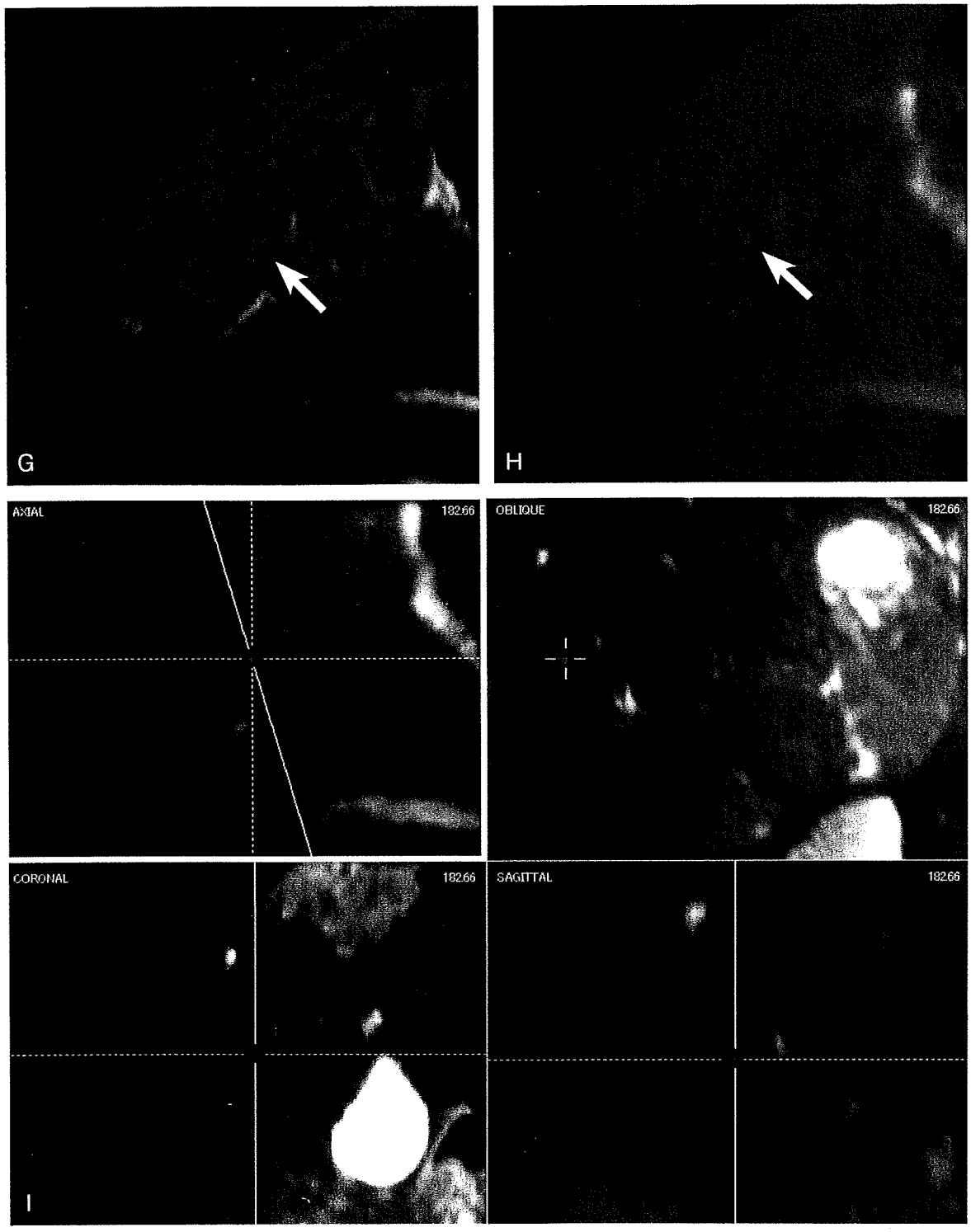


図6　つづき

G　SPIO 投与後脂肪抑制併用 T2 強調像（3T FSE 3976/76）　A と同時期。背景肝の線維化，脈管構造の描出が良好となっているが，病変（→）と脈管との分離同定は依然困難である。 H, I　SPIO 投与後脂肪抑制併用 T2 強調像（3T 3D-FSE 4150/134）　A と同時期。横断像（H）において背景肝の線維化，脈管構造の描出は FSE 法と比較して不明瞭となっているが，病変（→）と脈管との分離同定が可能である。再構成像（I）により多方向からの評価が可能である。高磁場装置における磁化率効果の増強が示唆される症例である。

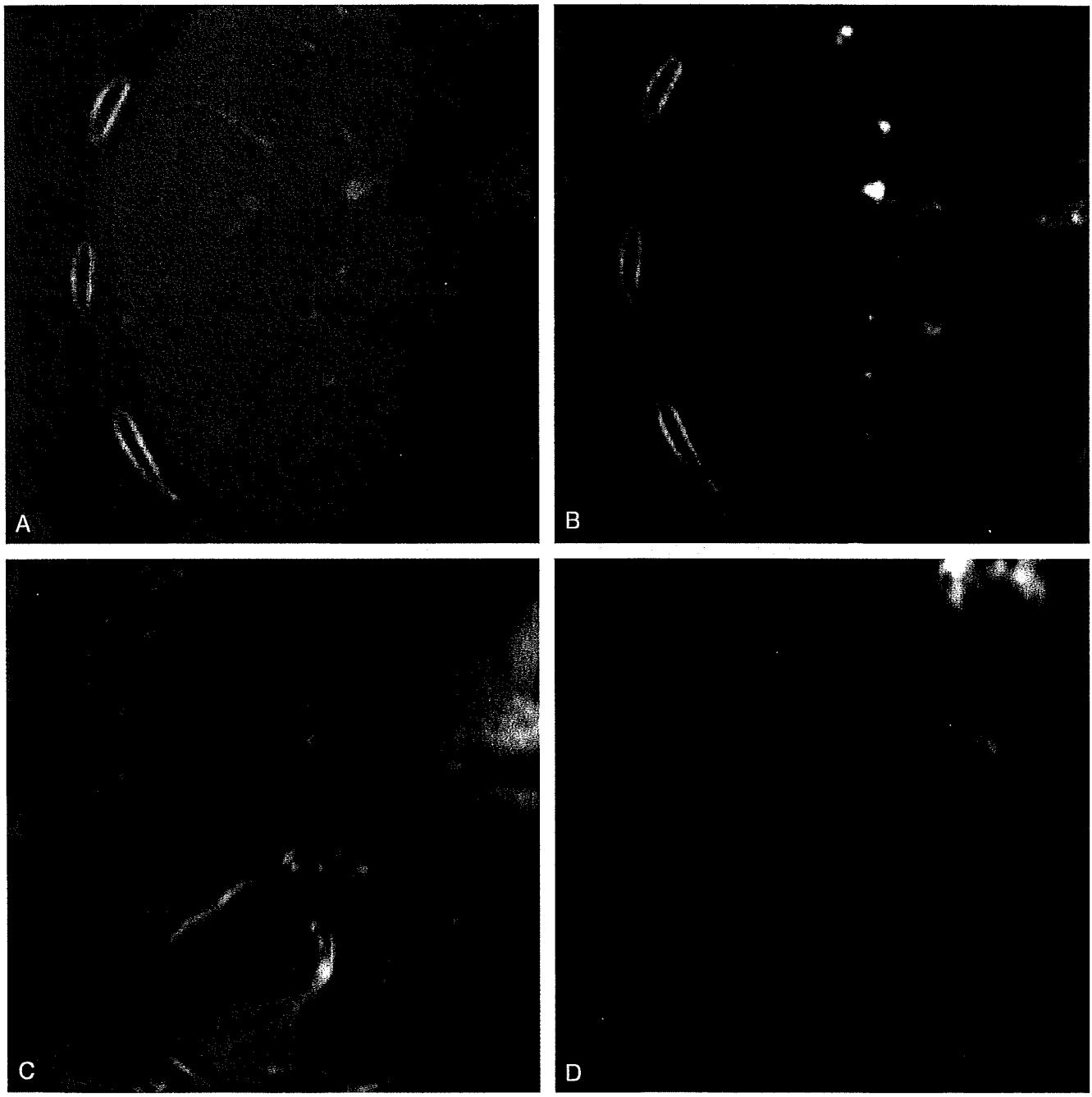
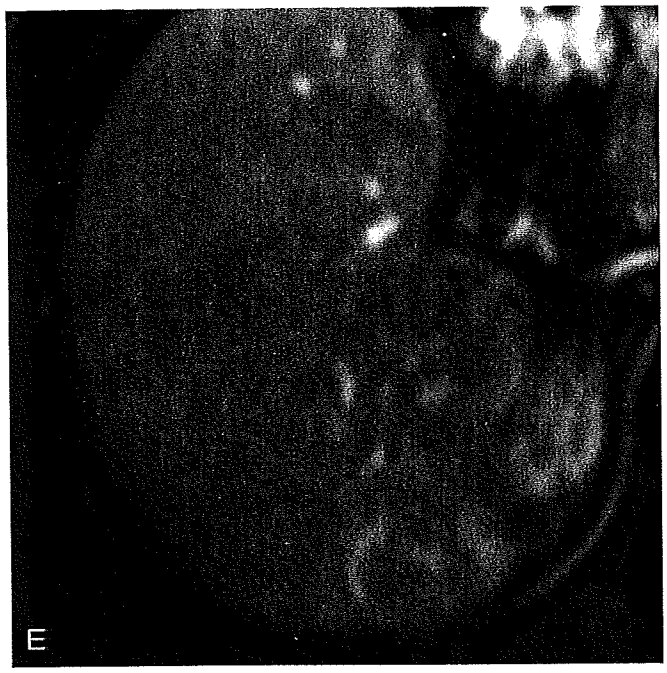
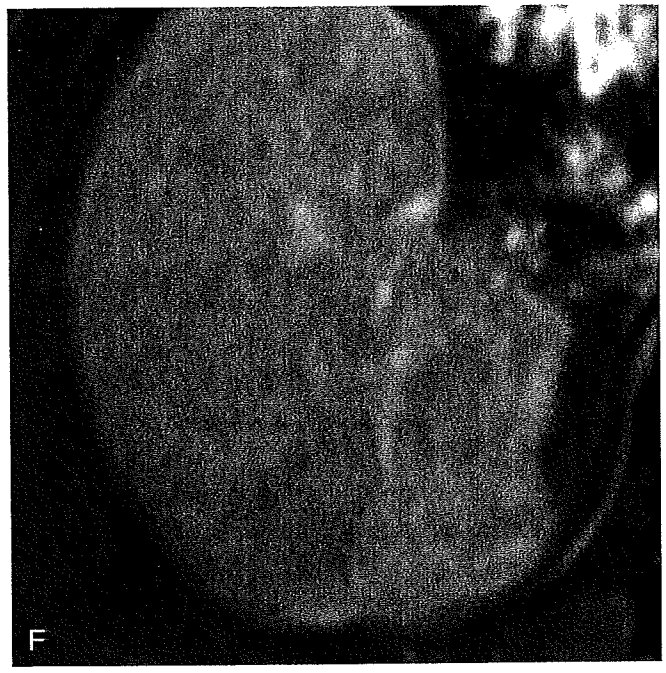


図 7　Gd-EOB-DTPA 造影 MRI

A　CTAP　肝右葉前区域に結節状の門脈血流欠損域を認める。 B　CTHA　門脈血流欠損域は動脈性濃染を示し，周囲に動脈血流低下域を認める。境界病変内に発生した肝細胞癌の所見である。 C　脂肪抑制併用 T2 強調像（FSE 3993/70）　肝細胞癌は高信号域，境界病変は等信号域として描出されている。 D　脂肪抑制併用 T1 強調像（GRE 3.5/1.4/15）　肝細胞癌は低信号域，境界病変は等信号域として描出されている。





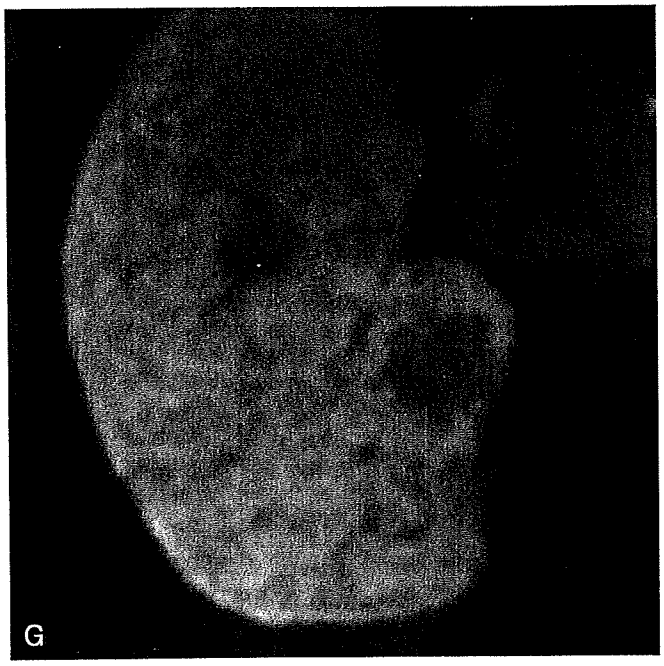


図7　つづき
E, F　Gd-EOB-DTPA造影 dynamic MRI（GRE 3.5/1.4/15）早期相（E）において肝細胞癌は高信号域，境界病変は低信号域として描出されているが，後期相（F）では周囲肝と等信号となり，病変の描出は不明瞭である。
G　Gd-EOB-DTPA造影肝実質相（GRE 140/1.69/60）周囲肝はGd-EOB-DTPAの取り込みにより高信号を呈し，病変とのコントラストが増強している。境界病変は軽度低信号域，肝細胞癌はより明瞭な低信号域として描出されており組織学的分化度の違いを反映している可能性がある。

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Summary

The diagnosis and therapy of hepatocellular carcinoma：the latest move and future view：MRI

The current state of MR imaging in hepatocellular carcinoma (HCC) was described. The T2-weighted imaging is useful in the estimation of malignancy grade of HCC. Gd-enhanced dynamic MR imaging is a less-invasive method that can visualize hemodynamics of HCC. SPIO-MR imaging is useful for the detecting small lesions, but there is the discrepancy between uptake and malignancy grade. As the leading-edge trends, diffusion-weighted imaging, 3.0 T MR imaging, 3D fast spin-echo imaging, and Gd-EOB-DTPA were also described.

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肝臓

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正常解剖(図1)

右の上腹部で横隔膜下面の凹みに存在している．肝後面の上部はbare areaと呼ばれ，腹膜を欠くため，肝は横隔膜に接している．肝鎌状靱帯により大きく解剖学的左葉と右葉とに区分される．また，胆嚢窩と下大静脈を結ぶ線(Cantlie's line)により外科的な左葉と右葉とに分けられ，この線上に中肝静脈が走行する．

門脈本幹は肝門部で右枝と左枝に分かれ，右枝はさらに前区域枝と後区域枝とに分かれる．肝動脈は，固有肝動脈から左肝動脈と右肝動脈とに分かれる．右肝動脈も前区域枝と後区域枝

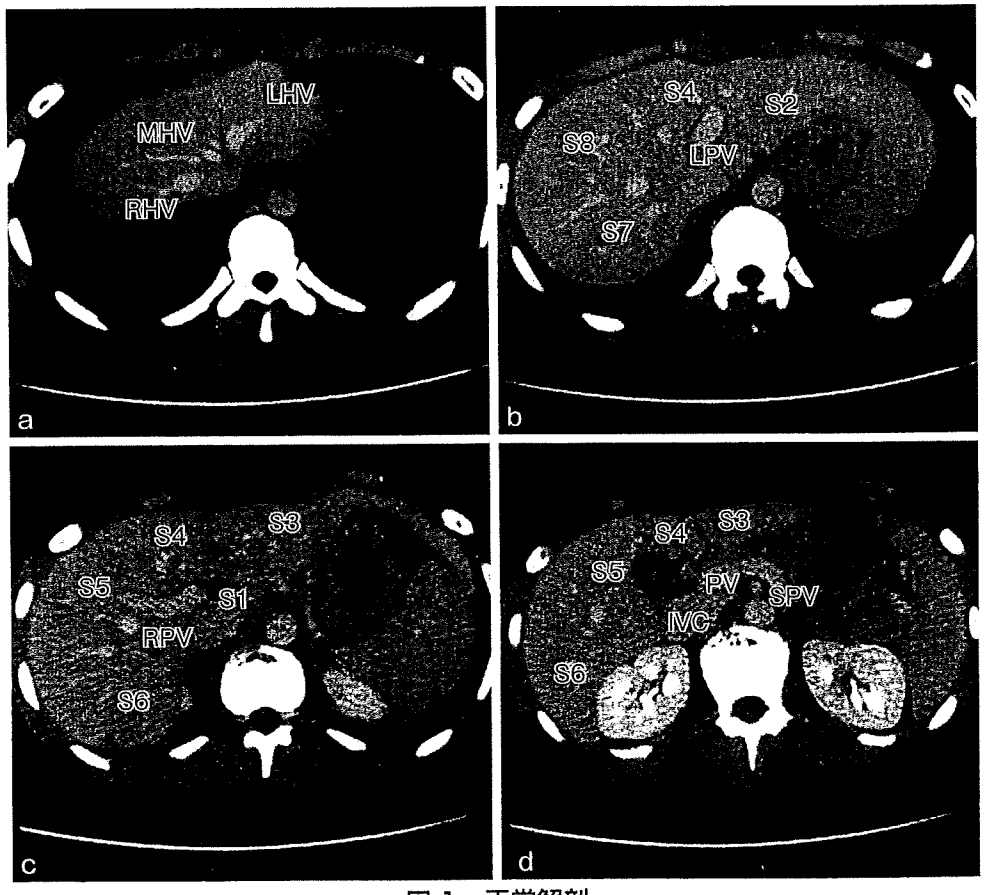

図1 正常解剖

a～d：造影CT(門脈相)
LHV：左肝静脈，MHV：中肝静脈，RHV：右肝静脈，PV：門脈本幹，LPV：門脈左枝，RPV：門脈右枝，SPV：脾静脈，IVC：下大静脈

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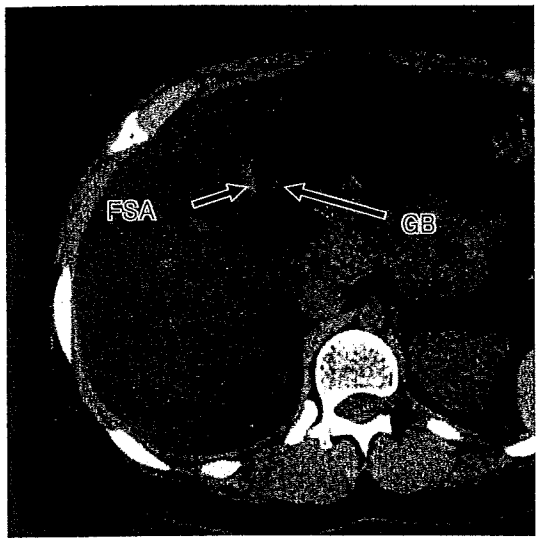

図2　高度脂肪肝

単純CT．肝実質は筋肉よりも低濃度を示している．肝内の脈管が肝実質よりも高濃度に描出されている．異所性静脈還流域である胆嚢床部には脂肪沈着が乏しく，相対的に高濃度に描出されている(focal spared area：FSA)．GB：胆嚢

Column　化学シフトによる位相コントラスト

グラディエントエコー(GRE)法で撮像した際にみられる現象である．脂肪と水のプロトンはわずかに共鳴周波数が違うため，歳差運動の速度も異なってくる．このため回転運動の位置，すなわち位相が同じ方向(in-phase)となったり，逆方向(opposed-phase)となったりを時間経過とともに繰り返す．

in-phaseでの信号は水と脂肪からの信号を足したものであるか，逆にopposed-phaseでは180度方向が違うため，両者の信号が打ち消しあってしまう．そのため，水と脂肪が混在する組織ではin-phaseに比してopposed-phaseで信号が低下する．軽度から中等度の脂肪沈着の検出に鋭敏な方法で，位相コントラスト法とも呼ばれている．

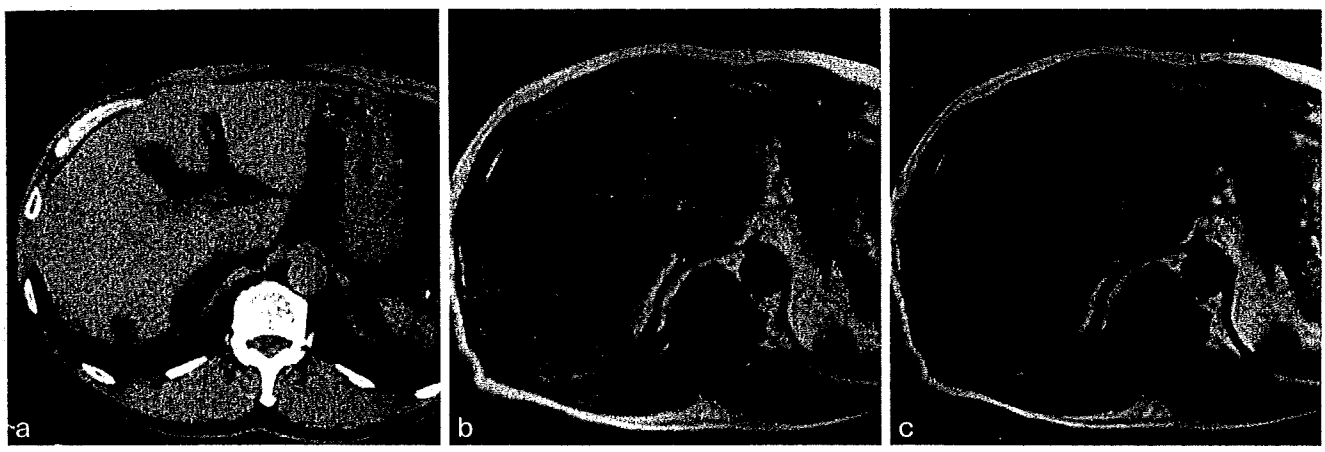

図3　中等度脂肪肝

a：単純CT，b：T1強調像(in-phase)，c：T1強調像(opposed-phase)
単純CTでは，肝実質の濃度が低下し，血管は不明瞭化している．T1強調像(in-phase)では，肝は筋肉よりも高信号を示している．T1強調像のopposed-phaseでは，肝脂肪沈着部位が信号の低下を示している．なお，S7に嚢胞を認める．

とに分かれ，肝内の動脈は門脈に伴走する．肝内胆管も門脈に伴走する．

肝臓の区域は，Couinaud分類が一般的である．門脈や肝静脈の走行により尾状葉(S1)，左葉背外側区域(S2)，左葉腹外側区域(S3)，左葉内側区域(S4)，右葉前下区域(S5)，右葉後下区域(S6)，右葉後上区域(S7)，右葉前上区域(S8)と8つに分けられる．

各種の画像/異常所見

●びまん性肝疾患

1．脂肪肝(fatty liver，図2，3)

肝細胞に中性脂肪が蓄積する病態である．原因としてはアルコール，過栄養，糖尿病，薬剤など多彩である．

CTでは，脂肪が沈着すると肝の濃度は低下する．正常肝のCT値(65±5 HU)は脾のCT

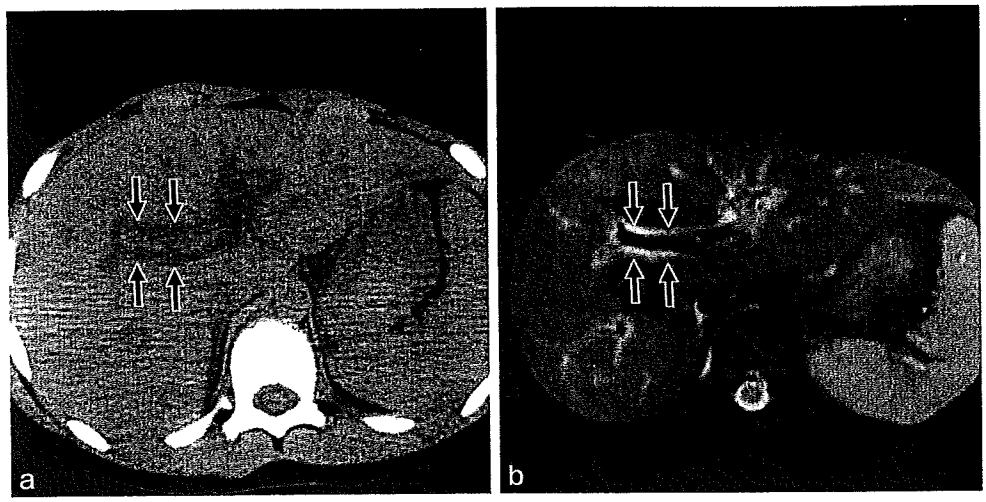

図4　急性肝炎

a：単純 CT，b：脂肪抑制併用 T2 強調像
門脈周囲には Glisson 鞘の浮腫性変化を反映し，tram-line 状に単純 CT で低濃度，T2 強調像では高信号域として認められる(矢印)．T2 強調像では肝実質の信号も上昇している．

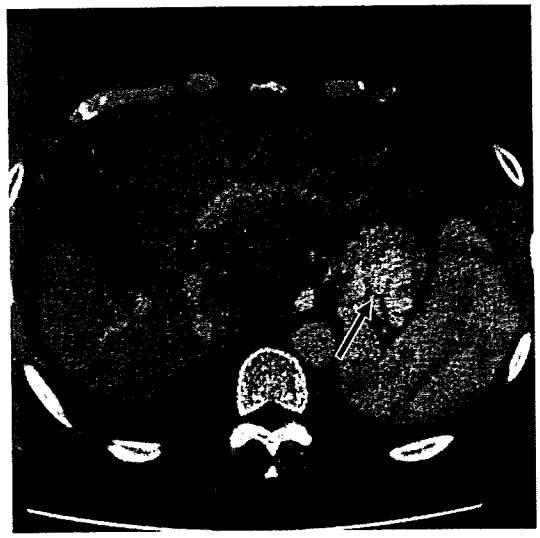

図5　肝硬変

造影 CT(門脈優位相)．肝左葉は腫大し，右葉は萎縮している．門脈圧亢進に伴い，脾腫や胃静脈瘤(矢印)を認める．

値(45±5 HU)より高いので，比較しながら診断する．軽度～中等度では肝内血管が不明瞭化する．高度脂肪肝では肝内血管が相対的に高濃度として描出される．MRI では，高度脂肪肝は SE 法による T1 強調像で高信号に描出されるが，軽度～中等度の場合は信号強度による判定は難しい．軽度～中等度の場合では，GRE を用いた位相コントラスト法(phase-shift)による T1 強調像が有用で，脂肪沈着部位は in-phase と比べ，opposed-phase で信号が低下する(コラム参照)．異所性静脈還流により内側区背側部や胆嚢床部，S4 鎌状間膜付着部周囲に限局的な非脂肪沈着部位(focal fat-spared area)をしばしば認め，肝内病変と誤認しないよう注意する必要がある．

2. 急性肝炎(acute hepatitis，図4)

病態はウイルス，アルコール，薬剤などの原因による肝細胞の変性壊死とその再生であり，炎症反応が門脈域にみられる．CT では，胆嚢の虚脱と壁の浮腫性肥厚が認められる．肝門から比較的太いレベルの門脈周囲に tram-line あるいは ring 状の低濃度域(periportal collar sign)を呈する．MRI では，門脈周囲は Glisson 鞘の浮腫性変化を反映して T1 強調像で低信号，T2 強調像で高信号に描出される(periportal abnormal high intensity：PAI)．これらは，Glisson 鞘の炎症細胞浸潤や浮腫性変化を反映しているものと考えられている．ただし，外傷や腫瘍によるリンパ系の拡張や閉塞，うっ血などによっても同様な所見を呈することがあるため，臨床所見を加味して診断する必要がある．

3. 肝硬変(cirrhosis of liver，図5)

肝炎などの慢性肝疾患の終末像で，肝実質にはびまん性に線維化と再生結節で置換される．右葉や内側区は萎縮し，外側区や尾状葉が代償性に腫大する．肝辺縁は鈍化し，肝表面は凹凸

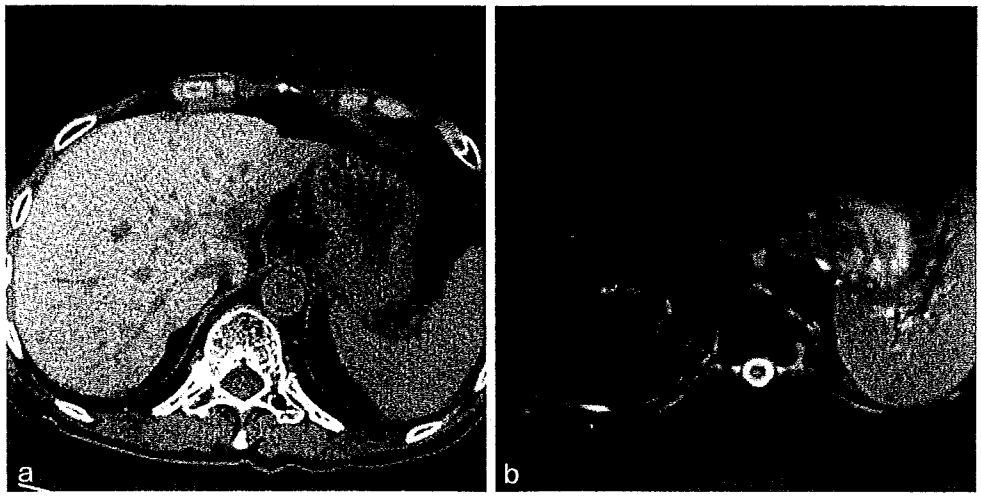

図6　ヘモジデローシス

a：単純CT，b：脂肪抑制併用T2強調像
肝は単純CTではびまん性に高濃度を呈し，T2強調像では筋肉よりも明らかに低信号に描出されている．

不整像を呈する．

再生結節によって，CTでは肝の濃度は多発結節状に不均一となる．また，MRIではT2強調像やGRE法で多発結節状に低信号を呈する．門脈圧亢進症に伴う脾腫に加え，食道・胃静脈瘤や胃腎・脾腎シャント，傍臍静脈の再開通などの遠肝性の門脈大循環シャントが形成される．非代償期では，腹水などの肝外の変化も認める．脾臓のうっ血に伴う出血巣にヘモジデリン沈着したGamna-Gandy bodyが脾臓内に認められ，T2強調像やT2*強調像で低信号を呈する．

4. ヘモジデローシス(hemosiderosis，図6)

肝を筆頭に，実質臓器に過剰の鉄がヘモジデリンとして沈着する病態である．実質臓器の機能障害をきたし，皮膚の色素沈着や糖尿病などの症状を呈するものをヘモクロマトーシス，機能障害を伴わないものをヘモジデローシスと呼ぶ．CTでは，沈着したヘモジデリンの影響で濃度が上昇する．MRIでは，T2強調像やT2*強調像で筋よりも明らかな低信号を呈する．

●腫瘤性病変

1. 肝細胞癌(hepatocellular carcinoma，図7)

肝細胞癌はB型やC型肝炎ウイルスの持続感染などによる慢性肝疾患を背景に発生することが多い．特にC型ウイルス感染例では肝細胞癌は多段階発癌することが知られており，分化度によって腫瘤内の血行支配が変化する．悪性度が高まるにつれて腫瘤内の門脈血流は減少する．腫瘤内の動脈血流は前癌病変では減少するが，その後次第に増加し，中分化型では著増する．低分化型では逆に動脈血流が減少する．

中分化型を主体とする古典的肝細胞癌は，単純CTでは低濃度で，造影CTでは動脈優位相でモザイク状に濃染し，門脈相から平衡相では周囲肝より低濃度となる．単純結節型では線維性被膜を伴うことが多く，単純CTや造影CTの動脈優位相では低濃度，門脈相から平衡相では高濃度に描出される．MRIのT1強調像では低，等，高信号とさまざまな信号強度を呈するのに対し，T2強調像では一般に高信号を呈する．T1強調像で高信号を呈する成因の25%は，癌細胞への脂肪沈着である．脂肪抑制を併用したT1強調像や，位相コントラストを利用するin-phaseおよびopposed-phaseのT1強調像を比較することで，脂肪沈着の有無や程度を推定できる点がMRIの優れた点である．

2. 転移性肝癌(metastatic liver tumor，図8)

肝は転移性腫瘍の好発部位で，消化器癌からの血行性転移によるものが多い．肺癌や消化管

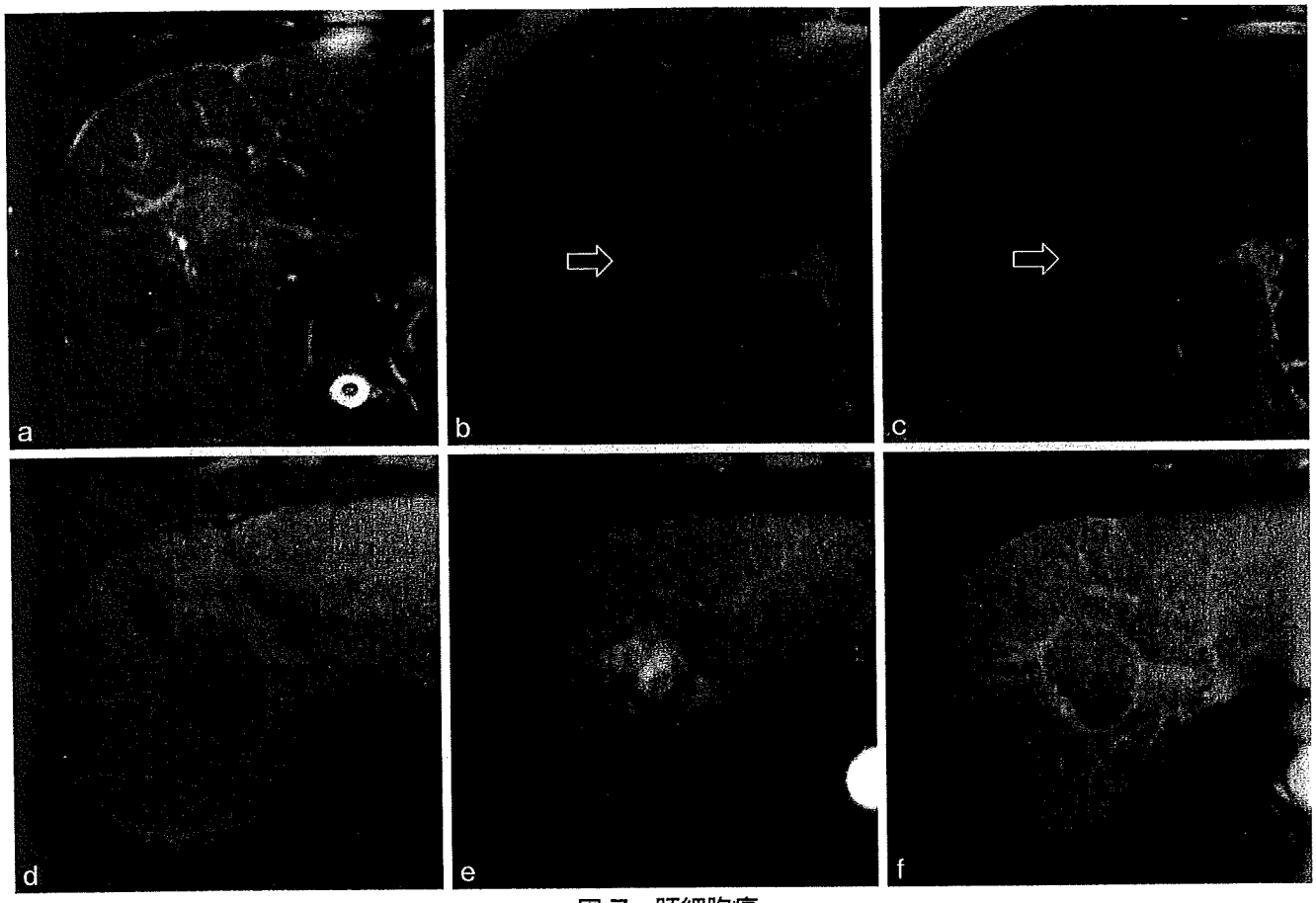

図7　肝細胞癌

a:脂肪抑制併用 T2 強調像，b:T1 強調像(in-phase)，c:T1 強調像(opposed-phase)，d:造影 MRI(造影前)，e:造影 MRI(動脈相)，f:造影 MRI(平衡相)
T2 強調像では高信号を呈している．T1 強調像(in-phase)ではほぼ等信号を呈し，腫瘤背側部は周囲肝より高信号を呈している．T1 強調像(opposed-phase)では病変全体の信号が低下し，脂肪成分の含有が示唆されるが，背景部の信号低下が著しい．造影前に低信号を呈していた病変は，動脈相で早期濃染によって高信号に描出され，平衡相では washout で信号が低下し，被膜がリング状濃染を示している．

癌，膵癌，乳癌由来の肝転移巣は乏血性で，膵内分泌腫瘍や腎細胞癌，甲状腺癌，カルチノイドなどは多血性を示す．

単純 CT では一般に低濃度を呈する．大腸癌で，特に粘液産生性腺癌では石灰化を伴い，転移巣内に高濃度域を認めることがある．乏血性腫瘍は造影 CT の動脈優位相で細胞成分に富む辺縁部がリング状に濃染し，平衡相で線維成分に富む中心部が遅延性に濃染することが多い．多血性腫瘍では，動脈優位相で病変全体が濃染し，平衡相では周囲肝と比較し等濃度ないし低濃度を呈する．

MRI では，T1 強調像で低信号，T2 強調像で高信号を呈する．超常磁性酸化鉄粒子(superparamagnetic iron oxide particle：SPIO) 造影剤投与後の T2 強調像では，造影剤を取り込まないため高信号に描出される．また，肝細胞胆道系造影剤(gadolinium ethoxybenzayl diethlene-triamine pentaacetic acid：Gd-EOB-DTPA)の肝細胞相では T1 強調像で低信号に描出され，微小な転移巣の把握に有用である．

3. 胆管細胞癌(肝内胆管癌，cholangiocellular carcinoma，図9)

肝内胆管上皮由来の悪性腫瘍で，線維性間質に富む腺癌である．肝門型と二次分枝より末梢の肝内胆管由来の末梢型に大別される．末梢型では腫瘤形成型が多く，肝門部型では胆管浸潤性発育や胆管内発育するタイプが多い．

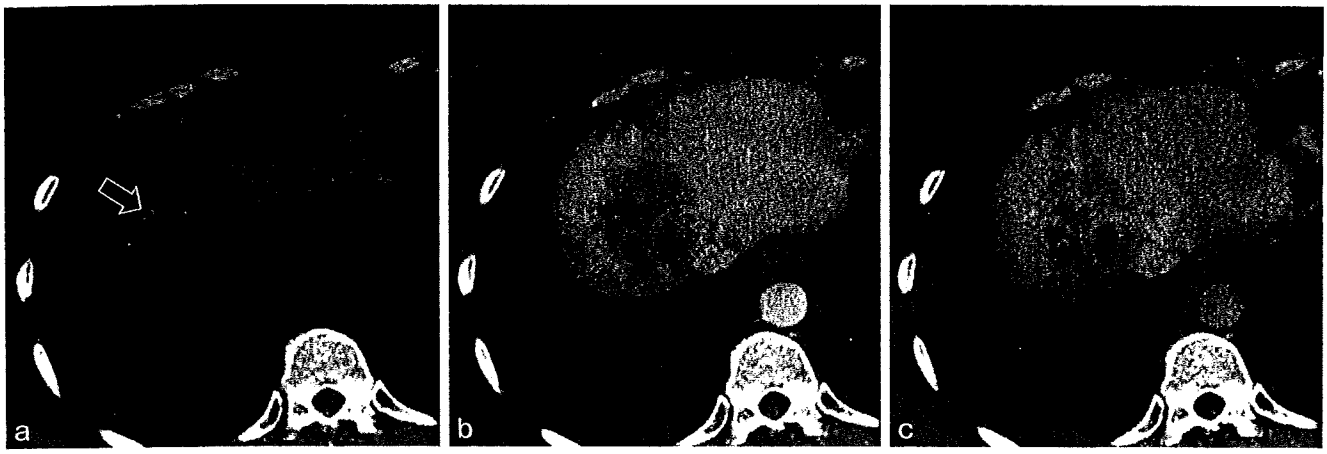

図 8　転移性肝腫瘍

a：単純 CT，b：造影 CT 動脈相，c：造影 CT 平衡相
単純 CT では，内部石灰化（矢印）を伴う低濃度腫瘤として描出されている．造影では動脈相で腫瘤辺縁優位に増強効果を認めて，平衡相では腫瘤内部に遅延性濃染を認める．

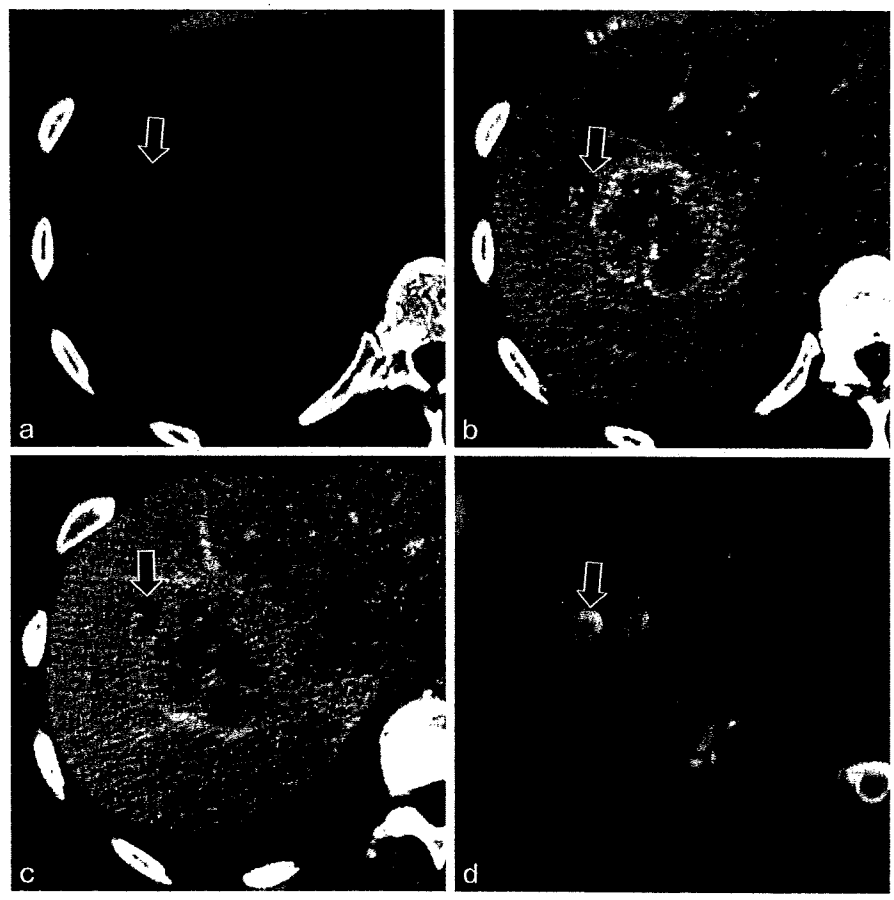

図 9　胆管細胞癌

a：単純 CT，b：造影 CT 動脈相，c：造影 CT 平衡相，d：脂肪抑制併用 T2 強調像
単純 CT では，低濃度腫瘤として描出されている．造影 CT では動脈相で辺縁優位に濃染し，平衡相で内部は遅延性濃染を示す．胆管細胞癌区域内の胆管枝に拡張（矢印）を認めるとともに，動脈相にて末梢側は区域性に淡い濃染を示す．T2 強調像では，腫瘤は高信号を示すが，中心部は軽度低信号となり，壊死に伴う線維化が示唆される．

腫瘤型は単純 CT で分葉状の低濃度腫瘤として描出され，大型になると腫瘍辺縁の肝表側に陥凹（癌臍）を伴うことがある．造影 CT の動脈優位相では腫瘍成分の多い辺縁部が濃染され，平衡相では線維性間質の豊富な中心部が遅延性に濃染される．MRI の T1 強調像で一般に低信号に描出される．一方，T2 強調像で高信号に描出されるが，線維性間質が豊富な場合は低信号，壊死を伴う場合は著明な高信号を呈する．消化器由来腺癌の転移性肝癌と，臨床所見も参考に鑑別する必要がある．

肝門型では腫瘍部で胆管壁の肥厚を認め，内

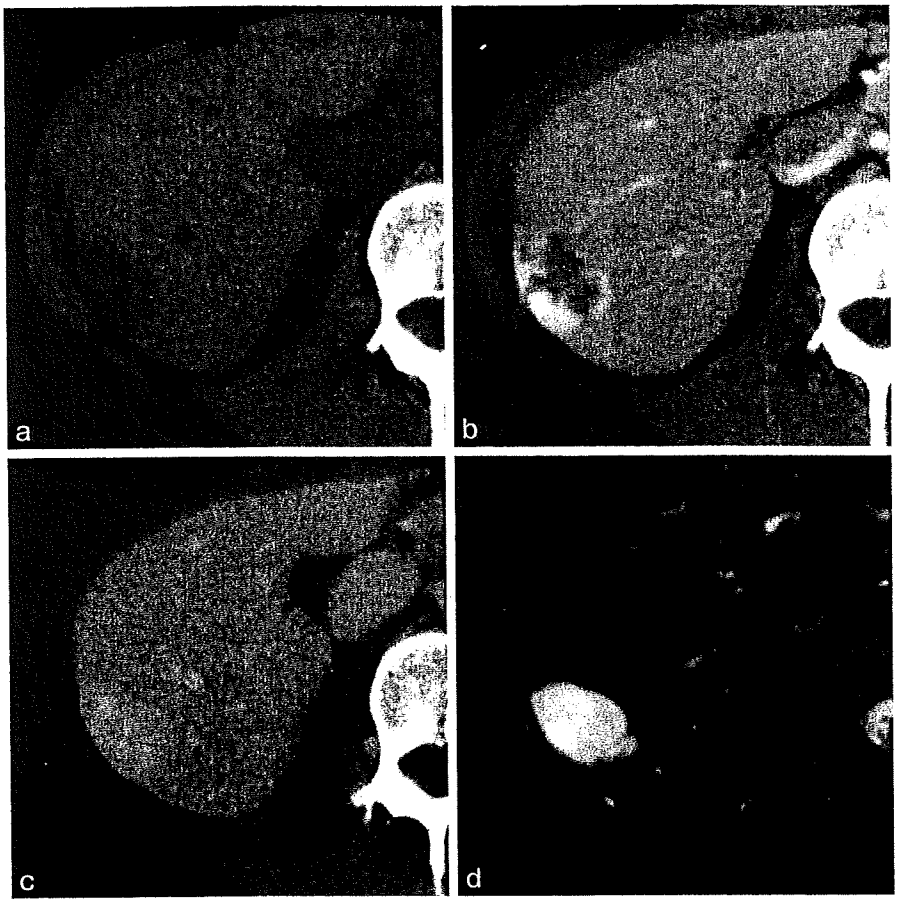

図 10　海綿状血管腫
a：単純 CT，b：造影 CT 動脈相，c：造影 CT 平衡相，d：脂肪抑制併用 T2 強調像
単純 CT では，下大静脈とほぼ等しい低濃度腫瘤を肝表直下に認める．膨隆を示さず形状は非円形である．造影 CT では動脈相で辺縁が結節状に濃染し始め，平衡相では病変全体が均一に濃染されている．T2 強調像では，均一で著明な高信号に描出されている．

腔は先細り状に狭窄し，肝内末梢域の胆管拡張を伴う．胆管の全体像の評価には，磁気共鳴胆道膵管撮影法(magnetic resonance chorangio pancreato-graphy：MRCP)が有用である．早期から所属リンパ節転移をきたすため，肝門部から大動脈周囲リンパ節の評価も重要である．

4. 肝海綿状血管腫(cavernous hemangioma, 図 10)

血管内皮細胞に覆われた血管腔が海面状に連続する良性腫瘍である．単純 CT では含有する血液が反映され，血管(大動脈や下大静脈腔)と等濃度を呈する．造影 CT では動脈相で腫瘤辺縁部から結節状に濃染(fill-in phenomenon)し，次第に中心部へと濃染が広がり，平衡相まで濃染が持続する(prolonged enhancement)．MRI では T1 強調像で低信号，T2 強調像では均一で著明な高信号を呈する．巨大な血管腫では血栓，瘢痕形成に加え，出血や石灰化といった変性により多彩な像を呈することがある．

5. 肝膿瘍(liver abscess, 図 11)

細菌および非細菌性の誘因により，肝内に膿汁の貯留域が形成される．感染経路として経胆道性が最も多い．肝膿瘍は中央の壊死部(膿瘍腔)と辺縁の炎症性肉芽で構成され，周囲肝には反応性浮腫を伴う．画像所見にはこの構造が反映される．

単純 CT では，膿瘍腔は境界明瞭な低濃度域として描出され，炎症や浮腫を反映する部分は不明瞭な軽度低濃度を呈する．造影 CT では，膿瘍腔は造影効果を示さない．膿瘍腔の隔壁構造や辺縁部は動脈相で明らかな濃染を示し，その外側は軽度低濃度を呈する(double target sign)．平衡相では辺縁の増強効果は持続し，周囲は正常肝と等濃度を呈する．MRI では，膿瘍腔は T2 強調像で著明な高信号を呈し，周囲の炎症や浮腫性変化は T2 強調像で淡い高信号を呈する．造影 CT や MRI の動脈相で，膿瘍が存在する区域の末梢域が楔状(区域性)に濃

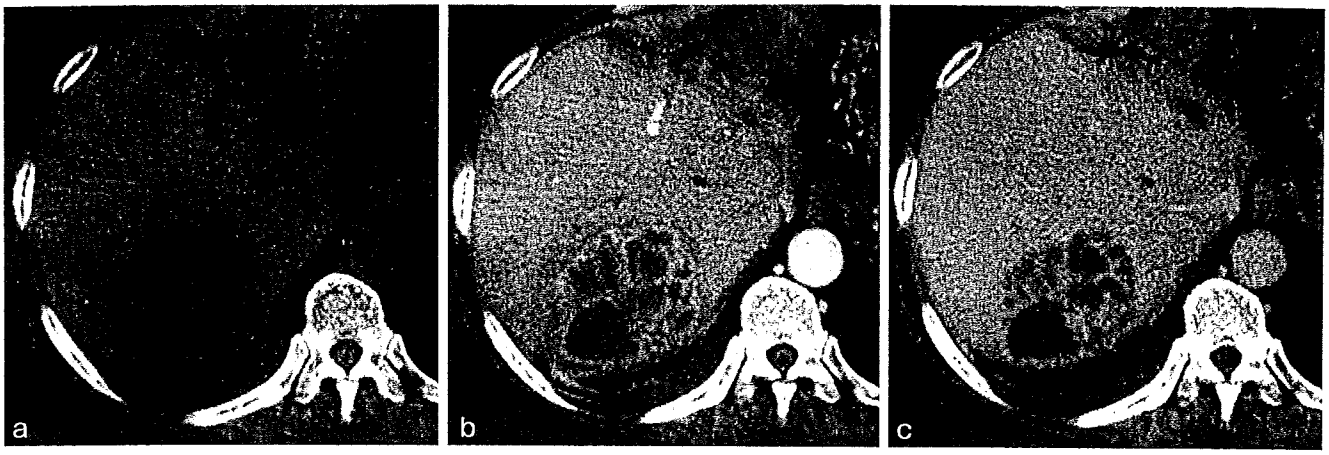

図 11　肝膿瘍

a：単純 CT，b：造影 CT 動脈相，c：造影 CT 平衡相
単純 CT では，不均一な低濃度腫瘤として描出されている．造影 CT では，動脈相で隔壁構造が濃染を示し多房性を呈する．腫瘤の外周は，正常肝より軽度低濃度を呈している．平衡相では，隔壁構造の濃染と同程度に腫瘤の外周部も濃染し，周囲肝実質と等濃度を呈している．

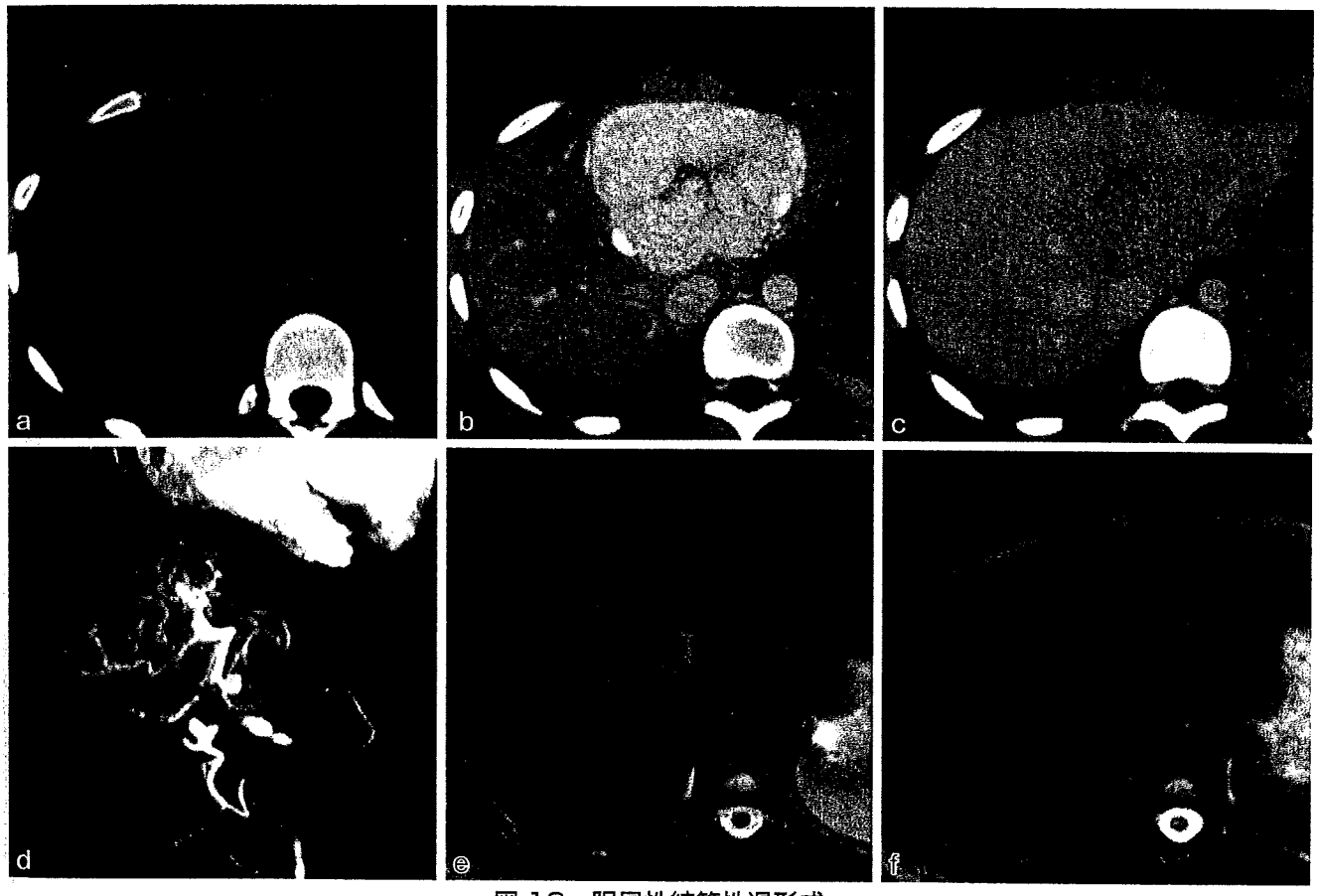

図 12　限局性結節性過形成

a：単純 CT，b：造影 CT 動脈相，c：造影 CT 平衡相，d：造影 CT 動脈相冠状断（MIP 像），e：脂肪抑制併用 T2 強調像，f：脂肪抑制併用 T2 強調像（SPIO 投与後）
単純 CT では，正常肝実質に比してわずかに低濃度を示す腫瘤性病変を左葉に認める．中心部はさらに濃度が低くなっている．造影 CT では，動脈相で著明な早期濃染を呈し，平衡相では正常肝と等濃度となっている．中心瘢痕部は一部遅延性に濃染されている．動脈優位相の MIP 像で，中心部から放射状に走行する動脈が明瞭に描出されている．T2 強調像では軽度高信号に描出される病変内に明らかな高信号を呈する中心瘢痕を認める．病変は SPIO を取り込み，周囲肝と同様，信号の低下を認める中心瘢痕部には SPIO の取り込みがないため，高信号に描出されている．

染を示すことがある．炎症が門脈炎を引き起こし，門脈血流低下を代償する動脈血流の増加を反映しているものと推測されている．

6. 限局性結節性過形成(focal nodular hyperplasia，図12)

肝内の血管奇形や外傷に起因する肝細胞の過形成と考えられている．60%に中心瘢痕を認め，線維性組織が中心から放射状に伸びる．被膜は存在しない．

単純CTでは，低もしくは等吸収を呈する．ダイナミックCTの動脈相では濃染を示し，平衡相では周囲肝と等吸収を呈する．中心瘢痕は遅延性濃染を示す．瘢痕の中心部から放射状に走行する(spoke-wheel appearance)拡張した供血動脈を認めることがある．MRIではT1強調像で低～等信号を呈し，T2強調像で等～軽度高信号を呈する．中心瘢痕部は，T2強調像で明らかな高信号を呈する．病変内にKupffer細胞が内包されていることからSPIO造影剤を取り込み，投与後のT2強調像で信号低下を認める．また，Gd-EOB-DTPA造影剤の肝細胞相でも取り込みを認め，造影T1強調像で高信号に描出される．

7. 肝細胞腺腫(hepatocellular adenoma)

良性肝腫瘍の1つで若年女性に多く，経口避妊薬や蛋白同化ホルモン，糖原病との関連が知られている．非硬変肝に発生し，通常は単発であるが，糖原病例では多発傾向がある．腫瘍内出血を伴いやすく，腹腔内へ出血し，急性腹症として発症することがある．

単純CTでは均一な低もしくは等濃度病変として描出される．脂肪沈着や壊死などが加わると不均一な低濃度域として描出される．また，出血の新鮮例では高濃度域として描出される．腫瘍が多血性であることを反映し，造影CTの動脈優位相では早期濃染を示す．平衡相では，周囲肝に比して等ないしは高濃度に描出されるが，壊死や出血部には造影効果を認めない．MRIでは，出血や脂肪の多寡によりT1強調像で低～高信号とさまざまな信号として描出される．T2強調像では，不均一な高信号域として描出されることが多い．

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Distribution of Hepatitis B Virus Genotypes among Patients with Chronic Infection in Japan Shifting toward an Increase of Genotype A▽

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Acute hepatitis B virus (HBV) infection has been increasing through promiscuous sexual contacts, and HBV genotype A (HBV/A) is frequent in patients with acute hepatitis B (AHB) in Japan. To compare the geographic distribution of HBV genotypes in patients with chronic hepatitis B (CHB) in Japan between 2005 and 2006 and between 2000 and 2001, with special attention to changes in the proportion of HBV/A, a cohort study was performed to survey changes in genotypes of CHB patients at 16 hospitals throughout Japan. Furthermore, we investigated the clinical characteristics of each genotype and examined the genomic characteristics of HBV/A isolates by molecular evolutionary analyses. Of the 1,271 patients, 3.5%, 14.1%, and 82.3% were infected with HBV/A, -B, and -C, respectively. In comparison with our previous survey during 2000 and 2001, HBV/A was twice as frequent (3.5% versus 1.7%; $P = 0.02$). The mean age was lower in the patients with HBV/A than in those with HBV/B or -C. Based on phylogenetic analyses of 11 full-length genomes and 29 pre-S2/S region sequences from patients, HBV/A isolates were imported from Europe and the United States, as well as the Philippines and India. They clustered with HBV/A from AHB patients and have spread throughout Japan. HBV/A has been increasing in CHB patients in Japan as a consequence of AHB spreading in the younger generation through promiscuous sexual contacts, aided by a tendency of HBV/A to induce chronic hepatitis. The spread of HBV/A infection in Japan should be prevented by universal vaccination programs.

Hepatitis B virus (HBV), a member of the *Hepadnaviridae*, is a circular, partially double-stranded DNA virus and is one of the major causes of chronic liver diseases, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC).

The HBV genome is composed of approximately 3,200 nucleotides. HBV is classified into eight genotypes, designated A to H, based on an intergroup divergence of 8% or more in the complete nucleotide sequence (3, 23, 26, 37). They have distinct geographical distributions and are associated with differences in clinical and virological characteristics, such as severity of liver disease and response to antiviral therapies (7, 8, 12, 13, 22, 28). Furthermore, subgenotypes have been reported for HBV/A, -B, and -C and named A1 to -3 (17, 38), B1 to -6 (31, 32, 40), and C1 to -6 (20, 31, 45). Equally, other genotypes are classified into subgenotypes. There have been increasing lines of evidence to indicate influences of HBV subgenotypes on the outcome of liver disease and the response to antiviral therapies (1, 39, 44).

In 2001, we reported the geographic distribution of HBV genotypes in Japan (27). Of the 720 Japanese patients with chronic HBV infection (CHB), 12 (1.7%) harbored HBV/A, 88 (12.2%) HBV/B, 610 (84.7%) HBV/C, 3 (0.4%) HBV/D, and 7 (1.0%) mixed genotypes. HBV/C was detected in over 94%

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of patients on the Japanese mainland, while HBV/B was found in 64% of those in Okinawa, the southernmost islands, and 44% of those in the Tohoku area in the northern part of the mainland.

Recently, acute HBV infection (AHB) has been increasing in Japan, predominantly through promiscuous sexual contacts. In addition, it was reported that HBV/A was more frequent in patients with acute hepatitis than in those with chronic hepatitis (29, 41, 49). Recent studies suggest that the chances for progression to chronic disease may differ among patients acutely infected with HBV of distinct genotypes (21, 25); patients infected with HBV/A run an increased risk of becoming HBV carriers. Hence, it is of utmost concern whether chronic HBV/A infection is increasing in Japan.

In the present study, we compared the geographic distribution of HBV genotypes in Japan during 2005 and 2006 with 2000 and 2001, with special attention to changes in the proportion of HBV/A. Furthermore, we investigated the clinical characteristics of each genotype and examined the genomic characteristics of HBV/A isolates by molecular evolutionary analyses.

MATERIALS AND METHODS

Patients. From September 2005 to October 2006, sera were collected from 1,370 consecutive patients with CHB at 16 representative hospitals that were liver centers in their respective regions throughout Japan for the purpose of investigating the geographic distribution of HBV genotypes in Japan. All of the patients were diagnosed after they had been followed for at least 12 months. Patients diagnosed with AHB were excluded from the study; they had a sudden onset of clinical symptoms of hepatitis, along with high-titer antibody to HBV core antigen of the immunoglobulin M class in serum. Their sera were tested for alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), and hepatitis B e antigen (HBeAg), as well as antibody to HBeAg (anti-HBe) (Dinabot, Tokyo, Japan). Four clinical diagnoses were established for them. The inactive carrier state was defined by the presence of HBV surface antigen (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 was defined by elevated ALT levels (>1.5 times the upper limit of normal [35 IU/liter]) persisting over 6 months (with at least three bimonthly tests). Cirrhosis was diagnosed principally by ultrasonography (coarse liver architecture, nodular liver surface, blunt liver edges, and hypersplenism), platelet counts of <100,000/cm^3, or a combination thereof. Histological confirmation by fine-needle biopsy of the liver was performed as required. HCC was diagnosed by ultrasonography, computerized tomography, magnetic resonance imaging, angiography, tumor biopsy, or a combination thereof.

The study protocol conformed to the 1975 declaration of Helsinki and was approved by the ethics committees of the respective institutions. Every patient or his/her next of kin gave informed consent to the purpose of the study.

Genotypes and subgenotypes of HBV. The six HBV genotypes (A to F) were determined serologically by enzyme immunoassay (EIA) using commercial kits (HBV Genotype EIA; Institutes of Immunology Co., Ltd., Tokyo, Japan). The method depends on the combination of epitopes on pre-S2 region products detected by monoclonal antibodies that were specific for each of them (46, 47). Subgenotypes of HBV/A, designated A1 and A2, were determined by direct sequencing of the pre-S2/S gene, followed by a phylogenetic analysis.

Quantification of HBV DNA and sequencing. HBV DNA levels in sera were quantitated with a commercial kit (Amplicor HBV Monitor; Roche Diagnostics, Basel, Switzerland) with a detection range from 2.6 to 7.6 log copies/ml. Nucleic acids were extracted from 100 μl of serum using the Qiaamp DNA Blood Minikit (Qiagen GmbH, Hilden, Germany). Eleven complete HBV/A genomes and 29 pre-S2/S region sequences were amplified by PCR with appropriate primer sets, as described previously (40). The amplified HBV DNA fragments were directly sequenced using the ABI Prism Big Dye kit version 3.0 (Applied Biosystems, Foster City, CA) in an ABI 3100 automated DNA sequencer (Applied Biosystems). All sequences were analyzed in both forward and reverse directions. Complete and partial HBV genome sequences were aligned using GENETYX version 11.0 (Software Development Co., Ltd., Tokyo, Japan).

TABLE 1. Characteristics of 1,271 CHB patients

Parameter	Value
Characteristic	
Male gender [no. (%)]	766 (60.3)
Age (yr; mean ± SD)	51.4 ± 14.0
Diagnosis	
Inactive carrier state [no. (%)]	206 (16.2)
Chronic hepatitis [no. (%)]	786 (61.8)
Cirrhosis [no. (%)]	175 (13.8)
HCC [no. (%)]	104 (8.2)
Antiviral treatment [no. (%)]	577 (45.4)
Blood tests	
Platelets (10^4/mm^3)	21.4 ± 30.2
ALT (IU/liter)	59.8 ± 103.0
ALP (IU/liter)	270.4 ± 136.0
γ−GTP (IU/liter)	47.4 ± 66.1
HBV markers	
HBeAg [no. (%)]	399 (31.4)
HBV DNA (median [range] [log copies/ml])	4.2 (<2.6 to >7.6)

Molecular evolutionary analysis of HBV. Reference sequences were retrieved from the DDBJ/EMBL/GenBank databases with their accession numbers for identification. To investigate the relationship between HBV isolates from patients with chronic and acute hepatitis B in Japan, HBV/A isolates (AH1 to -10) were randomly retrieved from them and sequenced in our previous study (29). Nucleotide sequences of HBV DNA were aligned by the program CLUSTAL X, and genetic distance was estimated by the six-parameter method (10) in the Hepatitis Virus Database (36). Based on these values, phylogenetic trees were constructed by the neighbor-joining method (30) with the midpoint rooting option. To confirm the reliability of the phylogenetic trees, bootstrap resampling tests were performed 1,000 times.

Statistical analysis. Categorical variables were compared between groups by the χ^2 test or Fisher's exact test and noncategorical variables by the Mann-Whitney U test. A P value of less than 0.05 was considered significant.

Nucleotide sequence accession numbers. The DDBJ/EMBL/GenBank accession numbers of the complete genome sequences of HBV isolates JPN_CH1 to -11 are AB453979 to AB453989.

RESULTS

Distribution of HBV genotypes among patients with CHB. Of the 1,370 serum samples, the genotype could not be determined for 99 (7.2%) by EIA due to low HBsAg levels, leaving 1,271 for analysis in this study (Table 1). Of these, 206 (16.2%) were inactive carriers, 786 (61.8%) had chronic hepatitis, 175 (13.8%) cirrhosis, and 104 (8.2%) HCC. They had a mean age of 51.4 ± 14.0 years and included 766 (60.3%) men. They had a median HBV DNA level of 4.2 log copies/ml, and 399 (31.4%) of them were positive for HBeAg. Antiviral treatment had been given to 577 (45.4%) of them with interferon, lamivudine, adefovir pivoxil, or entecavir.

The genotypes were HBV/A in 44 (3.5%), HBV/B in 179 (14.1%), HBV/C in 1,046 (82.2%), and HBV/D in 2 (0.2%) (Table 2). In comparison with our previous report on the distribution of genotypes in Japan in 2001 (27), HBV/A was more frequent in this study (3.5% versus 1.7%; $P = 0.02$). Of the 16 hospitals in this study, 10 overlapped with those in our previous report from 2001. In these 10 hospitals, HBV/A was more frequent in the present than in the previous survey (3.6% versus 1.7%; $P = 0.04$).

The distribution of HBV genotypes in Japan differed by

TABLE 2. Distribution of HBV Genotypes

Genotype	No. (%)	
	2005–2006 (n = 1,271)	2000–2001[a] (n = 720)
A	44 (3.5[b])	12 (1.7)
B	179 (14.1)	88 (12.2)
C	1,046 (82.3)	610 (84.7)
D	2 (0.2)	3 (0.4)
Mixed	0 (0.0)	7 (1.0)

[a] From Orito et al. (27).
[b] P = 0.02.

geographic location (Fig. 1). HBV/C was the most prevalent in the majority of areas. In the Tohoku area, the northern part of the Japanese mainland (Honshu), HBV/B was more prevalent than in the other areas of the Japanese mainland. In Okinawa, the southernmost islands of Japan, HBV/B was predominant. Of note, HBV/A was more frequent in the Kanto area (9.5%), the metropolitan area, and Okinawa (9.1%) than in the other areas.

Clinical differences among HBV/A, -B, and -C. Clinical backgrounds were compared among the patients infected with HBV/A, -B, and -C (Table 3). HBeAg was significantly less prevalent in the patients infected with HBV/B than in those infected with HBV/A or -C ($P < 0.01$ for each). When the positivity of HBeAg was stratified by age, HBeAg was markedly less common in patients infected with HBV/B than in those infected with HBV/A or -C who were older than 40 years of age (7/157 [4.5%] versus 4/19 [21.1%] [$P < 0.05$] or 215/755 [28.5%] [$P < 0.01$]) (Fig. 2). There were no significant differences in HBV DNA levels among patients infected with the three genotypes. As antiviral treatments might have influenced the severity of liver disease, clinical states were compared among patients infected with HBV/A, -B, and -C who did and did not receive it; antiviral treatments did not affect the above-mentioned trends represented in Table 3 in age, diagnosis, and HBeAg, as well as ALT and HBV DNA levels (data not shown).

Additionally, we compared the distributions of age and liver diseases in patients infected with HBV/A, -B, and -C. In patients infected with HBV/C, the prevalence of cirrhosis and HCC increased in those older than 50 years of age compared to younger patients (Fig. 3), whereas in the patients infected with HBV/B, cirrhosis and HCC were rare in elderly patients. The proportion of patients younger than 40 years of age was higher in those infected with HBV/A than in those infected with HBV/B or -C (25/44 [56.8%] versus 22/179 [12.3%] or 288/1,046 [27.5%]; $P < 0.01$ for each), while cirrhosis and HCC were also found in those older than 50 years of age infected with HBV/A.

Coinfection with human immunodeficiency virus type 1 (HIV-1) was found in 6 of the 44 (13.6%) patients infected with HBV/A compared to only 3 of the 1,046 (0.3%) patients infected with HBV/C ($P < 0.0001$); it occurred in none of the 179 patients infected with HBV/B.

Phylogenetic analyses. Among the 44 HBV/A isolates, the complete genome was sequenced successfully in 11 (JPN_CH1 to -11). Seven of them were classified as HBV/A2 and four as HBV/A1. A phylogenetic tree was constructed based on the complete genome sequences of these 11 isolates, along with those from two patients with AHB and those from 40 HBV/A isolates retrieved from the database (Fig. 4). Of the seven HBV/A2 isolates, the four from patients with CHB in this study formed a cluster with the Japanese isolates retrieved from the database and two from patients with AHB. Of the other three isolates, JPN_CH5 clustered with French and U.S. isolates, JPN_CH6 with German isolates, and JPN_CH7 with

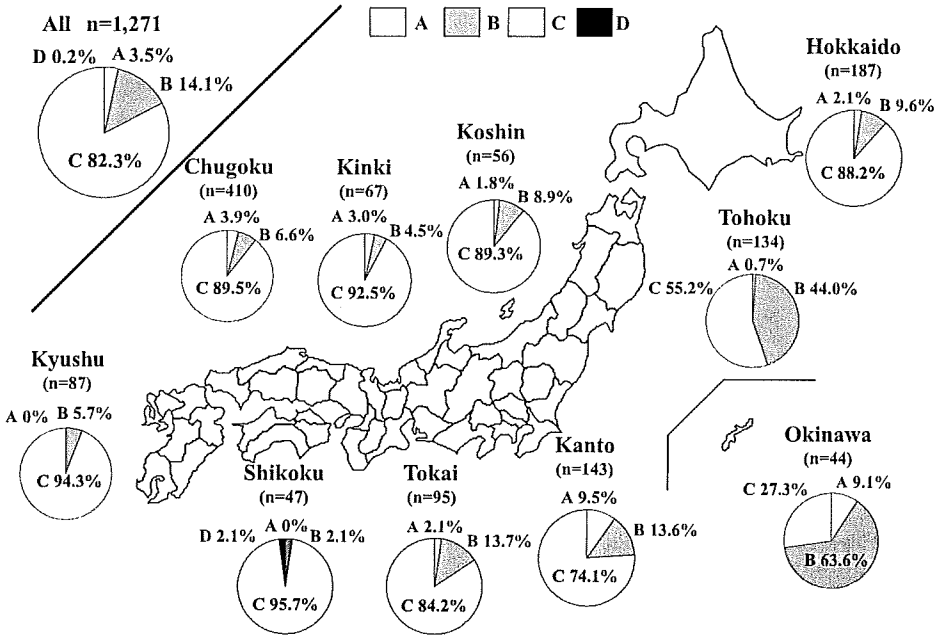

FIG. 1. Geographic distribution of HBV genotypes in patients with chronic HBV infection in Japan during 2005 and 2006.

TABLE 3. Clinical characteristics of individuals chronically infected with HBV of different genotypes

Parameter	Value for genotype:		
	A (n = 44)	B (n = 179)	C (n = 1,046)
Male gender [no. (%)]	32 (72.7)	112 (62.6)	621 (59.4)
Age (yr [mean ± SD])	41.3 ± 14.9[a]	55.8 ± 13.7[b]	48.8 ± 13.3
Diagnosis			
Inactive carrier state [no. (%)]	13 (29.5)[c]	63 (35.2)[b]	129 (12.3)
Chronic hepatitis [no. (%)]	26 (59)	103 (57.5)	656 (62.7)
Cirrhosis [no. (%)]	3 (6.8)	10 (5.6)[b]	162 (15.5)
HCC [no. (%)]	2 (4.5)	3 (1.7)[b]	99 (9.5)
Anti viral treatment [no. (%)]	13 (29.5)[d]	48 (26.8)[b]	516 (49.3)
Blood tests			
Platelet (10^4/mm^3)	23.3 ± 21.9	25.9 ± 35.9[e]	20.6 ± 29.5
ALT (IU/liter)	56.2 ± 83.8	42.2 ± 104.2[e]	63.0 ± 103.3
ALP (U/liter)	247.1 ± 123.0	255.5 ± 97.9	273.9 ± 141.9
γ-GTP (U/liter)	39.6 ± 34.6	49.3 ± 63.4	47.5 ± 67.6
HBV markers			
HBeAg [positive rate(%)]	15 (34.0)[f]	17 (9.5)[b]	367 (35.1)
HBV DNA (median [range]) (log copies/ml)	4.2 (<2.6–>7.6)	4.1 (<2.6–>7.6)	4.2 (<2.6–>7.6)

[a] $P < 0.01$, A versus B or C.
[b] $P < 0.01$, B versus C.
[c] $P < 0.01$, A versus C.
[d] $P < 0.05$, A versus C.
[e] $P < 0.05$, B versusC.
[f] $P < 0.01$, A versus B.

Spanish and Italian isolates. All four HBV/A1 isolates in this study formed a cluster with Philippine and Indian isolates.

In addition, the pre-S2/S region sequences of a total of 29 isolates were determined, including the 11 isolates whose complete genomes were sequenced. Of these, 21 (72%) were classified as HBV/A2 and the remaining 8 as HBV/A1. A phylogenetic tree was constructed based on the pre-S2/S region sequences from the 29 isolates, along with those from 10 patients with AHB infected with HBV/A and 47 HBV/A isolates retrieved from the database (Fig. 5). The 21 HBV/A2 isolates in the present study formed a cluster with Japanese, American, and European isolates retrieved from the database and those from patients with acute hepatitis. In addition, some of them were highly homologous with each other. Likewise, HBV/A1 isolates from eight patients with chronic hepatitis in this study were highly homologous with those from two patients with acute hepatitis and isolates from the Philippines and India. Based on the phylogenetic analyses, HBV/A isolates were imported from Europe and the United States, as well as the Philippines and India, and had infiltrated throughout Japan.

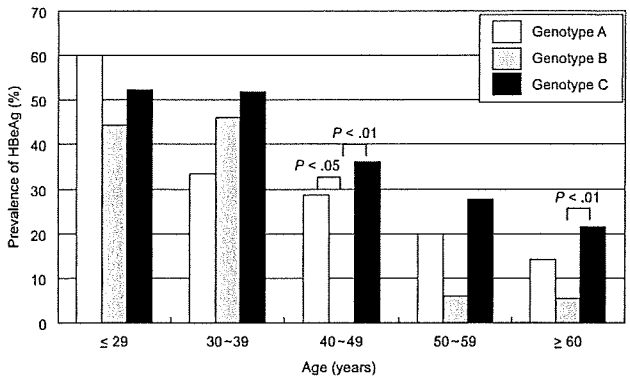

FIG. 2. Prevalence of HBeAg among patients infected with HBV of different genotypes stratified by the age.

DISCUSSION

Perinatal transmission from carrier mothers to their babies has been the principal route for establishing persistent HBV infection in Asian countries (19). In Japan, passive and active immunoprophylaxis with HBV immune globulin and vaccine has been mandated for babies born to HBeAg-positive carrier mothers since 1986; this was extended to HBeAg-negative carrier mothers in 1995. As a result, HBsAg has become rare in Japanese born after 1986; it was detected in only 0.2% of first-time blood donors younger than 19 years of age in 2000 (24). However, AHB has been increasing in Japan, predominantly through promiscuous sexual contacts.

In Japan, HBV/A is detected rarely among patients with CHB but is frequent in those with acute hepatitis (14, 25, 29, 41, 43). Yotsuyanagi et al. reported the distribution of genotypes in 145 Japanese patients with AHB and found HBV/A in 27 (19%), HBV/B in 8 (5%), and HBV/C in 109 (75%) (49). HBV/A is more frequent in metropolitan areas than other areas. The majority of patients with HBV/A infection in metropolitan areas have had extramarital sexual contacts with multiple irregular partners, through which they could have contracted infection. In support of this view, among men who have sex with men (MSM) who are coinfected with HBV and HIV-1 in Tokyo, most were infected with HBV/A (15, 35).

In Japan, AHB in adulthood becomes chronic in only ~1%

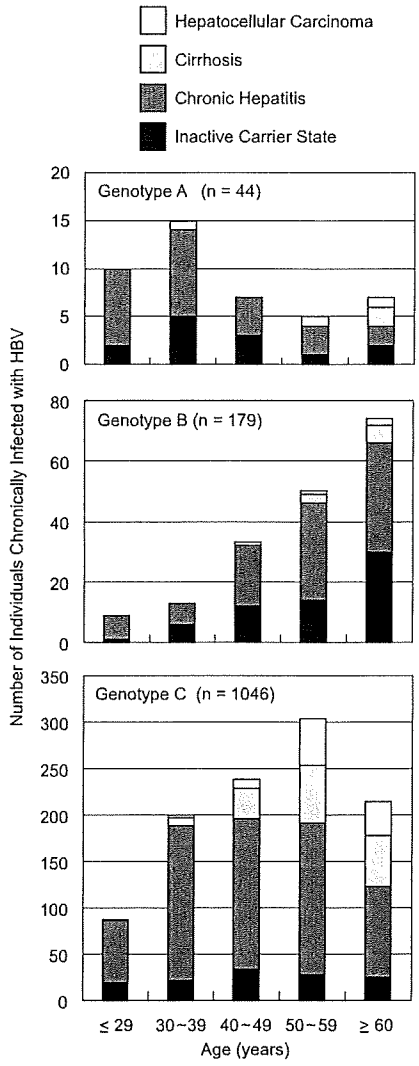

FIG. 3. Distribution of HCC, cirrhosis, chronic hepatitis, and inactive carrier state among the 1,271 patients infected with HBV of different genotypes stratified by the age.

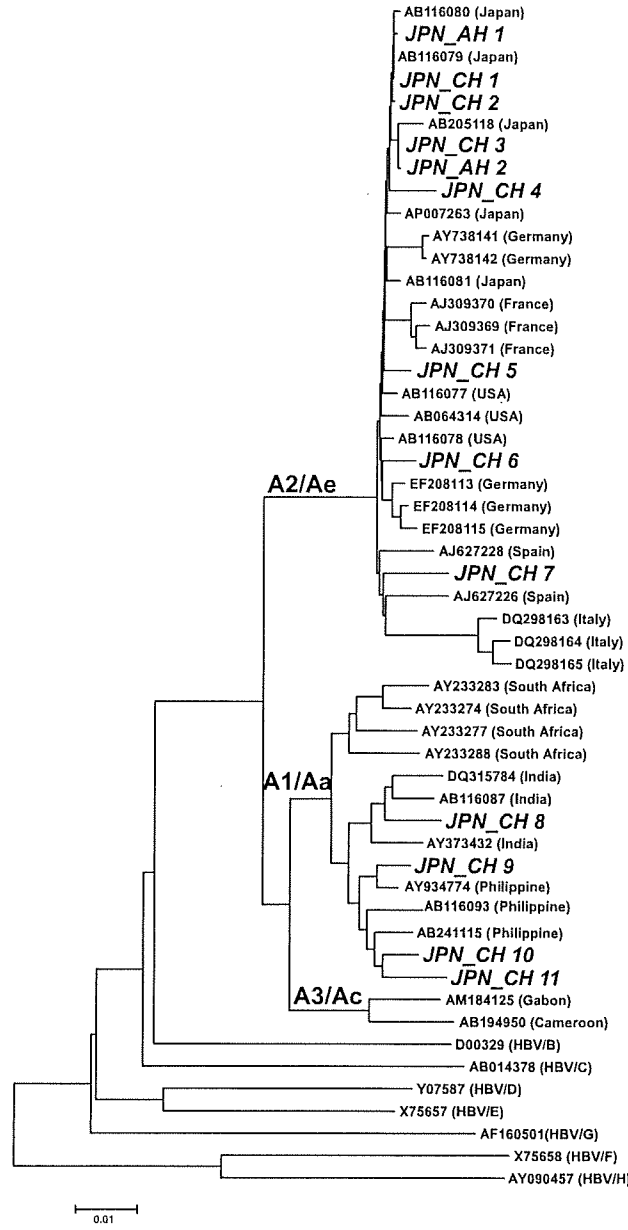

FIG. 4. Phylogenetic tree constructed based on the complete genome sequences of HBV/A isolates. Those from 11 patients with chronic infection in this study are shown in boldface italic (JPN_CH1 to -11), along with two isolates (JPN_AH1 and -2) from patients with acute hepatitis in Japan reported in our previous study (17). Representative isolates were retrieved from the DDBJ/EMBL/GenBank databases, including 21 HBV/Ae, 10 HBV/Aa, and 2 HBV/Ac isolates, along with 7 HBV isolates representative of the other seven genotypes. Isolates from the databases are identified by accession numbers, followed by the country of origin. The bar at the bottom spans 0.01 nucleotide substitutions per site.

of cases. This is much less than the progression to chronic disease (close to 10%) in Europe and the United States, where HBV/A prevails (34). Recent studies have suggested that the chances for persistence may differ among patients acutely infected with HBV of distinct genotypes (21, 25). In particular, acute infection with HBV/A may bring about an increased risk of progression to chronic disease. Therefore, an increase of acute infection with HBV/A would result in a surge of HBV/A among patients with CHB in Japan. In actuality, in comparison with our previous results during 2000 and 2001 (27), HBV/A was twice as frequent in this study (3.5% versus 1.7%; $P = 0.02$). HBV/A has been increasing in patients with CHB in the Kanto area, where HBV/A in patients with acute hepatitis is more frequent than in the other areas. In the islands of Okinawa, also, HBV/A was found to be prevalent in this study. Of the four patients infected with HBV/A there, two were coinfected with HIV-1. They were both MSM, and they were suspected to have been infected with HIV through sexual contacts on the Japanese mainland. It has been reported that HIV infection increases the probability that AHBs will become chronic (2, 11, 33, 48). Because they share routes of transmission and the risk for HIV-1 and HBV infections, approximately

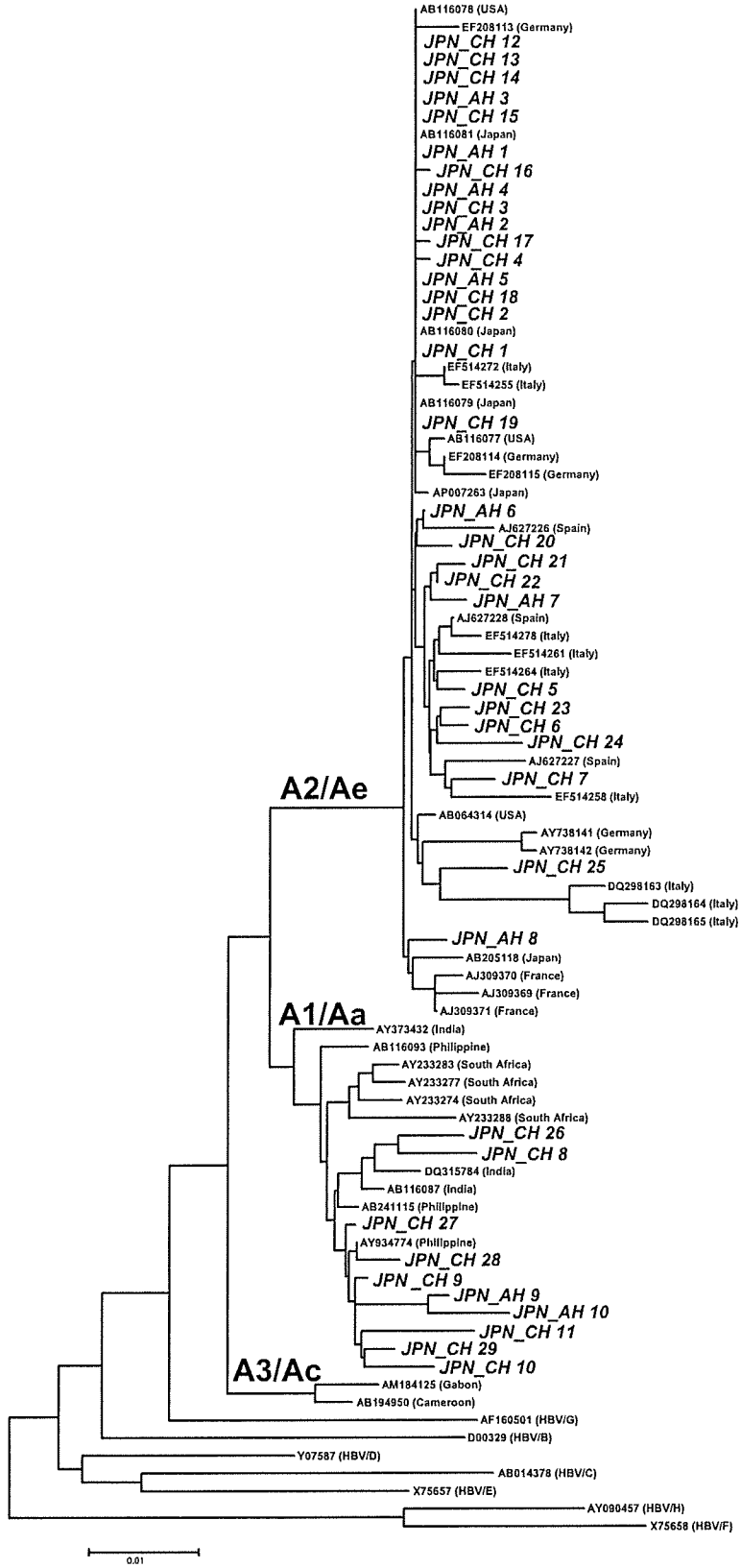

FIG. 5. Phylogenetic tree constructed based on pre-S2/S region sequences of HBV/A isolates. Those from 29 patients with chronic infection in this study are shown in boldface italic (JPN_CH1 to -29), along with 10 isolates (JPN_AH1 to -10) from patients with acute hepatitis in Japan reported in our previous study (17). Representative isolates were retrieved from the DDBJ/EMBL/GenBank databases, including 28 HBV/Ae, 10 HBV/Aa, and 2 HBV/Ac isolates and 7 HBV isolates representative of the other seven genotypes. Isolates from the databases are identified by accession numbers, followed by the country of origin. The bar at the bottom spans 0.01 nucleotide substitutions per site.

90% of patients with AIDS have markers of past or ongoing HBV infection (18). Thus, HBV carriers are more frequent in the HIV-1-positive than in the HIV-1-negative population (4, 9). Among patients with HIV infection in Japan, 6.3% are HBsAg positive, in particular, 8.3% of HIV-infected MSM (16). In this study, coinfection with HIV was found in 6 of the 44 (13.6%) patients infected with HBV/A. All of them were men. Their median age was 27.7 ± 4.1 years, and five patients were positive for HBeAg. Thus, there is a possibility that HIV-1 and HBV/A coinfections are increasing among young people in Japan, and the high rate of HBeAg positivity may be influenced by immune suppression due to HIV infection.

In the phylogenetic analysis, the HBV/A2 isolates recovered in this study were homologous to those from Europe and the United States, and some of them clustered with the Japanese isolates. On the other hand, there were HBV/A1 isolates that formed a cluster with those from the Philippines and India. Furthermore, some isolates from patients with acute hepatitis who were infected with HBV/A in Japan were highly homologous to HBV/A isolates from patients with chronic hepatitis. This invites speculation that some HBV/A isolates were introduced into Japan from foreign countries, while others have already settled down there and spread from patients with chronic infection to their contacts. HBV/A would have been infiltrating throughout Japan by these two different routes.

Clinical differences among patients infected with HBV/A, -B, and -C were observed. The mean age was lower in the patients infected with HBV/A than in those infected with HBV/B or -C. As mentioned above, AHB patients infected with HBV/A have been increasing in the younger generation in Japan, and around 10% of them would have progressed to chronic infection. This is one of the reasons why the patients infected with HBV/A are younger than those infected with HBV/B or -C. Most patients infected with HBV/B were negative for HBeAg, while a high proportion of the patients infected with HBV/A and -C had it. In particular, this difference was remarkable in the patients who were older than 40 years of age. Thus, the seroconversion rate for the loss of HBeAg among younger people may be higher in infection with HBV/B than in that with HBV/A or -C. Inactive carriers were commoner in HBV/A than in HBV/C infection, as well.

These lines of evidence indicate that the activity of hepatitis is lower in HBV/B than HBV/C infection, and patients with HBV/B seroconvert from HBeAg to anti-HBe at young ages. In addition, cirrhosis and HCC were less frequent in the patients infected with HBV/B than in those infected with HBV/C. Therefore, the prognosis would be better in the patients infected with HBV/B than in those infected with HBV/C. These results are in accord with previous reports (5, 13, 28, 42). There have been few reports on the clinical features of patients with chronic hepatitis infected with HBV/A in Japan. Chu et al. have reported the distribution of HBV genotypes with reference to clinical characteristics in the United States (6). They have shown that HBV/A and HBV/C infections are accompanied by a higher frequency of HBeAg than HBV/B infection, while HBV/B is associated with a lower rate of hepatic decompensation than HBV/A and -C. In our study, inactive carriers were commoner, while cirrhosis and HCC were found less often in HBV/A than in HBV/C infection. HBeAg was more prevalent in the patients infected with HBV/A than in those infected with HBV/B who were older than 40 years of age. Therefore, it can be said that the prognosis is better for patients infected with HBV/A than for those infected with HBV/C; it may be poorer than for those infected with HBV/B.

In conclusion, HBV/A has been increasing among CHB patients in Japan. On the basis of phylogenetic analyses, some HBV/A isolates appear to have been imported from foreign countries. They clustered with HBV/A from AHB patients and have infiltrated throughout Japan. It is very likely that acute and chronic infections with HBV/A have been increasing in Japan. Obviously, immunoprophylaxis of perinatal HBV infection, implemented since 1986 on a national basis, has been insufficient to prevent horizontal HBV/A infection diffusing among high-risk groups by transmission routes shared by HIV infection. The foreseeable spread of HBV/A infection in Japan should be prevented by universal vaccination programs extended to high-risk groups or the general population.

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