Genotype C of hepatitis B virus can be classified into at least two subgroups

Tran Thien-Tuan Huy,1,2,3 Hiroshi Ushijima,2 Vo Xuan Quang,3 Khin Maung Win,4 Pairoj Luengojanakul,5 Kaoru Kikuchi,6 Tetsutaro Sata1 and Kenji Abe1

1Department of Pathology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan
2Department of Developmental Medical Sciences, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
3Department of Gastroentero-Hepatology, Cho Ray Hospital, Ho Chi Minh City, Vietnam
4Department of Hepatology, Yangon General Hospital, Yangon, Myanmar
5Department of Gastroenterology, Mahidol University Siriraj Hospital, Bangkok, Thailand
6Gastroenterology Section, Okinawa Chubu Hospital, Okinawa, Japan

A genomic characterization of hepatitis B virus (HBV) was done for 56 pre-S1/pre-S2 genes and 10 full-length HBV genotype C isolates from five Asian countries. Phylogenetic analysis of the pre-S1/pre-S2 genes revealed two major groups within genotype C: one for isolates from southeast Asia including Vietnam, Myanmar and Thailand (named HBV/C1) and the other for isolates from Far East Asia including Japan, Korea and China (named HBV/C2). This finding was confirmed by phylogenetic analysis based on the full-length sequence of 32 HBV genotype C isolates, including 22 from database entries. Two isolates from Okinawa, the island off the southern end of Japan, formed a different branch. Specific amino acid sequence changes were identified in the large S protein (amino acids 51, 54, 60, 62 and 73) and P protein (amino acids 231, 233, 236, 248, 252 and 304). Our results indicate that genotype C of HBV can be classified into at least two subgroups.

INTRODUCTION

Hepatitis B virus (HBV) infection is a global health problem, with more than 350 million people chronically infected worldwide (Lee, 1997). The infection is associated with a wide spectrum of clinical symptoms, ranging from acute or fulminant hepatitis to various forms of chronic liver disease, such as chronic hepatitis, cirrhosis and hepatocellular carcinoma. HBV has been classified into genotypes A–G, with an intergenotypic diversity of at least 8% in the full genome sequence (Okamoto et al., 1988; Norder et al., 1994). HBV genotypes have a distinct geographical distribution and correlate with severity of liver disease (Kidd-Ljunggren et al., 2002). Genotypes B and C are prevalent in Asia, and genotype C causes more serious liver disease than genotype B (Shiina et al., 1991; Orito et al., 2001). Moreover, HBV strains in the same genotype may differ in their capacity to induce clinical liver disease. Subgroup Ba of genotype B, which is recombinant with genotype C, is found predominantly in southeast Asian countries and appears to have more detrimental effects than subgroup Bj (Sugauchi et al., 2002a). Recently, a novel genotype C variant has been found in Australian aborigines (Sugauchi et al., 2001). Therefore, it is possible that virological differences in HBV genotype C exist in Asian countries resulting in different clinical outcomes for patients. To analyse this further, we carried out genomic characterization of HBV genotype C isolates and found that they could be classified into at least two subgroups.

METHODS

Source of sera, genotyping and serotyping of HBV. HBV DNA-positive sera were obtained from 56 patients in five different Asian countries: 17 from Vietnam, 21 from Myanmar, four from Thailand, nine from China and five from Japan (three from Tokyo and two from Okinawa). All sera were found to be HBV genotype C by PCR genotyping using type-specific primers as reported previously (Naito et al., 2001). The HBV serotype was inferred from the
sequence of the S gene (Magnius & Norder, 1995). The serum samples were kept at −40°C or below until used. Informed consent for participation in this study was obtained from each individual.

**Extraction of DNA.** Viral DNA was extracted from 10 μl serum using a DNA/RNA extraction kit (SepaGene RV-R; Sanko Junyaku Co.). The resulting pellet was eluted in 50 μl RNase-free water and kept at −20°C until used.

**Amplification of HBV DNA.** The region of the HBV genotype C sequence covering the pre-S region (522 bases from the beginning of pre-S1 to the end of pre-S2) was amplified by heminested PCR in all 56 isolates. The full-length nucleotide sequences of 10 HBV isolates belonging to genotype C were also determined. HBV DNA was amplified by PCR with LA Taq (TaKaRa Shuzo) or AmpliTaq Gold DNA polymerase (Applied Biosystems). The sequences of oligonucleotide primers and their combinations used in this study are listed in Table 1. To obtain the entire sequence, the first round of PCR was carried out for 40 cycles (98°C for 10 s, 50°C for 20 s and 72°C for 2–5 min) followed by extension at 72°C for 10 min. The second round was carried out for 35 cycles (94°C for 1 min, 55°C for 1 min and 72°C for 1 min) followed by extension at 72°C for 7 min in order to amplify the five overlapping fragments that covered the full genome. The PCR products were separated by 1% agarose gel electrophoresis and purified using a QIA quick gel extraction kit (Qiagen) for sequence analysis.

**DNA sequencing.** Purified PCR products were subjected to direct sequencing using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Sequences of amplified DNA were determined using an automated DNA sequencer ABI 377 (Perkin Elmer).

**RESULTS**

### Nucleotide sequences and phylogenetic analysis

By phylogenetic analysis of pre-S1/pre-S2 genes from 56 isolates obtained in this study and 33 from database entries, two major groups were found to be clustered within genotype C (Fig. 1a). Specifically, all 42 isolates from southeast Asia, including Thailand, Vietnam and Myanmar, were clustered in one branch and 34 isolates from Far East Asia, including Japan, China and Korea, formed another branch. In addition, there was a branch containing two isolates from Okinawa prefecture (the island off the

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**Table 1. PCR primers for HBV DNA used in this study**

For the pre-S gene, P1/S2-2 were used for the first round of PCR and P1/S4R for the second round. For the complete sequence, five overlapping fragments were obtained by nested or heminested PCR using the following primer combinations for the second round of PCR: HBV-1/BG1R for fragment A, HBc1/PS8R for fragment B, P1/S1-2 for fragment C, S1-1/HBx2 for fragment D and HBx1/HBV-2 for fragment E. The first round of PCR was done with the primer combinations of HBV-1/S1-2 for fragments A to C and S1-1/HBV-2 for fragments D and E, respectively. Underlining of the primer sequence indicates the EcoRI site.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Nucleotide sequence (5′→3′)</th>
<th>Position*</th>
<th>Polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>For pre-S gene</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>TCACCATATTTCTGGGAAACAAGA</td>
<td>2817–2839</td>
<td>Sense</td>
</tr>
<tr>
<td>S2-2</td>
<td>GGCACGAGTAAAAAATGGGCCA</td>
<td>687–668</td>
<td>Antisense</td>
</tr>
<tr>
<td>S4R</td>
<td>AGAAGATGAAGGCATAGCAG</td>
<td>434–415</td>
<td>Antisense</td>
</tr>
<tr>
<td><strong>For complete sequence</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>HBV-1</td>
<td>CCGGAAAAGATTCTCTTCTACCTCTGCTAATCA</td>
<td>1821–1841</td>
<td>Sense</td>
</tr>
<tr>
<td>S1-2</td>
<td>CGAACACCAGAAACAATGGGCCA</td>
<td>704–685</td>
<td>Antisense</td>
</tr>
<tr>
<td>BG1R</td>
<td>ATAGGGGCTCTTCTGCTCTGCT</td>
<td>2316–2297</td>
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</tr>
<tr>
<td>HBc1</td>
<td>AGTGTTGAGTTCTGCTCT</td>
<td>2269–2287</td>
<td>Sense</td>
</tr>
<tr>
<td>PS8R</td>
<td>ARGCCCTGAGGCTAGGGCTCTCTCT</td>
<td>3098–3078</td>
<td>Antisense</td>
</tr>
<tr>
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<td>2817–2839</td>
<td>Sense</td>
</tr>
<tr>
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<tr>
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<td>Antisense</td>
</tr>
<tr>
<td>HBx2</td>
<td>AGTGCGAGGGTGAAGGCAG</td>
<td>1604–1584</td>
<td>Antisense</td>
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<td>HBx1</td>
<td>GTCCCTCTCTTCTGCGGT</td>
<td>1487–1507</td>
<td>Sense</td>
</tr>
</tbody>
</table>

*Nucleotide position based on the sequence of HBV genotype C (accession no. D50517).
†Degenerate nucleotide R = A/G.
Fig. 1. For legend see page 287.
**Fig. 1.** For legend see page 287.
Fig. 1. Phylogenetic tree constructed on the pre-S gene (522 nucleotides) (a), the full genome (b), and the S (c) and P (d) genes, respectively. Bootstrap values are shown at the beginning of each main node. The length of the horizontal bar indicates the number of nucleotide substitutions per site. The origin of each strain is also shown in parentheses.
southern end of Japan) and two from China. Two database-derived isolates found in Australian Aborigines and New Caledonia formed an outgroup of the genotype. Phylogenetic analysis based on full genomic sequences of 10 HBV isolates from Vietnam (3), Myanmar (3), Thailand (2) and Okinawa (2) also confirmed the existence of two major subgroups within genotype C (Fig. 1b). Similar clusters were also identified by analysis of the large S and the P genes (Fig. 1c, d). From these results, we designated the two subgroups within the HBV genotype C subgroup C1 (HBV/C1) for isolates mainly distributed in southeast Asia and subgroup C2 (HBV/C2) for isolates in Far East Asia.

Nucleotide divergence between HBV/C1, HBV/C2 and other genotypes

To define the magnitude of intergenotypic and intragenotypic differences, pairwise analysis of nucleotide comparisons was performed over the complete HBV genome and the large S gene of 45 HBV strains representing all genotypes. Over the complete genome from genotype A to G, the mean percentages of nucleotide divergence between HBV/C1 and other genotypes were always higher than 8%: 9·5% (genotype A), 9·3% (genotype B), 10·7% (genotype D), 14·5% (genotype E), 14·1% (genotype F) and 12·8% (genotype G) (Table 2). In contrast, the nucleotide difference between HBV/C1 and HBV/C2 was 5·2% and the intragenotypic difference in each group was 3·1% and 3·9%, respectively. The above results, together with the nucleotide divergences in the large S gene, support the concept that HBV/C1 and HBV/C2 belong to genotype C and that they can be considered as separate subgroups within this genotype.

Amino acid changes in HBV/C1 and HBV/C2

By comparison of the deduced amino acid sequences among the complete HBV genomes, we identified a number of amino acid differences between HBV/C1 and HBV/C2 in the large S gene and P gene, but not in the X and core genes (Table 3). HBV/C1 isolates had the following consensus sequence of amino acids: 51Q, 54A, 60V, 62S, 73S/N, 125S and 227L in the large S gene and 136T/I, 142Q, 231M, 233S, 236P, 248S, 252Q, 304Q, 354Y and 358H in the P gene. Interestingly, two HBV isolates from Okinawa had almost identical amino acid sequences to those of HBV/C1, except for 51Q and 231M, which matched those of HBV/C2.

Serotypes and the nucleotide change at nt 1858 in HBV/C1 and HBV/C2

All of the HBV/C1 isolates recovered in this study belonged to serotype adr based on the amino acid sequences at positions 122 and 160 of the S protein. The T→C nucleotide change at position 1858 (C-1858) in codon 15 of the pre-core gene was found in 6 out of 16 isolates (37·5%) of HBV/C1, but in none of the HBV/C2 isolates.

DISCUSSION

HBV genotypes are distributed geographically, but their virulence and pathogenicity differ in each location (Lok, 2000; Kidd-Ljunggren et al., 2002). In addition, subgroups of HBV genotype have also been reported in genotypes A (A’ and A-A’) (Bowyer et al., 1997; Kramvis et al., 2002) and B (Ba and Bj) (Sugauchi et al., 2002a). The genotyping of HBV is important for clarifying the route of infection with and virulence of the virus. In particular, examination of sequence diversity among different isolates of the virus is important since variants may differ in their patterns of serological reactivity, replication of the virus, activity of liver disease, prognosis and response to treatment. In fact, patients infected with genotype C have a more aggressive clinical phenotype than those with genotype B (Orito et al., 2001; Kao et al., 2002). Interestingly, however, isolates within the same genotype can cause different clinical manifestations, e.g. between subgroups Ba and Bj (Sugauchi et al., 2003). In the present study, we focused on genotype C because it is prevalent mainly in Asia and seems to contribute to progressive liver disease and poor clinical outcomes in infected patients. We found that genotype C detected in Asia could be classified into at least two subgroups, which we named HBV/C1 and HBV/C2. Notably, HBV/C1 was found only in southeast Asia including Vietnam, Myanmar and Thailand, while HBV/C2 was found in Far East Asia including Japan, Korea and China.

By means of phylogenetic analysis in the pre-S region containing the pre-S1 to the pre-S2 genes from 56 isolates in Asia, we identified two major subgroups within genotype C. In addition to these subgroups, there were additional small clusters consisting of two isolates from Okinawa and two isolates from China. It has been reported that HBV from Australian Aborigines showed 7·1% difference at the nucleotide level and belonged to a novel genotype C variant (Alestig et al., 2001a). These above findings were also confirmed by phylogenetic analysis of the full genome sequences of 32 isolates. Based on analysis of the full genome sequences, two HBV isolates from Okinawa showed a closer relationship to C2 than to C1, but formed a different branch with a 100% bootstrap value. Therefore, HBV isolates from Okinawa could be considered as variants of genotype C, but do not belong to the C1 or C2 subgroups.

The time that has elapsed since the divergence of HBV/C1 can be estimated on the basis of the complete genome differences compared with HBV/C2. From the assumed rate of 4·5×10⁻³ mutations per site per year (Orito et al., 1989), HBV/C1 would have diverged from genotype C about 160 years ago (data not shown). The geographical distribution of these subgroups could help us to understand how HBV is spreading in Asia.

The amino acid changes specific to HBV/C1 and HBV/C2 were concentrated in the pre-S1, pre-S2 and P regions, but not in the X and core regions. The pre-S1 region contains
**Table 2.** Mean percentage and range of nucleotide divergence over the complete genome and the large S gene among 43 isolates of HBV with different genotypes

Values above the diagonal correspond to pairwise comparisons of the complete genomes and those below the diagonal correspond to comparisons of the large S genes. Values in parentheses indicate the standard deviation (bootstrap value 1000; Kimura two-parameter method). Divergences in intragenotypes and/or intrasubgroups are shown in bold, the upper value corresponding to comparison of the complete genome and the lower to comparison of the large S genes.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>C1</th>
<th>A</th>
<th>B</th>
<th>C2</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>3.1</td>
<td>(3.0–3.2)</td>
<td>9.5 (9.0–10.0)</td>
<td>9.3 (8.8–9.8)</td>
<td>5.2 (4.9–5.5)</td>
<td>10.7 (10.2–11.2)</td>
<td>14.5 (13.9–15.1)</td>
<td>14.1 (13.5–14.7)</td>
</tr>
<tr>
<td>A</td>
<td>6.8</td>
<td>(6.2–7.4)</td>
<td>4.9 (4.5–5.3)</td>
<td>10 (9.6–10.4)</td>
<td>9.3 (8.7–9.8)</td>
<td>10.1 (9.6–10.6)</td>
<td>13.5 (13.0–14.0)</td>
<td>14.4 (13.7–15.1)</td>
</tr>
<tr>
<td>B</td>
<td>8.7</td>
<td>(7.9–9.5)</td>
<td>8.7 (7.9–9.5)</td>
<td>5.6 (5.2–6.0)</td>
<td>9.3 (8.9–9.7)</td>
<td>10.6 (10.1–11.1)</td>
<td>14.6 (13.9–15.3)</td>
<td>14.5 (13.9–15.1)</td>
</tr>
<tr>
<td>C2</td>
<td>4.0</td>
<td>(3.6–4.4)</td>
<td>6.8 (6.1–7.5)</td>
<td>8.5 (7.7–9.3)</td>
<td>3.9 (3.7–4.1)</td>
<td>10.4 (9.9–10.9)</td>
<td>14.0 (13.4–14.6)</td>
<td>14.3 (13.7–14.9)</td>
</tr>
<tr>
<td>D</td>
<td>9.7</td>
<td>(8.8–10.6)</td>
<td>9.3 (8.4–10.2)</td>
<td>9.1 (8.3–9.9)</td>
<td>1.2 (0.9–1.5)</td>
<td>1.2 (0.9–1.5)</td>
<td>11.1 (10.6–11.6)</td>
<td>14.2 (13.6–14.8)</td>
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<tr>
<td>E</td>
<td>9.9</td>
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<tr>
<td>F</td>
<td>13.8</td>
<td>(12.7–14.9)</td>
<td>13.7 (12.6–14.8)</td>
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<tr>
<td>G</td>
<td>9.8</td>
<td>(8.7–10.9)</td>
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<td>8.0 (7.2–8.8)</td>
<td>8.4 (7.6–9.2)</td>
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<tr>
<td>Genotype/isolate</td>
<td>S gene</td>
<td>P gene</td>
<td>Reference</td>
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<td>Pre-S1</td>
<td>Pre-S2</td>
<td>TP</td>
<td>Spacer</td>
<td>Pol</td>
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<tr>
<td>Amino acid...</td>
<td>51</td>
<td>54</td>
<td>60</td>
<td>62</td>
<td>125</td>
<td>227</td>
<td>136</td>
<td>142</td>
</tr>
</tbody>
</table>

### Genotype C

#### Subgroup C1 consensus
- Genotype C: Subgroup C1 consensus
  - Amino acid: Q A V S S/N S L T/I Q M S P S Q Q Y H
  - Reference: Yuasa et al. (2000)
  - Direct database submission

#### Genotype A
- Genotype A
  - Amino acid: H A V A G T S V Q L S P S R H H H
  - Reference: Alestig et al. (2001b)

#### Genotype B
- Genotype B
  - Amino acid: N D V A G T S V Q L G Q P/S G H H R
  - Reference: Alestig et al. (2001b)

#### Genotype C
- Genotype C Subgroup C1 consensus
  - Amino acid: H E A A G T S A K L R S P R H H H
  - Reference: Yuasa et al. (2000)
  - Direct database submission

#### Subgroup C2 consensus
- Genotype C: Subgroup C2 consensus
  - Amino acid: S M V G G T S A Q L N Q T W N H H

#### Genotype D
- Genotype D
  - Amino acid: T E V A G T S V Q L R Q P R H H H

#### Genotype E
- Genotype E
  - Amino acid: N D V A G T S V Q L G Q P/S G R H H
  - Reference: Direct database submission

#### Genotype F
- Genotype F
  - Amino acid: S M V G G T S A Q L N Q T W N H H

#### Genotype G
- Genotype G
  - Amino acid: P E V A G T S V Q L R Q P R Y H H

### Genotype C isolates

#### Subgroup C1
- Genotype C Subgroup C1 isolates
  - Amino acid: Q A V S S S S L T Q M S P S Q Q Y H
  - Reference: Yuasa et al. (2000)
  - Direct database submission

#### Genotype C
- Genotype C
  - Amino acid: Q A V S S S S L T Q M S P S Q Q Y H
  - Reference: Sugauchi et al. (2002b)

#### Genotype C isolates
- Genotype C isolates
  - Amino acid: Q A V S S S S L T Q M S P S Q Q Y H
  - Reference: Sugauchi et al. (2002b)

### Subgroup C2

#### D26861 (Japan)
- Genotype C: Subgroup C2
  - Amino acid: H E A A G T S A K L R S P R H H H N
  - Reference: Horikita et al. (1994)

#### D50517 (Japan)
- Genotype C: Subgroup C2
  - Amino acid: H E A A G T S A K L R S P R H H H N
  - Reference: Ashina et al. (1996)

#### D50520 (Japan)
- Genotype C: Subgroup C2
  - Amino acid: H E A A G T S A K L R S P R H H N
  - Reference: Ashina et al. (1996)

#### AY247030 (Korea)
- Genotype C: Subgroup C2
  - Amino acid: H E A A G T S V K L R S P R H H N

#### AY247031 (Korea)
- Genotype C: Subgroup C2
  - Amino acid: H E A A G T S A K L R S P R H H N

### Consensus

- Amino acid: Q A V S S S S L T Q M S P S Q Q Y H
  - Reference: Direct database submission
the HBV receptor for entering hepatocytes (Neurath et al., 1986) and also has sites for transcriptional factors (Melegari et al., 1994). It has been reported that mutations in the CCAAT motif located in the pre-S1 gene result in retention of the S protein and lead to the more aggressive form of HBV-related liver disease (Bock et al., 1999). Therefore, the relationship between HBV/C1 and HBV/C2 and their virulence in chronic liver diseases including hepatocellular carcinoma is of great interest, since the prevalence of hepatocellular carcinoma associated with HBV infection is extremely high in Asia compared with other regions. Of note, genotype C correlates well with the occurrence of hepatocellular carcinoma in Japanese patients, but not in Taiwanese patients younger than 50 years of age (Kao et al., 2000).

Although it is now well established that nt 1858 is critical for the emergence of a pre-core stop-codon mutant at codon 28, the role of the C-1858 variant on the course of HBV infection is still unclear. It has been reported that there was high prevalence of C-1858 strains of genotype C in southeast Asia (Lindh et al., 1997). Furthermore, Alestig et al. (2001b) reported that C-1858 strains of HBV found in southeast Asia showed a common phylogenetic origin and represented one of the subgroups of HBV genotype C. However, our study has shown that 10 out of 16 (62.5%) strains of HBV/C1 detected in southeast Asia had T-1858, whereas the remaining six isolates had C-1858. Furthermore, no cases with the pre-core stop codon mutation in HBV/C1 were seen (data not shown). Therefore, we believe that the C-1858 phylogeny entity in southeast Asia does not give a representative view of HBV prevailing in southeast Asia.

Several recent studies have shown the existence of recombination between different HBV genotypes (Morozov et al., 2000; Cui et al., 2002; Sugauchi et al., 2002a). We also looked for the possibility of such recombination in the HBV/C1 and HBV/C2 subgroups, but no evidence for such an event was found using SimPlot analysis (data not shown).

In conclusion, we have presented evidence for the existence of at least two subgroups within genotype C of HBV, designated HBV/C1 and HBV/C2. HBV/C1 prevails in the southeastern part of Asia including Vietnam, Myanmar and Thailand and HBV/C2 in the northeastern part of Asia including Japan, China and Korea. Conserved amino acid sequences between each subgroup were identified. Future studies are needed to determine whether these subtypes correlate with the progression of liver disease including hepatocellular carcinoma, which could influence treatment.

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REFERENCES


Sugauchi, F., Orito, E., Ichida, T. & 10 other authors (2003). Epidemiologic and virologic characteristics of hepatitis B virus genotype B having the recombination with genotype C. Gastroenterology 124, 925–932.