controls; Table 1). The associations for the original significant SNP (rs9277542) and marginal SNP (rs3077) on GWAS were replicated in both replication sets [replication-1 (Japanese); rs3077, $P = 2.70 \times 10^{-8}$, OR = 0.48 and rs9277542, $P = 3.33 \times 10^{-6}$, OR = 0.54; replication-2 (Korean); rs3077, $P = 2.08 \times 10^{-6}$, OR = 0.47 and rs9277542, $P = 8.29 \times 10^{-5}$, OR = 0.54, Table 2]. We conducted meta-analysis to combine these studies using the DerSimonian Laird method (random effects model) to incorporate variation among studies. As shown in Table 2, the odds ratios were quite similar across the three studies (GWAS and two replication studies) and no heterogeneity was observed ($P_{het} = 0.80$ for rs3077 and 0.40 for rs9277542). P_{meta} values were 4.40×10^{-19} for rs3077 (OR = 0.46, 95% confidence interval (CI) = 0.39–0.54), and 1.28×10^{-15} for rs9277542 (OR = 0.50, 95% CI = 0.43–0.60). Among the remaining 30 SNPs in the replication study, 27 SNPs were successfully genotyped by the DigiTag2 assay with SNP call rate ≥ 95% and HWE p-value ≥ 0.01. Two SNPs (rs9276431 and rs7768538), located in the genetic region including the *HLA-DQ* gene, were marginally replicated in the two sets of HBV carriers and healthy controls with Mantel-Haenszel P values of 2.80×10^{-7} (OR = 0.56, 95% CI = 0.45–0.70) and 1.09×10^{-7} (OR = 0.53, 95% CI = 0.42–0.67), respectively, when using additive, two-tailed Cochran Mantel-Haenszel (CMH) fixed-effects model with no evidence of heterogeneity ($P_{het} = 0.67$ for rs9276431 and 0.70 for rs7768538) (Table S1).

Meta-analysis using the random effects model across 6 independent studies, including 5 additional published data, showed $P_{meta} = 3.94 \times 10^{-45}$, OR = 0.55 for rs3077, $P_{meta} = 1.74 \times 10^{-21}$, OR = 0.61 for rs9277535 and $P_{meta} = 1.69 \times 10^{-15}$, OR = 0.51 for rs9277542, with the SNP rs9277535 being located about 4-kb upstream from rs9277542 and showing strong linkage disequilibrium of $r^2 = 0.955$ on the HapMap JPT (Table S2). As shown in Table S2, the odds ratio was very similar among the 6 studies, and heterogeneity was negligible with $P_{het} > 0.01$.

Moreover, based on GWAS using samples from 94 chronic HBV carriers with LC or HCC and 87 chronic HBV carriers without LC and HCC, we found no significant SNPs associated with CHB progression (Figure S1).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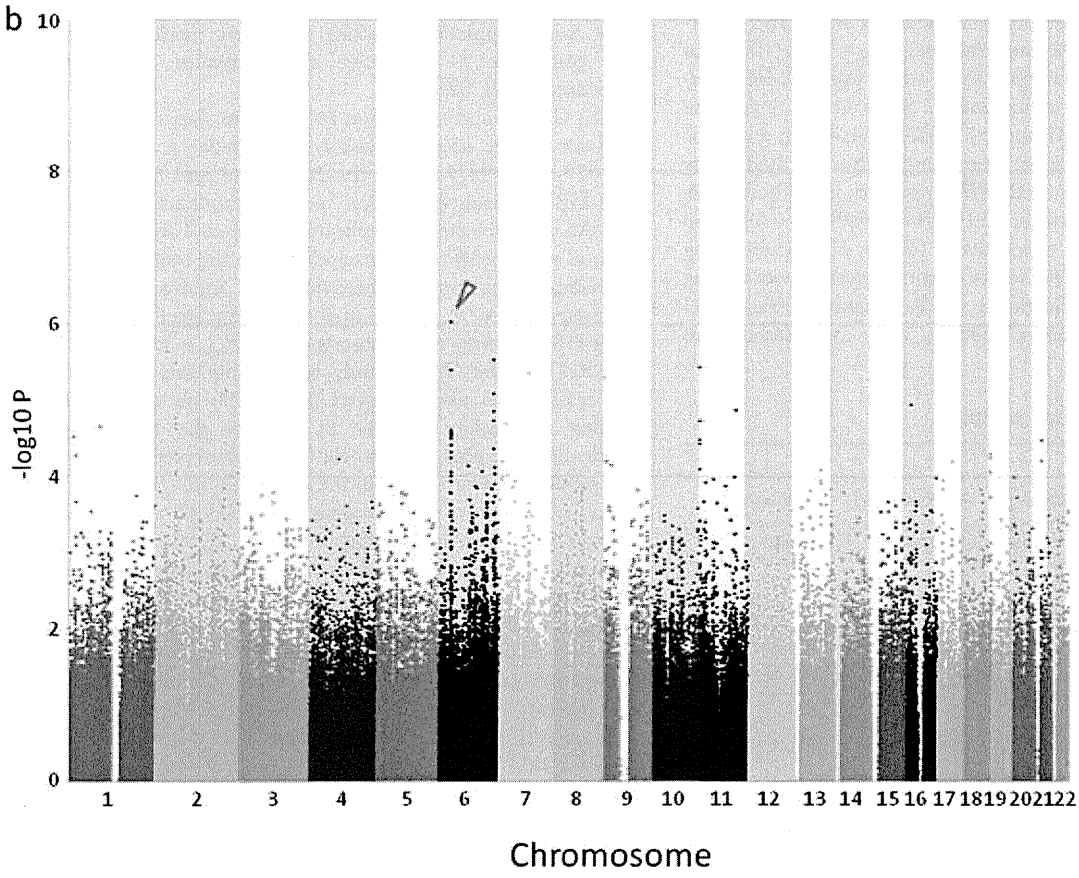
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Figure 1. Results of genome-wide association studies. a) HBV carriers and healthy controls, and b) HBV carriers and HBV-resolved individuals were compared. *P* values were calculated by chi-squared test for allele frequencies. Dots with arrows on chromosome 6 show strong associations with protective effects against persistent HB infection and with HBV clearance.
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Clearance of Hepatitis B virus in Japanese and Korean Individuals

We also conducted a GWAS to identify the host genetic factors related to clearance of HBV in the above 181 Japanese HBV carriers and 185 Japanese HBV-resolved individuals using a genome-wide SNP typing array (Affymetrix Genome-Wide Human SNP Array 6.0 for 900 K SNPs). The same two SNPs (rs3077 and rs9277542) showed strong associations in the allele frequency model ($P=9.24\times10^{-7}$ and $P=3.15\times10^{-5}$) with clearance of HBV (Figure 1b).

The above 32 SNPs, including the two associated SNPs (rs3077 and rs9277542), were selected for a replication study in two independent sets of HBV carriers and HBV resolved individuals (replication-1:256 Japanese HBV carriers and 150 Japanese HBV resolved individuals; and replication-2:344 Korean HBV carriers and 106 Korean HBV resolved individuals; Table 1). All 32 SNPs were genotyped using the DigiTag2 assay and 29 of 32 SNPs were successfully genotyped (Table S3). The associations of the original SNPs were replicated in both replication sets [replication-1 (Japanese): rs3077, $P=3.32\times10^{-2}$, OR $=0.72$ and rs9277542, $P=1.25\times10^{-2}$, OR $=0.68$; replication-2 (Korean): rs3077, $P=2.35\times10^{-7}$, OR $=0.41$ and rs9277542, $P=4.97\times10^{-6}$, OR $=0.46$; Table 3]. Meta-analysis using random effects model showed $P_{meta}=1.56\times10^{-4}$ for rs3077 (OR $=0.51$, 95% CI $=0.36$–0.72), and 5.91×10^{-7} for rs9277542 (OR $=0.55$, 95% CI $=0.43$–0.69). While there was evidence of heterogeneity between these studies for rs3077 ($P_{het}=0.03$) and no evidence for rs9277542 ($P_{het}=0.19$), significant associations with HBV clearance were observed with Mantel-Haenszel $P_{meta}=3.28\times10^{-12}$ for rs3077 and 1.42×10^{-10} for rs9277542, when using CMH fixed-effects model. Among the remaining 27 SNPs in the replication study, two SNPs (rs9276431 and rs7768538), located in a genetic region including *HLA-DQ* gene, were marginally replicated in the two sets of HBV carriers and HBV resolved individuals with Mantel-Haenszel *P* values of 2.10×10^{-5} (OR $=0.59$) and 1.10×10^{-5} (OR $=0.56$), respectively (Table S3), when using CMH fixed-effect model. Due to the existing heterogeneity among three groups (GWAS, Replication-1 and Replication-2) ($P_{het}=0.03$ for rs9276431 and 0.04 for rs7768538), weak associations were observed with $P_{meta}=0.03$ for rs9276431 and 0.02 for rs7768538 by the random effects model meta-analysis.

Meta-analysis across 6 independent studies, including 5 additional published data, showed $P_{meta}=1.48\times10^{-9}$, OR $=0.60$ for rs3077, $P_{meta}=1.08\times10^{-17}$, OR $=0.66$ for rs9277535 and $P_{meta}=5.14\times10^{-5}$, OR $=0.55$ for rs9277542 (Table S4). As shown in Table S4, the OR for the rs9277535 and rs9277542 were similar among the 6 independent studies, and heterogeneity was negligible ($P_{het}=0.03$ for rs9277535 and 0.14 for rs9277542). However, significant level of heterogeneity for rs3077 was observed with $P_{het}=9.57\times10^{-6}$ across 5 independent studies, including our study.

Table 1. Number of study samples.

		GWAS	Replication-1	Replication-2
population		**Japanese**	**Japanese**	**Korean**
HBV carriers	Total	**181**	**256**	**344**
	IC	20	94	–
	CH	67	101	177
	LC	3	10	–
	HCC	91	51	167
Healthy controls		**184**	**236**	**151**
Resolved individuals		**185**	**150**	**106**

Abbreviation: IC, Inactive Carrier; CH, Chronic Hepatitis; LC, Liver Cirrhosis; HCC, Hepatocellular Carcinoma.
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URLs

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

Discussion

The recent genome-wide association study showed that the SNPs located in a genetic region including *HLA-DPA1* and *HLA-DPB1* genes were associated with chronic HBV infection in the Japanese and Thai population [10,11]. In this study, we confirmed a significant association between SNPs (rs3077 and rs9277542) located in the same genetic region as *HLA-DPA1* and *HLA-DPB1* and protective effects against CHB in Korean and Japanese individuals. Mata-analysis using the random effects model across 6 independent studies including our study suggested that, widely in East Asian populations, variants in antigen binding sites of *HLA-DP* contribute to protective effects against persistent HBV infection (Table S2).

On GWAS and replication analysis with Japanese and Korean individuals, we identified associations between the same SNPs (rs3077 and rs9277542) in the *HLA-DPA1* and *HLA-DPB1* genes and HBV clearance; however, no new candidate SNPs from the GWAS were detected on replication analysis (Table S3). When the data of reference#18 was excluded from the meta-analysis across 6 independent studies, heterogeneity among 4 studies was estimated to be $P_{het}=0.15$ and significant association of rs3077 with HBV clearance was observed with $P_{meta}=5.88\times10^{-24}$, OR $=0.56$ (Table S4). In our study, a negligible level of heterogeneity for rs3077 was also observed ($P_{het}=0.03$) on meta-analysis by adding replication-1 (Table 3). Despite the heterogeneity in replication-1, a marginal association was observed for rs3077 with the same downward trend in the odds ratio ($P=3.32\times10^{-2}$, OR $=0.72$). Moreover, meta-analysis using GWAS and replication-2 showed significant association of $P_{meta}=1.89\times10^{-12}$, OR $=0.43$ for rs3077 with no evidence of heterogeneity ($P_{het}=0.75$). Although the reason why heterogeneity was observed in replication-1 is unclear, one possible reason is the clinical heterogeneity due to different kits being used for antibody testing. The associations of *HLA-DPA1/−DPB1* with CHB and HBV clearance showed the same level of significance in the comparison of HBV patients with HBV resolved individuals (OR $=0.43$ for rs3077 and 0.49 for rs9277542) as the one with healthy controls (OR $=0.46$ for rs3077 and 0.50 for rs9277542), when the replication-1 was excluded in the analysis (Table 2 and Table 3). The results of meta-analysis across 6 independent studies including our study also showed the same or slightly weaker associations in the

Table 2. Results of replication study for protective effects against CHB.

	Position		MAF[a]	Allele	Stage	HBV carriers			Healthy controls				OR[b]		
dbSNP rsID	Chr	Buld 36.3	Nearest Gene	(allele)	(1/2)	(population)	11	12	22	11	12	22	HWEp	95% CI	*P*-value[c]	P_{het}[d]
rs3077	6	33141000	HLA-DPA1	0.44	T/C	GWAS	13	51	117	28	88	67	0.919	0.42	1.14×10^{-7}	
				(T)		(Japanese)	(7.2)	(28.2)	(64.6)	(15.3)	(48.1)	(36.6)		(0.30–0.58)		
						Replication-1	26	95	134	46	125	65	0.309	0.48	2.70×10^{-8}	
						(Japanese)	(10.2)	(37.3)	(52.5)	(19.5)	(53.0)	(27.5)		(0.37–0.62)		
						Replication-2	23	81	111	31	74	40	0.767	0.47	2.08×10^{-6}	
						(Korean)	(10.7)	(37.7)	(51.6)	(21.4)	(51.0)	(27.6)		(0.35–0.65)		
						Meta-analysis[e]								0.46	4.40×10^{-19}	0.80
														(0.39–0.54)		
rs9277542	6	33163225	HLA-DPB1	0.45	T/C	GWAS	18	53	110	29	102	52	0.073	0.42	5.32×10^{-8}	
				(T)		(Japanese)	(9.9)	(29.3)	(60.8)	(15.8)	(55.7)	(28.4)		(0.31–0.58)		
						Replication-1	30	106	118	54	114	67	0.681	0.54	3.33×10^{-6}	
						(Japanese)	(11.8)	(41.7)	(46.5)	(23.0)	(48.5)	(28.5)		(0.42–0.70)		
						Replication-2	30	87	94	35	72	36	0.933	0.54	8.29×10^{-5}	
						(Korean)	(14.2)	(41.2)	(44.5)	(24.5)	(50.3)	(25.2)		(0.40–0.74)		
						Meta-analysis[e]								0.50	1.28×10^{-15}	0.40
														(0.43–0.60)		

[a]Minor allele frequency and minor allele in 198 healthy Japanese (ref#19).
[b]Odds ratio of minor allele from two-by-two allele frequency table.
[c]P value of Pearson's chi-square test for allelic model.
[d]Heterogeneity was tested using general variance-based method.
[e]Meta-analysis was tested using the random effects model.
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comparison of HBV patients with HBV resolved individuals (OR = 0.56 for rs3077, 0.66 for rs9277535 and 0.55 for rs9277542) than in the one with healthy controls (OR = 0.55 for rs3077, 0.61 for rs9277535 and 0.51 for rs9277542), which was the opposite result as we expected (Table S2 and Table S4). These results may suggest that other unknown immune system(s) exist to eliminate the HBV in the HBV resolved individuals.

Among the HLA class II loci (*HLA-DPA1*, *HLA-DPB1* and *HLA-DQB2*), which were associated with CHB and HBV clearance, a weak linkage disequilibrium ($r^2<0.1$) was observed between *HLA-DQB2* locus and *HLA-DPA1/−DPB1* loci in Japanese and Korean populations (Figure S2). We also found that similar linkage disequilibrium blocks (r^2) were observed among three subgroups (HBV carriers, HBV resolved individuals and Healthy controls). Moreover, logistic regression analysis of *HLA-DP* (rs3077 and rs92775542) with use of *HLA-DQ* (rs9276431 and rs768538) as covariates showed that the same level of significant associations of *HLA-DP* with CHB and HBV clearance as shown in the single-point association analysis, while no associations of *HLA-DQ* with $P_{log}>0.05$ were detected both in Japanese and in Korean (Table S5). These results show that *HLA-DP* is the main genetic factor for susceptibility to CHB and HBV clearance, and the associations of *HLA-DQB2* would result from linkage disequilibrium of *HLA-DP*A1/−DPB1.

In this study, we confirmed the significant associations between *HLA-DPA1* and *HLA-DPB1*, and protective effects against CHB and HBV clearance in Japanese and Korean individuals. These results suggest that the associations between the *HLA-DP* locus, CHB and HBV clearance are widely replicated in East Asian populations, including Chinese, Thai, Japanese and Korean individuals; however, there have been no similar GWAS performed in Caucasian and African populations. Moreover, there were no significant SNPs associated with HCC development in this study, thus suggesting that it is necessary to increase the sample size. To clarify the pathogenesis of CHB or the mechanisms of HBV clearance, further studies are necessary, including a functional study of the *HLA-DP* molecule, identification of novel host genetic factors other than *HLA-DP*, and variation analysis of HBV.

Materials and Methods

Ethics Statement

All study protocols conform to the relevant ethical guidelines, as reflected in the *a priori* approval by the ethics committees of all participating universities and hospitals. The written informed consent was obtained from each patient who participated in this study and all samples were anonymized.

Genomic DNA Samples and Clinical Data

All of the 1,793 Japanese and Korean samples, including individuals with CHB, healthy controls and HBV-resolved individuals (HBsAg-negative and anti-HBc-positive), were collected at 20 multi-center hospitals (liver units with hepatologists) throughout Japan and Korea. The 19 hospitals in Japan were grouped into the following 8 areas: Hokkaido area (Hokkaido University Hospital, Teine Keijinkai Hospital), Tohoku area (Iwate Medical University Hospital), Kanto area (Musashino Red Cross Hospital, Saitama Medical University, Kitasato University Hospital, University of Tokyo), Koshin area (Shinshu University Hospital, Kanazawa University Hospital), Tokai area (Nagoya City University Hospital, Nagoya Daini Red Cross Hospital), Kinki area (Kyoto Prefectural University of Medicine Hospital, National Hospital Organization Osaka National Hospital, Osaka

Table 3. Results of replication study for clearance of hepatitis B virus.

	Position		MAF[a]	Allele	Stage	HBV carriers			Resolved individuals			OR[b]		
dbSNP rsID	Chr	Build 36.3 Nearest Gene	(allele)	(1/2)	(population)	11	12	22	11	12	22	95% CI	*P*-value[c]	P_{het}[d]
rs3077	6	33141000 HLA-DPA1	0.44	T/C	GWAS	13	51	117	29	82	74	0.44	9.24×10^{-7}	
			(T)		(Japanese)	(7.2)	(28.2)	(64.6)	(15.7)	(44.3)	(40.0)	(0.32–0.61)		
					Replication-1	26	95	134	20	64	60	0.72	3.32×10^{-2}	
					(Japanese)	(10.2)	(37.3)	(52.5)	(13.9)	(44.4)	(41.7)	(0.53–0.97)		
					Replication-2	23	81	111	29	48	28	0.41	2.35×10^{-7}	
					(Korean)	(10.7)	(37.7)	(51.6)	(27.6)	(45.7)	(26.7)	(0.29–0.58)		
					Meta-analysis[e]							0.51	1.56×10^{-4}	0.03
												(0.36–0.72)		
					Meta-analysis[e]							0.43	1.89×10^{-12}	0.75
					(GWAS+replication-2)							(0.34–0.54)		
rs9277542	6	33163225 HLA-DPB1	0.45	T/C	GWAS	18	53	110	28	88	69	0.51	3.15×10^{-5}	
			(T)		(Japanese)	(9.9)	(29.3)	(60.8)	(15.1)	(47.6)	(37.3)	(0.37–0.70)		
					Replication-1	30	106	118	28	62	52	0.68	1.25×10^{-2}	
					(Japanese)	(11.8)	(41.7)	(46.5)	(19.7)	(43.7)	(36.6)	(0.51–0.92)		
					Replication-2	30	87	94	30	53	22	0.46	4.97×10^{-6}	
					(Korean)	(14.2)	(41.2)	(44.5)	(28.6)	(50.5)	(21.0)	(0.33–0.64)		
					Meta-analysis[e]							0.55	5.91×10^{-7}	0.19
												(0.43–0.69)		
					Meta-analysis[e]							0.49	9.69×10^{-10}	0.65
					(GWAS+replication-2)							(0.39–0.61)		

[a]Minor allele frequency and minor allele in 198 healthy Japanese (ref#19).
[b]Odds ratio of minor allele from two-by-two allele frequency table.
[c]P value of Pearson's chi-square test for allelic model.
[d]Heterogeneity was tested using general variance-based method.
[e]Meta-analysis was tested using the random effects model.
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City University), Chugoku/Shikoku area (Tottori University Hospital, Ehime University Hospital, Yamaguchi University Hospital, Kawasaki Medical College Hospital) and Kyushu area (Kurume University Hospital). Korean samples were collected at Yonsei University College of Medicine.

HBV status was measured based on serological results for HBsAg and anti-HBc with a fully automated chemiluminescent enzyme immunoassay system (Abbott ARCHITECT; Abbott Japan, Tokyo, Japan, or LUMIPULSE f or G1200; Fujirebio, Inc., Tokyo, Japan). For clinical staging, inactive carrier (IC) state was defined by the presence of HBsAg with normal ALT levels over 1 year (examined at least four times at 3-month intervals) and without evidence of portal hypertension. Chronic hepatitis (CH) was defined by elevated ALT levels (>1.5 times the upper limit of normal [35 IU/L]) persisting over 6 months (at least by 3 bimonthly tests). Liver cirrhosis (LC) was diagnosed principally by ultrasonography (coarse liver architecture, nodular liver surface, blunt liver edges and hypersplenism), platelet counts <100,000/cm^3, or a combination thereof. Histological confirmation by fine-needle biopsy of the liver was performed as required. Hepatocellular carcinoma (HCC) was diagnosed by ultrasonography, computerized tomography, magnetic resonance imaging, angiography, tumor biopsy or a combination thereof.

The Japanese control samples from HBV-resolved subjects (HBsAg-negative and anti-HBc-positive) at Nagoya City University-affiliated healthcare center were used by comprehensive agreement (anonymization in an unlinkable manner) in this study. Some of the unrelated Japanese healthy controls were obtained from the Japan Health Science Research Resources Bank (Osaka, Japan). One microgram of purified genomic DNA was dissolved in 100 μl of TE buffer (pH 8.0) (Wako, Osaka, Japan), followed by storage at −20°C until use.

SNP Genotyping and Data Cleaning

For GWAS, we genotyped a total of 550 individuals, including 181 Japanese HBV carriers, 184 Japanese healthy controls and 185 spontaneously HBV-resolved Japanese individuals (HBsAg-negative and anti-HBc-positive), using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Inc., Santa Clara, CA), in accordance with the manufacturer's instructions. The average QC call rate for 550 samples reached 98.47% (95.00–99.92%), which had an average sample call rate of 98.91% (93.55–99.74%) by determining the genotype calls of over 900 K SNPs using the Genotyping Console v4.1 software (with Birdseed v1 algorithm) provided by the manufacturer [19]. We then applied the following thresholds for SNP quality control in data cleaning: SNP call rate ≥95% and MAF ≥1% for three groups (HBV carriers, healthy controls and HBV-resolved individuals), and HWE *P*-value ≥0.001 for healthy controls [20]. Here, SNP call rate is defined for each SNP as the number of successfully genotyped samples divided by the number of total samples genotyped. A total of 597,789 SNPs and 590,278 SNPs on autosomal chromosomes