The Molecular Epidemiology of Varicella-Zoster Virus: Evidence for Geographic Segregation

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Of 75 varicella-zoster virus (VZV) isolates obtained from patients in Africa, Asia, and the Far East, 74 (98.6%) were found to be positive for a BglII restriction site in gene 54. By contrast, <22% of strains from patients in the United Kingdom and in North and South America were positive for the BglII restriction site. Viruses positive for BglII were significantly more common in zoster occurring in patients of nonwhite origin (P < .05). Irrespective of the country in which the sample was obtained, 98% of strains positive for BglII clustered within a single phylogenetic group, which we termed “group A”; the exception was 1 strain that appeared to be recombinant genotype C/A. We used the BglII site to examine both the spread of type A viruses in the United Kingdom and the patterns of VZV infections within persons from different ethnic groups who grew up in the United Kingdom or abroad.

Primary infection with varicella-zoster virus (VZV) causes chicken pox, after which immunity to clinical reinfection is usually lifelong [1]. The virus remains latent in the dorsal root ganglia, reactivating to cause shingles (zoster) under conditions of declining cellular immunity, most commonly in association with age [2]. Many studies have shown the virus to be genetically stable. Low rates of mutation do occur in tissue culture, as evidenced by differences between the live attenuated vOka vaccine strain and the parental wild-type virus from which it is derived [3]. However, strains recovered after low passage are indistinguishable, at several loci, from the parent strain [4], as are viruses transmitted directly from one person to another [5]. The strains of virus causing varicella and, subsequently, zoster in 1 patient were found to be identical by restriction-enzyme analysis [6]. Geographically related strain variation, however, has been well described, and differences between Japanese and US genotypes have been used to distinguish between vOka, which is derived from a Japanese strain, and circulating wild-type US and UK viruses [7–10]. All US and UK strains were positive for the PstI site (PstI+) in gene 38, which distinguishes them from the Oka vaccine strain [9, 10]. In addition, the Oka strain is positive for a BglII site (BglII+) in gene 54, which is present in only 20% of US and UK strains overall [10, 11]. More recently, polymorphisms unique to vOka have been identified, and a SmaI restriction site in open reading frame (ORF) 62 has been used to distinguish between the vaccine strain and all wild-type strains, including Japanese strains [3, 12]. In a previous study, we had shown that BglII+ strains were present in 60% of patients with zoster whose primary infection had occurred in either the Indian subcontinent, Africa, or the Caribbean [13], compared with a prevalence of 10% in patients of white origin who had grown up in the United Kingdom. Moreover, among varicella cases in east London, an area of high immigration from these areas, there had been a significant in-

Received 26 February 2002; revised 20 May 2002; electronically published 13 September 2002.

Presented in part: 26th International Herpesvirus Workshop, Ravensburg, Germany, 28 July–3 August 2001 (abstract 10.23).

Informed consent was obtained from all patients, and the study was performed with approval from the East London City and Hackney Ethics Committee (P96/046).

Financial support: National Health Service Research and Development (grant RFG609); Special Trustees of St. Bartholomew’s Hospital (grant XMNL).

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The Journal of Infectious Diseases 2002; 186:888–94

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crease in $BglI^+$ strains during the preceding 25 years. At the
time of that previous study, we had hypothesized that the $BglI^-$
allele was likely to be prevalent among circulating wild-type
strains in these areas and that this virus may have been im-
ported into the United Kingdom.

More recently, the mapping of single-nucleotide polymor-
phisms (SNPs) across the VZV genome has been used to dif-
ferentiate at least 3 major clades of VZV [14]. Strains belonging
to 2 of the clades, which we have termed “B” and “C,” appear
to be common in subjects living in Europe, whereas strains
from Asia, the Far East, and Africa group within a third large
clade, termed “A.” Preliminary data had suggested that clade
A strains were positive for the $BglI$ allele, whereas clades B
and C were negative for it ($BglI^-$) [14]. To investigate this find-
ing and to examine more closely (1) the distribution of the
$BglI^+$ and $BglI^-$ alleles in relation to disease (varicella or zoster), (2)
geographic origin of the sample, and (3) demographic charac-
teristics of the host, we typed $>$300 virus strains collected
within the United Kingdom and 98 virus strains collected from
around the world, for the presence or absence of the $BglI^+$
allele. The relationship of the $BglI$ allele to phylogenetic subtype
was examined in 33 UK strains and in 40 strains from around the
world.

Patients, Materials, and Methods

Patients and samples. Viruses were collected opportunistic-
ally from patients with varicella ($n = 105$) or zoster ($n = 151$) who
presented to general practitioners and university hospitals during
1979–1996 (table 1). The origins of these samples have been de-
scribed elsewhere [13]. In brief, 69% of samples were from patients
presenting to university hospitals in London, Edinburgh, and Bel-
fast. Of these patients, 40% were female, and 85% were adult (>15
years of age). Overall, 32% of these patients were known to be
immunocompromised, and 11% were pregnant. In most cases
(92%), the varicella or zoster was uncomplicated. A total of 79
(31%) of the samples came from patients presenting, with uncom-
licated varicella or zoster, to general practitioners in either rural
(44 samples) or urban practices (35 samples) throughout the United
Kingdom.

A further 183 virus strains were obtained from 2 prospective studies
of patients with zoster who presented to family doctors in south and
east London during 1997–2000 (table 1). In both studies, detailed
demographic data were obtained—including ethnic origin, country
of birth, age at which chicken pox was diagnosed, country in which
chicken pox was contracted, and, when appropriate, age at which
immigration to the United Kingdom occurred. These strains were
analyzed to determine whether there was any association between
genotype and the four variables of sex, ethnicity, immunosuppression,
and age. In addition, the relationship between $BglI$ genotype, ethnic
origin, and geographic origin of the sample was evaluated. Samples
were divided by ethnic origin (white [$n = 160$] or nonwhite [$n =
23$, comprising 10 Afro-Caribbean, 8 Asian from the Indian sub-
continent, and 5 Far East Asian from China/Hong Kong and Viet-
nam]) and by country (United Kingdom [UK] or non-UK) in which
the patient had contracted varicella or zoster. Of the 23 nonwhite
patients, 2, who had been born in Hong Kong and Vietnam and
who had emigrated to the United Kingdom at ages 9 and 11 years,
were excluded from this analysis, because neither could remember
having contracted varicella and because there was uncertainty re-
garding their location at the time of VZV infection. To increase the
numbers, an additional 21 samples from nonwhite patients (11 Afro-
Caribbean and 10 Asian [6 from the Indian subcontinent and 4 from
the Far East]), who presented during the subsequent year (2000–2001)
of the east London zoster study, were included in the analysis. Thus,
a total of 202 (160 white and 42 nonwhite) patients in the United
Kingdom who presented with zoster were available for this analysis.
The data on these samples and on 38 samples collected from nonwhite
patients outside the United Kingdom who presented with zoster were
compared.

A total of 98 strains collected from patients with varicella or
zoster were obtained from collaborators in Africa (Zambia and
Guinea Bissau), Asia (Bangladesh and India), the Far East (Hong
Kong, Singapore, and Japan), and North and South America.

<table>
<thead>
<tr>
<th>Origin</th>
<th>No. of patients</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>United Kingdom</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General</td>
<td>105</td>
<td>151</td>
<td>256</td>
<td>32</td>
<td>56</td>
</tr>
<tr>
<td>London</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>East</td>
<td>0</td>
<td>106</td>
<td>106</td>
<td>15</td>
<td>49</td>
</tr>
<tr>
<td>South</td>
<td>0</td>
<td>77</td>
<td>77</td>
<td>9</td>
<td>49</td>
</tr>
<tr>
<td>Additional (nonwhite)</td>
<td>0</td>
<td>21</td>
<td>21</td>
<td>5</td>
<td>51</td>
</tr>
<tr>
<td>Zambia</td>
<td>0</td>
<td>16</td>
<td>16</td>
<td>100</td>
<td>56</td>
</tr>
<tr>
<td>Guinea Bissau</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>60</td>
<td>—</td>
</tr>
<tr>
<td>Singapore</td>
<td>14</td>
<td>14</td>
<td>28</td>
<td>14</td>
<td>50</td>
</tr>
<tr>
<td>Japan</td>
<td>2</td>
<td>6</td>
<td>8</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>India</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Brazil</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>United States</td>
<td>18</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

* —, Unknown.
Figure 1. Restriction fragment-length–polymorphism analysis of polymerase chain-reaction (PCR) products from strains of varicella-zoster virus that are either negative (lanes S1–S6) or positive (lane P) for BglI. PCR amplification was performed with a forward primer into which, to control for the enzyme reaction, a BglI restriction site had been engineered. Each product was digested with BglI endonuclease and was analyzed by electrophoresis on 4% NuSieve agarose gel. M, 100-bp marker; NTC, nontemplate control; P, positive control; Undig, undigested product; S1–S6, digested products.

(United States and Brazil) (table 1). The strains from India, Guinea Bissau, and Bangladesh were collected from patients presenting during varicella epidemics in each country. No direct epidemiologic link between patients in each country was reported. Varicella viruses from the United States, Japan, Hong Kong, Brazil, and Singapore were collected from patients presenting in different years. The Zambian cases of zoster were in patients with human immunodeficiency virus infection who presented to a dermatology clinic.

BglI genotyping. All strains were analyzed for BglI and PstI restriction sites by published methods [4, 9]. VZV DNA was extracted from patients’ vesicle fluid, and polymerase chain reaction (PCR) was used to amplify, in gene 54, a 234-bp region that includes a BglI restriction site. PCR products were digested with BglI restriction endonuclease and were analyzed on 4% NuSieve agarose gels stained with ethidium bromide. As described elsewhere [4], a BglI site was engineered into the reverse primer used to amplify across the BglI allele. This acted as an internal control to ensure that the enzymatic reaction worked in samples containing the BglI/H11002 allele (figure 1).

SNP typing of strains. A total of 33 randomly selected strains from the United Kingdom and 40 randomly selected strains from other countries were typed according to SNPs, and a phylogenetic tree was produced (figures 2 and 3). The method has been described elsewhere [5, 14]; in brief, of 58 SNPs identified, by heteroduplex-mobility assay and sequencing, across the VZV genome, 13, in ORFs 1, 21, 50, and 54, were used to type strains [5, 14]. These 13 SNPs were selected because they were scattered, showed a high probability of linkage disequilibrium, and were informative in differentiating the 3 genotypes, A–C, which grouped with high bootstrap values (figure 3) [14].

Statistical analysis. Data were analyzed by the SPSS statistical package, release 6.1.3. The t test was used to compare mean ages of participants in the 2 prospective studies, and the χ² test and Fisher’s exact test (2-tailed) were used to compare proportions.

Results
The results of genotyping at known restriction sites are shown in table 2. Except for 6 (75%) of the 8 Japanese strains, all of the strains were PstI+. More than 70% of the virus strains circulating in the United Kingdom, United States, and Brazil were BglI+. By contrast, almost 100% of strains collected from patients in Asia, Africa, and the Far East were BglI−. To investigate the possibility that genotyping might have been biased by the way in which some of the UK and all of the non-UK samples were collected, the opportunistically collected UK strains from patients with zoster were compared with zoster strains collected prospectively in the south- and east-London studies. Overall, the 3 independent UK collections of samples from patients with zoster showed good agreement (table 2), with 12%–15% of strains being BglI+. The prevalence of BglI genotypes was also similar among patients with varicella or zoster occurring within a larger geographic area—that is, Africa, Asia, and the Far East. Differences were observed among
UK strains: 31% of strains from patients with varicella were \(Bgl\text{I}^-\), compared with 15% of strains from patients with zoster \((P < .01)\) (table 2).

The SNP pattern and the phylogenetic tree based on the genotyping of 73 strains by the SNP methodology are shown in figures 2 and 3, respectively. Of the 73 virus strains genotyped in this study, 41 were found to be genotype A (figure 3). All were \(Bgl\text{I}^-\) (figure 2). Seven strains were genotype B, and 22 were genotype C. None of the genotype B and genotype C strains were \(Bgl\text{I}^-\) (figure 2). One genotypically mixed, C/A strain from Brazil was found to be \(Bgl\text{I}^-\) (figure 2). An additional 2 recombinant, B/C strains were identified (figures 2 and 3). Using these results, we calculated that 41 (98%) of 42 strains that were \(Bgl\text{I}^-\) belonged to genotype A and that all strains that were \(Bgl\text{I}^-\) negative belonged to either genotype B or genotype C; the 1 \(Bgl\text{I}^-\) strain that was not genotype A was, in fact, a recombinant, C/A strain.

Of the 183 patients from the 2 prospective, community-based studies, 23 had \(Bgl\text{I}^-\) strains and 160 had \(Bgl\text{I}^-\) strains. Patients with \(Bgl\text{I}^-\) strains and patients with \(Bgl\text{I}^-\) strains were similar with respect to sex (male/female proportion, 10/23 vs. 80/160, respectively), immunosuppression (4/23 vs. 21/160, respectively), and mean age (56 vs. 53 years, respectively) \((P > .05)\). A total of 23 of the patients were nonwhite, and, among these, the proportion who reactivated a \(Bgl\text{I}^-\) strain was significantly higher than that in whites (8/23 vs. 15/160, respectively, \(P < .01\)).

For the 202 UK patients, the results of analysis of VZV genotype in relation to patient ethnicity and country where varicella or zoster were acquired are shown in table 3. Group 1 subjects were white and had lived their entire lives in either the United Kingdom or Ireland. None had lived abroad or had contracted varicella abroad. Of the 160 patients in this group, 73% (117) remembered having contracted varicella in the United Kingdom, although most did not remember the age at which it had occurred. As expected, 91% of this group were infected with a \(Bgl\text{I}^-\) strain. Group 2 included 16 nonwhites who had been born and had lived their entire lives in the United Kingdom, and 14 of these 16 remembered having contracted varicella in the United Kingdom, although only 4 remembered the age at which it had occurred. In addition, 3 subjects who had immigrated at ages <3 years and who remembered having

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**Figure 2.** Genotyping of 33 United Kingdom (UK) and 40 non-UK strains of varicella-zoster virus, at 13 informative loci in open reading frames (ORFs) 1, 21, 50, and 54. Single-nucleotide polymorphisms (SNPs), compared with the reference strain, Dumas [15], are denoted by black boxes. Major genotypes (A–C), 3 putative recombinant strains (1 genotype C/A and 2 genotype B/C), and the \(Bgl\text{I}\) restriction site are shown.

**Figure 3.** Phylogenetic tree of 33 United Kingdom (UK) and 40 non-UK varicella-zoster virus strains (parsimony method) [16]. Bootstrap values and genotypes A–C are shown; countries of origin of strains are indicated. Numerals (e.g., × 2) indicate the number of viruses from a particular country that have the same single-nucleotide-polymorphism sequence.
contrasted varicella in the United Kingdom were included in this group. Despite having contracted varicella in the United Kingdom, a significantly higher proportion of this group were infected with a $Bgl^+$ strain ($P < .01$) (table 3). Group 3 comprised 23 nonwhite subjects who had immigrated to the United Kingdom as adults (age $\geq 16$ years). Of these 23 subjects, 20 (87%) remembered having contracted varicella as children in their country of origin, although only 5 remembered the exact age at which it had occurred. None remembered having contracted varicella since arrival in the United Kingdom. A total of 30% (7/23) of the subjects in group 3, despite remembering that they had contracted varicella since arrival in the United Kingdom, a significantly higher proportion of this group were infected with a $Bgl^+$ strain ($P < .01$) (table 3). This finding is in line with the 40% reactivation of $Bgl^-$ strains among nonwhite immigrants with zoster who were sampled in Africa (Zambia and Guinea Bissau), the Indian subcontinent (India and Bangladesh), and the Far East (Japan, Hong Kong, and Singapore) were $Bgl^+$.

The numbers of strains tested from each country were small and, in some cases (Guinea Bissau, Brazil, India, and Bangladesh), were obtained during a single outbreak of varicella, albeit from epidemiologically unrelated sources; thus, they may not be representative of the prevalent strain in each country. Be that as it may, the viral genotypes causing varicella in Brazil were strikingly different from those sampled in varicella outbreaks in Guinea Bissau, India, and Bangladesh. Moreover, we have shown that the $Bgl^+$ genotype in varicella outbreaks in the United Kingdom is broadly similar from year to year [13]. Our data therefore support previous findings suggesting geographic segregation of VZV genotypes and indicate that viruses from the African and Asian subcontinents carry the $Bgl^+$ allele, whereas those from the United Kingdom and the Americas seem more likely to be $Bgl^-$ negative.

Results from a previous study had led us to hypothesize that $Bgl^+$ strains might be prevalent in Africa, the Indian subcontinent, the Far East, and the West Indies and that immigration from these areas might have led to increased circulation of $Bgl^+$ strains in the United Kingdom [13]. These data were based on opportunistically collected samples, for which incomplete demographic data were available. More-recent data, from strains collected prospectively, confirm the prevalence of $Bgl^+$ strains found in that previous study (12%–13% vs. 15%, respectively). This suggests that, in the United Kingdom, opportunistically collected samples of virus strains are genetically representative of the population of circulating wild-type strains and that the prevalent strain of VZV in the United Kingdom is $Bgl$ negative. A similar distribution of genotypes was seen in North America, with 11% of the samples that we tested being $Bgl^+$. This is consistent with previous reports, which found, albeit in low numbers of subjects, that $\sim 20\%$ of North American strains were $Bgl^+$ [11]. Our data also showed that 4 of 5 samples from South America (Rio de Janeiro) were $Bgl^-$ negative. By contrast, all but 1 of the strains from Africa (Zambia and Guinea Bissau), the Indian subcontinent (India and Bangladesh), and the Far East (Japan, Hong Kong, and Singapore) were $Bgl^+$. The prevalence of varicella-zoster virus strains positive for each $Bgl$ allele, $I$ and $Pst$ genotypes (table 2). Group 3 comprised 23 nonwhite subjects who had immigrated to the United Kingdom as adults (age $\geq 16$ years). Of these 23 subjects, 20 (87%) remembered having contracted varicella as children in their country of origin, although only 5 remembered the exact age at which it had occurred. None remembered having contracted varicella since arrival in the United Kingdom. A total of 30% (7/23) of the subjects in group 3, despite remembering that they had contracted varicella since arrival in the United Kingdom, a significantly higher proportion of this group were infected with a $Bgl^+$ strain ($P < .01$) (table 3). This finding is in line with the 40% reactivation of $Bgl^-$ strains among nonwhite immigrants with zoster who were sampled in Africa (Zambia and Guinea Bissau), the Indian subcontinent (India and Bangladesh), and the Far East (Japan, Hong Kong, and Singapore) were $Bgl^+$.

Our data therefore support previous findings suggesting geographic segregation of VZV genotypes and indicate that viruses from the African and Asian subcontinents carry the $Bgl^+$ allele, whereas those from the United Kingdom and the Americas seem more likely to be $Bgl^-$ negative.

Previously published data have shown that the strain of virus causing zoster in a patient was identical to the primary varicella strain infecting that patient [5]. The similarity of viral genotypes causing varicella and zoster is supported by the similar pattern of $Bgl$ genotypes among virus strains from varicella and zoster cases in Singapore and Japan. However, in the United Kingdom.

Table 2. Prevalence of varicella-zoster virus strains positive for either a $Bgl$ site ($Bgl^+$) or a $Pst$ site ($Pst^+$).

<table>
<thead>
<tr>
<th>Origin (n)</th>
<th>Overall</th>
<th>Varicella</th>
<th>Zoster</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$Bgl^+$</td>
<td>$Pst^+$</td>
<td>$Bgl^+$</td>
</tr>
<tr>
<td>United Kingdom</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General (256)</td>
<td>22</td>
<td>100</td>
<td>31</td>
</tr>
<tr>
<td>London</td>
<td>13</td>
<td>100</td>
<td>NA</td>
</tr>
<tr>
<td>East (106)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>South (77)</td>
<td>100</td>
<td>100</td>
<td>NA</td>
</tr>
<tr>
<td>Guinea Bissau (5)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Zambia (16)</td>
<td>100</td>
<td>100</td>
<td>NA</td>
</tr>
<tr>
<td>Hong Kong (3)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Singapore (28)</td>
<td>96</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>Japan (8)</td>
<td>100</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>India (6)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Bangladesh (9)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>United States (18)</td>
<td>11</td>
<td>100</td>
<td>11</td>
</tr>
<tr>
<td>Brazil (5)</td>
<td>20</td>
<td>100</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 3. Varicella-zoster virus genotype, by ethnicity and country of primary varicella, in patients presenting with zoster either in the United Kingdom (London) or elsewhere (Zambia, Singapore, Hong Kong, and Japan).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acquisition, location</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken pox</td>
<td>In United Kingdom</td>
<td>In United Kingdom</td>
<td>Outside United Kingdom</td>
<td>Outside United Kingdom</td>
</tr>
<tr>
<td>Zoster</td>
<td>White</td>
<td>160</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Nonwhite</td>
<td>91</td>
<td>145/160</td>
<td>63</td>
</tr>
<tr>
<td>No. of patients</td>
<td></td>
<td>145/160</td>
<td>63</td>
<td>12/19</td>
</tr>
<tr>
<td>$Bgl^+$ negative, % (proportion)</td>
<td></td>
<td>0/38</td>
<td>0/38</td>
<td></td>
</tr>
</tbody>
</table>

a Ethnic origin: Afrocaribbean, 12; Asian (Chinese/Vietnamese), 3; Asian (Indian subcontinent), 4.

b Country of origin: Africa, 6; Far East, 4; Indian subcontinent, 10; West Indies, 3.

c Ethnic origin: Afrocaribbean, 9; Asian (Chinese/Vietnamese), 1; Asian (Indian subcontinent), 2.

d Country of origin: Africa, 3; Far East, 2; Indian subcontinent, 1; West Indies, 1.
dom, the prevalence of $B_{gl}^{-}$ strains is significantly higher in samples from cases of varicella. This probably reflects an increase in $B_{gl}^{-}$ strains from patients with varicella in east London since the early 1980s [13]. In an earlier study, we had speculated that this was due to these strains’ recent importation in immigrants from Africa and Asia [13]. The data generated by the present study support this hypothesis. A total of 41 (98%) of 42 $B_{gl}^{-}$ strains tested belong to 1 major genotype, A; the remaining 1 strain was a mixed, C/A genotype. Genotype A strains appear to predominate among strains sampled in Africa, Asia, and the Far East (figures 2 and 3) [14]. Genotype B and genotype C $B_{gl}^{-}$ strains have been found in Europe, the United States, and South America [14]. Similar findings in 2 other studies (although each used different genotyping SNPs, as well as different nomenclatures to describe the genotypes) confirm the robustness of these data [14, 17–19]. Thus, the presence of a $B_{gl}$ restriction site in gene 54 appears to be a reliable marker of genotype A and may provide a useful screening tool for monitoring the spread of VZV globally.

In keeping with this, we found that all 6 of the $B_{gl}^{-}$ strains from the United Kingdom were genotype A. Although it is not possible, on the basis of the few loci typed, to determine the exact geographic origin of the UK genotype A strains, preliminary analysis suggests that it is diverse. Figures 2 and 3 show that at least 1 of the UK genotype A strains groups closely with Japanese strains, whereas others cluster with African and Indian strains. Although 2 of the African/Asian strains were isolated from subjects of Bangladeshi origin and 1 strain was isolated from a subject of Nigerian origin, the Japanese-like strain and 2 of the African/Asian strains were isolated from whites who have lived their entire lives in the United Kingdom. This suggests that genotype A strains are circulating in the United Kingdom.

Recombination between wild-type strains has not previously been described, although recombination has been observed in vitro [20]. In the present study, we found 3 putative recombinant strains, giving an overall recombination frequency of 4% (3/73). This figure is much higher than what normally would be expected for herpesviruses, even under in vitro conditions, and remains to be verified. All 3 of these strains were recovered from locations (London and Brazil) in which coexistence of $>1$ genotype is apparently common. Because none of these strains 3 would have been detected by typing at the $B_{gl}$ locus alone, more-extensive genotyping may be required in areas of mixed genotypes, and this is likely to be particularly important in countries where vaccination with Oka is routine.

The data generated in the present study have enabled us to analyze more closely the relationship between viral genotype, patient ethnicity, and country in which the patient has become infected with VZV. Overall, $B_{gl}^{-}$ strains were significantly more common among nonwhites than among whites ($P < .01$). This remained true even when we analyzed only those who had contracted chicken pox in the United Kingdom; that is, 37% of UK nonwhites had $B_{gl}^{-}$ strains, compared with 9% of whites (table 3; group 1 vs. group 2). It is possible that this difference reflects high intrafamilial transmission of VZV, with nonwhites in the United Kingdom being more likely to acquire a $B_{gl}^{-}$ strain from family members who themselves were infected in a country with a high prevalence of circulating $B_{gl}^{-}$ strains. Alternatively, different ethnic groups may be more susceptible to a particular strain—or, within a given geographic area, 1 strain may be more virulent than the other.

Surprisingly, 30% (7/23) of nonwhites who were likely to have had varicella in a country with a high $B_{gl}^{-}$ prevalence reactivated $B_{gl}^{-}$ strains (table 3). Three explanations are possible. First, in Africa, Asia, and the Far East, $B_{gl}^{-}$ strains may be more common than previously had been thought but, because of biased sampling or random error, were not detected in the 38 zoster and 37 varicella strains. Sampling of greater numbers of prospectively collected strains will be necessary to exclude this possibility. Second, it is possible that the 7 subjects with $B_{gl}^{-}$ strains had not, in fact, had varicella in their country of origin but had contracted asymptomatic primary varicella in the United Kingdom; this is somewhat less likely, because varicella in adults is usually symptomatic [21] and because collection of having contracted varicella as an adult is good [22]. Third, it is possible that the patients positive for $B_{gl}$, despite preexisting immunity from primary varicella in their country of origin, became reinfected with a UK strain of virus, which then reactivated (table 3, group 3). Reinfection of latently infected persons has been well described [23, 24]. Replication and antigen presentation within local lymphoid tissue are likely to be important for boosting of cell-mediated immunity and consequent protection of latently infected persons against virus reactivation [23]. The notion that reinfecting wild-type strains might cause systemic infection with spread to ganglia is not really consistent with our current understanding of VZV pathogenesis. However, there is 1 scenario in which a similar phenomenon may have occurred, in a few vaccine recipients in whom wild-type zoster developed without any obvious break-through varicella after vaccination [25]. In vitro experiments support the notion that VZV latency may result from infection of sensory-nerve endings by free virus in the epithelium, which infects in a retrograde fashion to infect dorsal root ganglia [26]. If this theory is true, then superinfecting virus, which does not produce a rash, is unlikely to lead to latent infection and subsequent reactivation. In monkeys, however, seeding of the ganglia with simian VZV has been shown to occur before rash development, suggesting that retrograde spread may not always be necessary for ganglionic infection [27]. In addition, autopsy reports from some immunocompromised children who died early during varicella infection have shown the presence of virus in ganglia in the absence of skin rash [1]. Further work, to examine our findings more closely, is now needed.

Our data demonstrate that the $B_{gl}$ site is a good screening marker for genotype A VZV strains and that it may prove to
be a powerful tool in the investigation of questions of molecular epidemiology. We have already shown that Bgl I genotype A strains are spreading in parts of the United Kingdom and that these may have been imported from countries with a higher prevalence of genotype A strains. Screening for the Bgl I allele in different populations may also shed light on patterns of VZV infection, reinfection, and recombination. Such data may have important implications for the use and monitoring of VZV vaccines.

References