Little is known about the pathogenic potential of individual strains in the varicella vaccine. We analyzed genomic variation among specimens obtained from vaccine recipients with postvaccination rash or herpes zoster (HZ), focusing on polymorphisms between live attenuated varicella vaccine virus and wild-type varicella-zoster virus. Eleven of 18 postvaccination HZ specimens contained >1 strain, and 7 of 18 appeared to be clonal. All 21 postvaccination rash specimens contained mixtures of vaccine strains. Four single-nucleotide polymorphisms (SNPs) consistently occurred in every isolate; all were polymorphisms in open-reading frame (ORF) 62, and 2 confer amino acid substitutions in the immediate-early protein 62. Four wild-type SNPs occurred in every isolate: one each occurred in ORF 10, ORF 21, ORF 62, and a noncoding region upstream of ORF 64. The frequencies of the remaining wild-type SNPs were variable, with the SNPs uniformly expressed (even in mixtures) in 20.5%–97.4% of isolates (mean frequency, 67.7%). No 2 clinical isolates had identical SNP profiles; as such, vaccine latency usually involves >1 strain.

On first exposure, varicella-zoster virus (VZV) causes varicella (chickenpox), a typically mild rash illness that is sometimes complicated by bacterial sepsis, pneumonia, encephalitis, intrauterine infection, or death [1–3]. Complications are more frequent and severe in immunocompromised patients [4]. VZV establishes lifelong latency in the dorsal root and cranial nerve ganglia [4] and can reactivate as herpes zoster (HZ), typically in immuno-compromised and elderly persons; the lifetime risk for HZ is ~15%–30% [4–6].

A live attenuated varicella vaccine (V-Oka) was developed [7] from wild-type VZV isolate (P-Oka) and is produced by several manufacturers. All marketed V-Oka preparations are known to contain multiple variants [8–12], and V-Oka genomic variation has been characterized [11, 12]. Some clonal vaccine variants have been isolated and have been found to have variable properties in tissue culture [9, 10], specifically the altered efficiency of transactivation of VZV promoters by immediate-early protein 62. Vaccine manufacturing protocols differ, and some DNA sequence variation occurs among commercial preparations, including alterations at loci previously identified as being different between P-Oka and V-Oka [11].

The United States recommended universal vaccination against varicella in 1996. More than 50 million doses have been distributed since licensure. Some countries have added varicella vaccination to their routine childhood immunization schedules, and others recommend vaccination of targeted groups (e.g., adolescents and adults, family contacts of immunocompro-
mised persons, and health care workers) [13–16]. The vaccine is widely available in the private sector worldwide. Vaccination is 80%–85% effective against chickenpox of any severity, and it is >95% effective in preventing severe disease [2, 17–22]. Since universal varicella vaccination began, the numbers of varicella cases and associated hospitalizations and deaths have decreased dramatically [3, 23–25].

The safety of varicella vaccine has been described in both prelicensure and postlicensure studies. In the only randomized, placebo-controlled trial evaluating the safety of Oka vaccine in children 1 to <12 years of age, generalized varicella-like rash occurred among 2% of vaccine recipients within 6 weeks after vaccination [26]. A low percentage of vaccine recipients (<5%) develop a papular or vesicular rash, usually at the injection site, within the same time frame. In the early years of varicella vaccination in the United States, generalized, varicella-like rashes developing within 14 days after inoculation were often caused by wild-type VZV incidentally acquired in temporal proximity to vaccination, whereas rashes of later onset were usually caused by vaccine strain VZV [27, 28]. V-Oka also establishes latent infection in vaccine recipients, which is clinically apparent from the reactivation of V-Oka to cause HZ [12, 17, 29, 30]. Zoster due to V-Oka reactivation commonly occurs in the dermatome in which the vaccine was administered. Vaccine-related HZ apparently occurs less frequently and is less severe than HZ associated with wild-type VZV. Among children with acute lymphocytic leukemia, the risk of HZ developing after vaccination was approximately one-third of that for similar children with a history of varicella [31]. Documented serious adverse events associated with V-Oka occur rarely and include pneumonia and Stevens-Johnson syndrome. Other serious adverse events, such as encephalitis and ataxia, have been reported to occur in temporal proximity to vaccine administration, although V-Oka strains have not been isolated in such cases [3, 27, 28].

The association of the specific variants in the vaccine with the clinical events of postvaccination rash and HZ is poorly understood. Quinlivan et al. [11] reported genetic analyses of VZV isolated from 12 vaccine recipients with postvaccination rash and from 3 vaccine recipients with V-Oka–related HZ. These vaccine recipients received prelicensure strains of V-Oka, and the VZV DNA analyzed was obtained from VZV isolated from tissue culture. Isolates recovered from postvaccination rash and vaccine-related HZ were clonal in that study.

In the present study, we sought (1) to define the single-nucleotide polymorphisms (SNPs) most commonly associated with rash due to varicella vaccine, emphasizing SNPs that confer changes to V-Oka proteins, and (2) to compare V-Oka DNA sequences with those in VZV DNA obtained directly from vaccine-related rashes (varicella-like and HZ rashes) in the absence of expansion/selection in culture.

MATERIALS AND METHODS

Viral strains and their propagation. The National VZV Laboratory of the Centers for Diseases Control and Prevention performs VZV strain identification for specimens submitted from patients with suspected vaccine-related adverse events, including rash and HZ occurring after vaccination. Specimens in the present study were stripped of identifying data and were exempt from institutional review board approval. Swab specimens of lesional fluid/basal cells and scabs were collected and were shipped dry at ambient temperature. V-Oka DNA was recovered from 21 patients with postvaccination varicella-like rash and from 18 patients with vaccine-associated HZ. The age range of patients with postvaccination varicella-like rash was 1–53 years (median, 6 years), and that of patients with HZ was 1–9 years (median, 3 years). Isolates were identified as strains originating from Oka vaccine via polymerase chain reaction (PCR) amplification and restriction fragment–length polymorphism analysis of DNA fragments located in open-reading frames (ORFs) 38, 54, and 62, as described elsewhere [32–35]. The ORF 62 SNP at position 106262 (the Dumas strain) clearly distinguishes V-Oka from wild-type strains; the sites in ORF 38 and 54 do not definitively discriminate V-Oka, but they are useful for the classification of global VZV genomic variance. All of the patients in the present study received varicella vaccine (Varivax; Merck).

Oka varicella strains. Biken V-Oka sequences are those determined and published elsewhere by Gomi et al. [8–10]. Production vials representing multiple lots of Varivax V-Oka were provided by Drs. Barbara Watson and Karl Heath (City of Philadelphia Department of Public Health, Philadelphia, PA). Varivax V-Oka–associated SNPs were invariant among all 5 vials provided, so only a single data set is shown. The parental strain (P-Oka) was provided by Dr. Jeffrey Cohen (National Institutes of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD), with permission from Drs. Michiaki Takahashi and Koichi Yamanishi (Osaka University, Osaka, Japan). P-Oka was propagated in human embryonic lung fibroblasts by cocultivation of trypsinized uninfected cells and VZV-infected cells.

DNA isolation, primer sequences, and PCR. DNA was purified from lysates of VZV-infected cells by use of the Easy DNA kit (Invitrogen) or from clinical lesions by use of MagniPure (Roche Diagnostics), according to the manufacturer’s instructions, and it was collected in 100–200 μL of elution buffer. DNA from uninfected human embryonic lung fibroblast cells was used as a negative control. Primer pairs new to this study are presented in table 1; the remainder have been published elsewhere [12]. The SNP profiles for isolates were examined in separate experiments to reduce the risk of error and contamination. PCR amplifications were performed on 2–5 μL of total DNA and 100 pmol of each forward and reverse primer. Primers were designed to amplify 100–250-bp frag-
ments around previously characterized vaccine SNPs. DNA amplification reactions were performed with a GeneAmp PCR System 9700 (Applied Biosystems) in 50-μL reaction volumes, by use of AmpliTaq Gold PCR Master Mix. Thermal cycling included a hot start at 96°C for 15 min, followed by 30 cycles at 94°C for 30 s, at 60°C for 30 s, and then at 72°C for 30 s, with a final extension at 72°C for 4 min. PCR products were separated on 4% precast acrylamide gels prestained with ethidium bromide (Invitrogen) and were visualized under UV irradiation alongside a 100-bp ladder (Invitrogen).

DNA was purified by electrophoresis on 2% precast agarose gels (BMA). Amplicons of expected size were excised, and DNA was extracted with a Gel Extraction Kit (Qiagen). The amplification protocol used was described elsewhere [36].

DNA sequencing and analysis. Automated DNA sequencing was performed with a capillary Genetic Analyzer 3100 (Applied Biosystems). Sequencing reactions of purified PCR products were performed, according to the manufacturer’s instructions, with BigDye Terminator Cycle Sequencing Ready Reaction Mix, version 3.1 (Applied Biosystems). The resulting products were purified using magnetic beads (Agencourt Bioscience) and eluted with template buffer (Applied Biosystems). The sequences of the PCR products were determined on both strands. The Sequencher software package (Gene Codes) was used for primary DNA sequence assembly and to compare the DNA sequences of clinical isolates with those of P-Oka and V-Oka (DNA Data Bank of Japan/GenBank accession numbers AB097932 and AB097933). All nucleotide sequence positions are referenced to the published nucleotide sequence for the Dumas strain (GenBank accession number: 9625875). Results of DNA sequencing were separately confirmed using fluorescent resonance energy transfer–based LightCycler real-time PCR (Roche Diagnostics); for this purpose, acceptor probes (end-labeled with Cy3R Red 704) and fluorescein-labeled detection probes overlapping the SNP were used. The presence of >1 peak revealed the presence of ≥2 variants in clinical isolates. This approach is an extension of previously reported VZV fluorescent resonance energy transfer PCR analysis to the SNPs studied here [35].

RESULTS

Comparison of P-Oka and V-Oka. We prepared a database representing all published DNA sequence variation between P-Oka and V-Oka as a background for the evaluation of clinical specimens obtained from patients with vaccine-related adverse events. Twenty regions containing V-Oka–associated polymorphisms in ORFs 6, 9A, 10, 21, 31, 39, 50, 51, 52, 54, 55, and 59, as well as 8 mutations in ORF 62, were tabulated [9] (P.R. Krause, personal communication; A. Shaw, N. Kraiouchkin, and D. DeStefano, personal communication). Published sequence data from previous studies provided most of the basis for selection of the SNPs used in this study [8, 34, 35]. Additional guidance came from our targeted sequencing of 5 lots of Varivax V-Oka, which presented a different profile of genetic variation than did the Biken vaccine. Sixteen of the 27 selected SNPs were associated with amino acid changes, and they provided the basis for our analysis of clinical isolates (figure 1).

Comparison of Varivax and Biken V-Oka vaccines with the Oka parental strain. Analysis of the Varivax and Biken V-Oka vaccines revealed that only a fraction of vaccine SNPs are present as unmixed markers in vaccine lots (4 SNPs and 9 SNPs, respectively) (figure 1). Three of the SNPs identified in the original genomic sequence comparison conducted by Gomi et al. [10] were not examined for these studies. A new, uniformly present SNP was identified in Biken V-Oka, located at position 89734 in ORF 51; this SNP was identified as a mixed SNP in the previously published sequence comparison. This same ORF 51 SNP was uniformly wild type in Varivax V-Oka (figure 1). All 4 uniform vaccine SNPs in Varivax V-Oka are
Figure 1. Variability of varicella-zoster virus (VZV) isolates at 27 targeted single-nucleotide polymorphism (SNP) loci. Red boxes denote SNPs uniformly expressed as wild type; blue boxes denote SNPs uniformly expressed as vaccine type; and yellow boxes denote a mixed genotype. Specimen identification numbers that are preceded with a “C” denote control strains. C, clonal; E, European genotype (Dumas strain); J, Japanese genotype (Oka parental strain); M, mixed; M1, Mosaic 1 genotype (California 123J); Mosaic 2 genotype, (M2_Mex); NC, noncoding; Oka Biken, Oka vaccine (Biken); Oka Merck, Oka vaccine (Varivax; Merck); R, purine; Y, pyrimidine.

also uniformly displayed in the Biken V-Oka vaccine (nt 105705, 106262, 107252, and 108111). Seven of the 27 SNPs identified in the Biken V-Oka (uniform and mixed) are consistently wild type in Varivax V-Oka (nt 10900, 12779, 59287, 89734, 107599, 108838, and 111650). These observations agree with findings from earlier studies from our laboratory [12], and they suggest that Varivax V-Oka has undergone some changes in variant content, compared with the original Biken seed lots. SNP analysis of J- and E-genotype wild-type isolates was identical to that of P-Oka at all SNPs studied here; the wild-type M-genotype isolates (M1 and M2) displayed the V-Oka SNP at ORF 54.

Characterization of DNA sequence variation among samples of postvaccination rash. On the basis of targeted SNP analysis, all 21 isolates obtained from confirmed V-Oka postvaccination rash contained >1 vaccine variant, because both wild-type– and vaccine-associated bases were observed at >1 individual loci (figure 1). The display of specific mixed markers differed for all 21 clinical specimens. We cannot determine from these data how many variants were present in individual specimens, whether the same variants exist in vaccine preparations, or whether any identical variants were present in clinical specimen. Four V-Oka SNPs (all located in the ORF 62 gene) were uniformly displayed across all 21 clinical isolates, even though all of these specimens contained mixtures of variants.

Characterization of DNA sequence variation in clinical specimens of vaccine-associated HZ. We observed no variability in the analyzed regions of 7 (39%) of the 18 isolates...
from laboratory-confirmed Oka strain–related HZ specimens, in contrast to postvaccination rash specimens (figure 1). In addition, individual HZ isolates generally contained fewer loci with mixed wild-type and vaccine SNPs, suggesting the possibility that fewer variants are present in vaccine-related HZ lesions than in postvaccination rash lesions. As with the rash isolates, all changes were pyrimidine-to-pyrimidine shifts or purine-to-purine shifts.

**Analysis of uniformly displayed vaccine SNPs.** Four V-Oka SNPs were uniformly displayed in every specimen (at nt 105705, 106262, 107252, and 108111) (figure 1). All 4 of these SNPs were located in ORF 62, and they include the position used by our laboratory and others to distinguish V-Oka from wild-type VZV (nt 106262). Two of the SNPs (at nt 106262 [Gly to Arg] and nt 107252 [Gly to Ser]) lead to an amino acid substitution in the ORF 62 protein; the other 2 are silent.

The relative frequencies at which V-Oka SNPs were expressed at each of the 27 loci (for postvaccination rash and HZ, respectively) are displayed in figure 2. Apart from the 4 invariant vaccine loci, V-Oka SNPs consistently occurred in >40% of the isolates at only 4 additional sites: nt 105310 in ORFs 54 and 62, nt 105544 in ORF 62, and nt 109838 in a noncoding region. Figure 3 displays the SNP frequencies (uniform vaccine, mixed, and uniform wild type) for each of the varicella and zoster isolates as aggregate fractions of the 27 markers. Figure 2 suggests that the most stably maintained vaccine loci are concentrated at the right end of the genome. These data demonstrate that most V-Oka SNPs are not useful for discriminating vaccine virus from wild-type strains.

**Analysis of stably displayed wild-type SNPs.** Four wild-type SNPs (for ORF 10, ORF 21, ORF 62, and ORF 63) were uniformly present in 100% of the specimens obtained from
Figure 3. Relative expression of uniformly wild-type, uniformly vaccine, and mixed markers among isolates recovered from persons with varicella-zoster virus–associated adverse events. The data are displayed as aggregate counts for each category for each isolate. Absence of mixed (yellow) loci suggests clonality of the isolate. SNP, single-nucleotide polymorphism.

DISCUSSION

We report a detailed genomic analysis of V-Oka variants present in specimens obtained from persons with postvaccination rash or HZ (table 2). Six additional SNPs were wild type in >90% of the isolates (nt 107599 in ORFs 9A, 50, 51, 52, 55, and 62). The remaining 12 SNPs were consistently wild type in 21%–87% of the specimens. The 3′ end of the VZV genome was more likely to have wild-type SNPs than was the 5′ end. Overall, 17 (63%) of 27 targeted SNPs were consistently wild type in >50% of the isolates, and 10 wild-type SNPs were associated with vaccine-related adverse events (including mixed specimens) in >90% of the isolates. The proportion of uniform wild-type, mixed, and uniform vaccine SNPs is displayed for each of the 39 clinical isolates (figure 3). Thirty isolates (77%) bore wild-type or mixed wild-type/vaccine SNPs at two-thirds or more of the loci examined.

persons with vaccine-associated adverse events, whether postvaccination rash or HZ (table 2). Six additional SNPs were wild type in >90% of the isolates (nt 107599 in ORFs 9A, 50, 51, 52, 55, and 62). The remaining 12 SNPs were consistently wild type in 21%–87% of the specimens. The 3′ end of the VZV genome was more likely to have wild-type SNPs than was the 5′ end. Overall, 17 (63%) of 27 targeted SNPs were consistently wild type in >50% of the isolates, and 10 wild-type SNPs were associated with vaccine-related adverse events (including mixed specimens) in >90% of the isolates. The proportion of uniform wild-type, mixed, and uniform vaccine SNPs is displayed for each of the 39 clinical isolates (figure 3). Thirty isolates (77%) bore wild-type or mixed wild-type/vaccine SNPs at two-thirds or more of the loci examined.
of in vitro–cultured isolates, perhaps with preferential expansion of one variant. Our analysis was performed on DNA extracted directly from clinical specimens. Differences in the methodological approach could also account for these contrasting observations. Comparison of material directly isolated from lesions with virus cultured from the same patients would help to resolve this issue.

The V-Oka SNPs tended to be more consistently displayed in the 3′ end of the genome, particularly SNPs in ORF 54 and ORF 62. The opposite trend was observed at SNP sites in the 5′ end of the genome, where most individual vaccine SNPs appeared in fewer than one-third of the clinical isolates. All of the SNPs examined here have been observed in vaccine preparations; some may be more prevalent among vaccine-related adverse event specimens than others (table 2). Among wild-type SNPs displayed in these isolates recovered from patients with adverse events, 15 of 22 base changes result in amino acid substitutions in their respective proteins.

All 3 commercial Oka-derived varicella preparations comprise mixtures of multiple variants, and most of the SNPs distinguishing V-Oka and P-Oka occur as a mixed genotype in vaccine [8–12]. Previously published sequence comparisons of Biken V-Oka with P-Oka indicated that only 11 base substitutions were uniform vaccine SNPs, with 1 located in a non-coding region at position 560, and with the remainder located in ORFs 6, 18, 54, and 62 (7 loci) [10]. The remaining 31 loci that were variable between P-Oka and V-Oka were mixtures of 2 alternative nucleotides in vaccine, indicating the presence of >1 variant.

To date, the vaccine variants identified in postvaccination rash or HZ probably preexist in the distributed product, although this has not been formally demonstrated. Because >75% of the isolates in this study had wild-type or mixed-genotype SNPs in two-thirds or more of the 27 loci studied, it will be important to determine the relative proportion at which such clones are represented in manufactured vaccine lots.

We found mixtures of variants in most of the HZ specimens and in all of the postvaccination rash specimens. The “hot spots” of variation that we observed were comparable to those reported by Quinlivan et al. [11]. The 4 invariant V-Oka loci, all located in ORF 62 (nt 105705, 106262, 107252, and 108111), were the same in both studies. In general, loci that tended to be stably wild type were also comparable. The loci nt 31732 and nt 107797 were 100% wild-type genotype in both studies. Two other loci, nt 12179 and nt 97796, were 100% wild type in the current study and 93.3% and 86.6% wild type, respectively, among the isolates analyzed by Quinlivan and coworkers. One notable contrast between the 2 studies occurred at nt 108838, which was uniformly wild type in the study by Quinlivan et al. [11] but was mixed (~50% vaccine or mixed genotype) in our study. These data thus suggest that specific wild-type SNPs are commonly associated with varicella vaccine–related adverse events. A careful evaluation of vaccine variants will be needed to assess which of them preexist in vaccine preparations and in what proportion.

Our data do not support the hypothesis that there are readily identifiable variants with enhanced virulence present in the vaccine. If we had detected individual clonal variants in lesions, or a small number of variants, it would have implied that certain vaccine variants were better equipped to replicate and disseminate. Similarly, for HZ, the detection of individual clones would have suggested a greater propensity for establishing latency or reactivating to cause disease. Our results indicate that only a minority (39%) of HZ lesions had evidence of a single clonal variant, and all 7 clonal variants differed from each other. Furthermore, in the majority of cases of both HZ and rash (17 of 39 specimens), variable SNPs (nonclonality) were revealed at ≥2 loci. Moreover, no 2 genetic profiles for any of the 39 specimens were identical. These data indicate that a large number of variants are associated with vaccine-related adverse events. Nonvaccine determinants (e.g., host and environmental factors) probably combine to result in an adverse event. Varicella vaccine is a live attenuated vaccine; as

### Table 2. Frequency and location of wild-type polymorphisms associated with vaccine-related adverse events.

<table>
<thead>
<tr>
<th>ORF</th>
<th>Dumas nucleotide reference</th>
<th>Isolates with wild-type marker, %</th>
<th>Specific substitution</th>
</tr>
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<tr>
<td>10</td>
<td>12779</td>
<td>100.0</td>
<td>Ala to Val</td>
</tr>
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<td>21</td>
<td>31732</td>
<td>100.0</td>
<td>Thr to Ile</td>
</tr>
<tr>
<td>62</td>
<td>107797</td>
<td>100.0</td>
<td>Leu to Pro</td>
</tr>
<tr>
<td>63a</td>
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<td>100.0</td>
<td>No change</td>
</tr>
<tr>
<td>62</td>
<td>107599</td>
<td>97.4</td>
<td>Val to Ala</td>
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</tr>
<tr>
<td>55</td>
<td>97796</td>
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<td>62</td>
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<tr>
<td>54</td>
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</table>

**NOTE.** ORF, open-reading frame.  
* Noncoding.
such, it may cause a spectrum of illness similar to that associated with the wild-type virus. Although serious adverse events have been reported after vaccination, they are rare compared with complications from natural disease [3, 28].

Using previously identified variant VZV SNPs between the P-Oka strain and V-Oka (Biken), we analyzed vaccine variability in 2 ways. First, we compared a commercial lot of Biken Oka vaccine with multiple lots of Varivax V-Oka. The 2 vaccines varied from each other at 10 (37%) of 27 SNPs that were analyzed. We also examined the variability of viral DNA from postvaccination rash and HZ lesions and compared these with the commercial vaccine used in vaccination (i.e., Varivax). Of 7 SNPs that were consistently found to be wild type in Varivax vaccine, only 1 (at nt 12779 in ORF 10) was consistently found to be wild type in the clinical specimens. For the remaining SNPs, clinical specimens contained vaccine genotype or mixed genotype. This does not preclude the possibility that variants with vaccine loci at these SNPs occur in V-Oka preparations, because variants may be present at concentrations below the limit of PCR detection, reflecting the relative proportion of variants in the 2 preparations.

Three of 4 consistently wild-type SNPs among clinical specimens confer an amino acid change to the gene products in ORFs 10, 21, and 62. The fourth SNP occurs in a noncoding region (nt 109200); it is unknown whether the locus participates in gene regulation. Thirteen additional SNPs were displayed uniformly as wild type in >50% of the clinical specimens. Ten of these SNPs confer amino acid changes to ORFs 9A, 31, 50, 52, 55, 62, and 64. Thus, most SNPs commonly found to be wild type in clinical specimens of postvaccination rash and HZ result in amino acid substitutions and, therefore, could affect protein function. Because these changes result in expression of the wild-type phenotype, all have the potential to contribute to VZV pathogenicity.

It is unclear whether the variability present in V-Oka vaccine arose during the original attenuation process or during routine vaccine production. Our recent study suggests that the array of vaccine variants in preparations may still be changing, at least with respect to their relative proportion in vaccine preparations [12]. Additional studies should resolve the potential for specific changes in VZV sequence to confer pathogenicity and/or reactogenicity. The analyses conducted here should facilitate that process, having narrowed the list of promising targets. In addition, currently marketed formulations of V-Oka should be evaluated for changes occurring with time and to determine the proportion that contain possibly more pathogenic variants.

References


