Carbamazepine (CBZ) is one of the most frequently used anti-convulsants in adults and children for the treatment of epilepsy, trigeminal neuralgia, and bipolar disorder. In particular, CBZ and its derivative oxcarbazepine (OXC) are the drugs of choice in the treatment of complex partial seizures in children. CBZ-induced hypersensitivity reactions of varying clinical presentation and severity are common and occur in ~3–10% of patients, with similar frequencies reported for adults and children. The majority of hypersensitivity reactions are relatively mild skin rashes that often require the discontinuation of CBZ for symptoms to resolve. However, CBZ also causes severe and life-threatening hypersensitivity reactions, which include the Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) spectrum and drug-induced hypersensitivity syndrome (HSS). SJS/TEN is characterized by a blistering rash and hemorrhagic erosions of mucous membranes, with TEN being the more severe form with more extensive skin detachment. HSS is characterized by a skin eruption, fever, and involvement of at least one internal organ, most frequently the liver. Although rare, the morbidity and mortality associated with these dramatic hypersensitivity reactions are substantial (long-term complications in 45% and mortality of 2% in children with SJS/TEN; mortality of up to 10% for SJS and HSS, and up to 50% for TEN in adults). A genetic basis of CBZ-induced hypersensitivity reactions has previously been investigated, primarily in adult patients. In these studies, strong associations of two genetic variants in the human leukocyte antigen (HLA) region—HLA-B*15:02 and HLA-A*31:01—with CBZ hypersensitivity were identified. Patients carrying HLA-B*15:02 were shown to be at strongly increased risk of CBZ-induced SJS/TEN. However, HLA-B*15:02 is observed primarily in certain Asian populations and only very rarely in patients outside of Asia.

The occurrence of hypersensitivity reactions including rare but life-threatening SJS and drug-induced hypersensitivity syndrome (HSS) limits the use of the anticonvulsant carbamazepine (CBZ). Human leukocyte antigen-B (HLA)-B*15:02 and HLA-A*31:01 have been identified as predictive genetic markers for CBZ hypersensitivity in Asian and European patients. To replicate these genetic associations in pediatric patients from North America with a diverse ethnic background, we investigated HLA-A*31:01 and HLA-B*15:02 in 42 children with CBZ hypersensitivity and 91 CBZ-tolerant children from across Canada. HLA-A*31:01 was significantly associated with CBZ-HSS (odds ratio (OR): 26.4, \( P = 0.0025 \)) and maculopapular exanthema (MPE) (OR: 8.6, \( P = 0.0037 \)) but not with CBZ-SJS. Conversely, HLA-B*15:02 was associated with CBZ-SJS (OR: 38.6, \( P = 0.002 \)) but not HSS or MPE. This study is the first to demonstrate the association of HLA-A*31:01 with CBZ hypersensitivity in children, providing important replication of this association and highlighting the importance of HLA-A*31:01 as a predictive biomarker across various ancestries.
Correspondingly, a higher incidence of CBZ-induced SJS/TEN in countries where HLA-B*15:02 is common has been suggested. More recently, HLA-A*31:01 was reported to be associated with various CBZ-induced hypersensitivity reactions, including HSS, SJS/TEN, and skin-specific maculopapular exanthema (MPE) in European and Asian patients.

HLA-B*15:02 and HLA-A*31:01 have been clearly established as risk factors for CBZ hypersensitivity in adult patients from Asia and Europe. However, even though some of the studies on HLA-B*15:02 included pediatric patients, to our knowledge these genetic associations have not been replicated in an entirely pediatric patient cohort. Because children often metabolize drugs differently than adults, dedicated pediatric pharmacogenomic studies are of value to confirm these associations, although to date no pediatric-specific effects have been reported for HLA-dependent adverse drug reactions. Furthermore, no studies have investigated these genetic risk variants in North American patients with diverse and admixed ancestries. In particular, the more recently reported association of HLA-A*31:01 requires further replication in both patients of European decent and those with non-European or non-Asian ancestries. This study aimed to address these gaps by investigating HLA-B*15:02 and HLA-A*31:01, two important pharmacogenetic markers with consistent evidence from adult-based studies, in Canadian children with diverse ancestries.

**RESULTS**

**Patient characteristics**

A total of 42 children experiencing CBZ-induced hypersensitivity reactions (CBZ cases) and 91 pediatric CBZ-tolerant control patients (CBZ controls) were genotyped. The CBZ cases included 9 children with SJS/TEN, 6 patients with HSS, 26 children with MPE, and 1 patient with acute generalized exanthematous pustulosis (AGEP) (Table 1). The median age at the start of CBZ treatment was higher in CBZ cases than in CBZ controls (P = 0.006; Table 1), whereas no differences were observed for mean age during treatment and age at the end of treatment or follow-up. With a total daily dose of 750 mg, the median CBZ dose was higher in CBZ controls than in CBZ cases (P < 0.001; Table 1). A larger proportion of CBZ cases were of non-European origin (60 vs. 38%; P = 0.03; Table 1). All patients received CBZ for the treatment of a seizure disorder.

Median time to onset of hypersensitivity reactions in CBZ cases was 13 days (range: 1–48 days; Table 1). In all but one patient, CBZ was the only possible causative drug with the appropriate temporal relationship between therapy initiation and onset of symptoms. One patient was taking CBZ and phenytoin but developed similar symptoms upon rechallenge with CBZ only. Of the nine children with CBZ-SJS/TEN, eight were classified as having SJS and one as having SJS/TEN overlap. Four of the children with CBZ-SJS/TEN were of European origin, two were of Southeast Asian origin, and one patient each reported First Nations, Sri Lankan, or Caribbean ancestry. All SJS/TEN patients had involvement of skin and oral mucosa, with eight patients also experiencing conjunctivitis. Genital mucosa was affected in two patients. Fever was frequent in children with CBZ-SJS/TEN (seven of nine patients). Elevation of liver enzymes was observed in two CBZ-SJS/TEN patients. All patients with CBZ-HSS experienced a bodywide skin eruption, fever, and elevation of liver enzymes. Eosinophilia was observed in two patients with CBZ-HSS, with one patient also experiencing lymphadenopathy. In one patient with CBZ-SJS, serology suggested an acute Mycoplasma pneumoniae infection. However, this patient had widespread skin lesions, which are less common in mycoplasma-induced reactions than in drug-induced reactions. In the other patients, no clear infectious etiology that could have contributed to the clinical manifestation and severity of hypersensitivity reactions was identified, although it should be noted that full screens for infections or virus reactivation were not routinely performed. The presence of mucous membrane erosions in all children with CBZ-SJS allowed for the exclusion of staphylococcal scalded skin syndrome as a possible differential diagnosis in these patients.

**Genotyping results**

Overall, 20 (15%) children treated with CBZ carried the T allele of rs1061235, a single-nucleotide polymorphism (SNP) previously shown to be in complete linkage disequilibrium with HLA-A*31:01 in Europeans. However, only 12 (9%) of these patients also carried HLA-A*31:01, as determined by direct sequence-based typing. Of the remaining patients, five carried HLA-A*33:03 and three carried HLA-A*33:01 (Supplementary Table S1 online). HLA-A*31:01 was therefore very strongly associated with CBZ-HSS or MPE in a combined analysis of the two reactions (OR: 26.4; P = 2.6 × 10⁻⁴; Table 2). A significant association was also observed with CBZ-induced MPE, with 6 of 26 (23.1%) CBZ-MPE cases carrying HLA-A*31:01 (OR: 8.6; P = 0.0037; Table 2). HLA-A*31:01 was therefore very strongly associated with CBZ-HSS or MPE in a combined analysis of the two reactions (OR: 11.2; P = 2.6 × 10⁻⁴; Table 2), with nine (28.1%) of the cases carrying HLA-A*31:01.

A highly significant association with CBZ-HSS and MPE was also observed for the HLA-A*31:01 proxy SNP rs1061235, with 14 (33%) CBZ hypersensitivity cases and only 6 (6.6%) control patients carrying the T allele (combined OR: 9.45; P = 2.6 × 10⁻⁵; Table 2).
Interestingly, rs1061235T was also overrepresented in hypersensitivity cases after HLA-A*31:01-positive children were excluded (OR: 5.83; \( P = 0.033 \); Supplementary Table S1 online), resulting in very similar effect sizes for the associations with HSS and MPE for HLA-A*31:01 and rs1061235. None of the patients with CBZ-HSS or MPE carried HLA-B*15:02 (Table 2). Therefore, in agreement with previous studies,24,31 no significant association of this risk variant with CBZ-HSS or MPE was observed.

### SJS/TEN

In contrast to the strong associations with HSS and MPE, no HLA-A*31:01 or rs1061235T carriers were detected among the nine patients with CBZ-induced SJS/TEN, and no significant association with CBZ-SJS/TEN was observed (OR: 1.33; \( P = 1.00 \); Table 2).

By contrast, three of the nine patients with CBZ-SJS/TEN were carriers of HLA-B*15:02, resulting in a significant association of HLA-B*15:02 with CBZ-SJS/TEN (OR: 38.6; \( P = 0.002 \); Table 2). All three HLA-B*15:02-positive patients with CBZ-SJS/TEN were of Asian origin; among these was the patient with concurrent mycoplasma infection. In this patient, even though mycoplasma infection can also be associated with oral and ocular inflammation and blistering,36 the genetic result was thus consistent with a CBZ-induced reaction.

---

Table 2

| Hypersensitivity reaction | CBZ cases (n = 42) | CBZ controls (n = 91) | \( P \) value\(^a\) | OXC cases (n = 5) |
|--------------------------|-------------------|----------------------|----------------|-----------------|
| Age, years               |                   |                      |                 |                 |
| Therapy start, median (range) | 9.9 (0.64–16.9)  | 7.4 (0.62–18.7)      | \( 0.006 \)     | 6.6 (3.4–15.3)  |
| Therapy mean, median (range) | 9.6 (0.92–21.2)  |                      | 0.52            |                 |
| Therapy end, median (range) | 10.8 (1.22–23.8) |                      | 0.21            |                 |
| Sex, n (%)               |                   |                      |                 |                 |
| Female                   | 19 (45)           | 43 (47)              | 4 (80)          |                 |
| Male                     | 23 (55)           | 48 (53)              | 1 (20)          |                 |
| CBZ duration, days \( n = 38^b \) |                   | \( n = 88^b \)       | \( <0.001 \)    |                 |
| Median (range)           | 14 (4–55)         | 728 (58–6,601)       | 14 (10–22)      |                 |
| CBZ dose, mg/day \( n = 35^b \) |                   | \( n = 88^b \)       | \( <0.001 \)    |                 |
| Median (range)           | 400 (80–1,000)    | 750 (100–2,000)      | 600 (525–900)   |                 |
| Ancestry, n (%)\(^c\)   |                   |                      |                 |                 |
| Europe                   | 17 (40)           | 56 (62)              | 3 (60)          |                 |
| Asia                     | 6 (14)            | 6 (7)                | —               |                 |
| Africa                   | 1 (2)             | 1 (1)                | —               |                 |
| Aboriginal\(^d\)        | 2 (5)             | 3 (3)                | —               |                 |
| Latin America/Caribbean  | 4 (10)            | 1 (1)                | —               |                 |
| Mixed                    | 10 (24)           | 14 (15)              | —               |                 |
| Unknown                  | 2 (5)             | 10 (11)              | 2 (40)          |                 |
| Time to onset of reaction, days \( n = 37^b \) |                   |                      |                 |                 |
| Median (range)           | 13 (1–48)         | —                    | 13 (10–22)      |                 |
| HSS                      | 16.5 (10–48)      | —                    | —               |                 |
| SJS/TEN                  | 14 (10–24)        | —                    | 16 (10–22)      |                 |
| MPE\(^e\)               | 11 (1–28)         | —                    | 13 (11–14)      |                 |
| AGEP                     | 45                | —                    | —               |                 |

\( ^a \)Test between CBZ cases and CBZ controls. \( ^b \)n, number known; indicated only if not known for all patients. \( ^c \)Country of origin; patients were classified as European if the country of origin of all four grandparents was European or Canada. Mixed origin was defined as ≥1 grandparent having a different origin from the other grandparents. Origin was classified as unknown if country of origin was not known for ≥1 grandparent. \( ^d \)Aboriginal Canadian (First Nations, Inuit, Métis). \( ^e \)Exact time to onset of reaction was not known for five patients. Significant \( P \) values are indicated in bold.
AGEP

Interestingly, the single patient with CBZ-induced AGEP tested positive for the proxy SNP rs1061235 but did not carry HLA-A*31:01 (Supplementary Table S1 online). The rs1061235T variant has also been reported in one adult patient with CBZ-AGEP of European descent.\textsuperscript{30} Direct typing of HLA-A*31:01 was not performed in this patient, but a perfect concordance between CBZ-AGEP and rs1061235T (21.4) was observed here, was estimated as 0.69 (0.04–13.27) and using normal approximation.\textsuperscript{30} HLA-B*15:02 genotyping failed for four CBZ-tolerant patients.

Combined analyses

When considering all CBZ hypersensitivity reactions together, a highly significant association with HLA-A*31:01 was observed (OR: 7.85; \( P = 0.0016\); Table 2), although the effect size was reduced as compared with an analysis that included only HSS and MPE cases. On the other hand, HLA-B*15:02 was not significantly overrepresented in the CBZ cases overall. In a combined analysis of both risk variants, a strong association was observed with all CBZ-induced hypersensitivity reactions (OR: 8.14; \( P = 2.6 \times 10^{-4}\); Table 2). Overall, 12 of 42 (28.6%) CBZ cases carried HLA-A*31:01 or HLA-B*15:02, whereas only 4 (4.6%) CBZ-tolerant patients were positive for either risk variant. Using an estimated incidence of CBZ-induced hypersensitivity reactions (pretest probability) of 5–10%, the positive posttest probability of a combined genetic test for both risk variants, based on the positive likelihood ratio of 6.21 observed here, was estimated as 25–41%, with a negative posttest probability of 3.8–7.7%.

HLA-A*31:01 in European patients

Given the differences in origin between CBZ cases and controls, we performed a subgroup analysis for HLA-A*31:01 in patients with three or more grandparents of European origin. A total of 20 CBZ cases and 65 controls were included in this analysis. The frequency of HLA-A*31:01 in European CBZ-tolerant children (3.1%; Table 3) was similar to the frequency reported previously in a European study (3.9%). Results observed for the association of HLA-A*31:01 with CBZ hypersensitivity were similar to those for the full cohort, with 20% of CBZ cases carrying HLA-A*31:01 (OR: 7.62; \( P = 0.025\); Table 3). As in the full cohort, a stronger association was observed when only patients with CBZ-HSS or MPE were considered (OR: 10.05; \( P = 0.004\); Table 3).

Table 2 Association of HLA-A*31:01, proxy SNP rs1061235, and HLA-B*15:02 with CBZ hypersensitivity

|                  | HLA-A*31:01 | rs1061235T | HLA-B*15:02 | Combined\textsuperscript{a} |
|------------------|-------------|------------|-------------|-----------------------------|
|                  | Total n     | n Positive | OR (95% CI) | P value\textsuperscript{b} | Total n     | n Positive | OR (95% CI) | P value\textsuperscript{b} |
| CBZ-HSS          | 6           | 3 (50)     | 26.36 (2.53–307.89) | 0.0025                     | 20          | 4 (20.0)   | —           | —                           |
| CBZ-MPE          | 26          | 6 (23.1)   | 8.57 (1.67–57.50)  | 0.0037                     | 50          | 9 (18.0)   | 3.32 (0.50–21.66) | 0.017         |
| CBZ-SJS/TEN      | 9           | —          | —            | —                           | 16          | 4 (25.0)   | 7.40 (2.91–34.50) | 0.0022        |
| CBZ-AGEP         | 1           | —          | —            | —                           | 1           | 1 (100)    | —            | —                           |

Significant \( P \) values and ORs are indicated in bold.

Table 3 Subgroup analysis of HLA-A*31:01 in patients of European origin only

|                  | Total n | n Positive | OR (95% CI) | \( P \) value\textsuperscript{a} |
|------------------|---------|------------|-------------|----------------------------------|
| CBZ-HSS          | 2       | 1 (50.0)   | 25.92 (0.27–243.7) | 0.088                           |
| CBZ-MPE          | 14      | 3 (21.4)   | 8.23 (0.84–109.23) | 0.037                           |
| CBZ-SJS/TEN      | 4       | —          | —            | —                                |
| CBZ-AGEP         | —       | —          | —            | —                                |
| All CBZ cases    | 20      | 4 (20.0)   | 7.62 (0.99–91.40) | 0.025                           |
| HSS/MPE          | 16      | 4 (25.0)   | 10.05 (1.28–122.88) | 0.013                           |
| CBZ-tolerant     | 65      | 2 (3.1)    | —            | —                                |

Significant \( P \) values and ORs are indicated in bold.

Table 3 Subgroup analysis of HLA-A*31:01 in patients of European origin only

\( P \) = 0.0016 (Table 2), although the effect size was reduced as compared with an analysis that included only HSS and MPE cases. On the other hand, HLA-B*15:02 was not significantly overrepresented in the CBZ cases overall. In a combined analysis of both risk variants, a strong association was observed with all CBZ-induced hypersensitivity reactions (OR: 8.14; \( P = 2.6 \times 10^{-4}\); Table 2). Overall, 12 of 42 (28.6%) CBZ cases carried HLA-A*31:01 or HLA-B*15:02, whereas only 4 (4.6%) CBZ-tolerant patients were positive for either risk variant. Using an estimated incidence of CBZ-induced hypersensitivity reactions (pretest probability) of 5–10%, the positive posttest probability of a combined genetic test for both risk variants, based on the positive likelihood ratio of 6.21 observed here, was estimated as 25–41%, with a negative posttest probability of 3.8–7.7%.
not HLA-A*31:01. None of the three children with OXC-MPE carried HLA-A*31:01 or rs1061235T. The number of cases is too small to draw conclusions from, but we did not observe any evidence of a strong association of HLA-A*31:01 with OXC hypersensitivity. Similarly, no carriers of HLA-B*15:02 were observed among the patients with OXC hypersensitivity. However, this was not unexpected, given that none of the patients with OXC hypersensitivity reported Asian ancestry.

Origin of risk variant carriers
All four HLA-B*15:02-positive children reported Asian countries of origin. Eleven of the 16 grandparents of these children were of Chinese origin, and 1 grandparent originated from the Philippines—both populations in which HLA-B*15:02 is common.38 Interestingly, one HLA-B*15:02-positive child with CBZ-SJS/TEN was of Sri Lankan origin, where, to our knowledge, no HLA-B*15:02-positive cases have been reported so far.

In contrast to children carrying HLA-B*15:02, those carrying HLA-A*31:01 or rs1061235T had a variety of origins (Figure 1). Owing to the high proportion of European ancestry in the overall cohort, European origin was also common in HLA-A*31:01 carriers. However, significant proportions of patients also had Aboriginal Canadian and Latin American origins (Figure 1a). Interestingly, a majority of patients with west Asian or south Asian origin who carried rs1061235T did not carry HLA-A*31:01, suggesting that rs1061235 alone is not an optimal proxy SNP in some populations.

DISCUSSION
Hypersensitivity reactions are a significant problem in the treatment of children with CBZ. In this study, we replicated important associations of two genetic risk factors—HLA-B*15:02 and HLA-A*31:01—in pediatric patients. Overall, our findings were in agreement with previous studies, suggesting similar genetic associations in children and adults and a higher risk of CBZ hypersensitivity in children carrying these risk variants. This being the first study in ethnically diverse North American patients, we replicated the association of HLA-A*31:01 with CBZ hypersensitivity and demonstrated the relevance of this risk variant across a broader range of ancestries; we also provided further replication in European patients.

Even though the number of HSS cases included in our study was small, our observation of a stronger association of HLA-A*31:01 with HSS as compared with MPE is in agreement with most previous findings,30,31 again suggesting a similar association in children and adults. Overall, the frequency of HLA-A*31:01 in children with CBZ-HSS or MPE was similar to the frequencies reported previously in European patients but lower than frequencies reported in Japanese and Korean populations.31,32 This is consistent with the large proportion of patients in our study reporting European origins and suggests that the overall frequency of HLA-A*31:01 in our multiethnic patient cohort was similar. Of importance, we showed that the proxy SNP rs1061235 is not an optimal surrogate marker for HLA-A*31:01 in ancestrally diverse populations. Our observation of rs1061235T also being overrepresented among CBZ hypersensitivity cases in patients not carrying HLA-A*31:01 suggests that further investigation of this proxy SNP, particularly in multiethnic populations, may still be of interest.

We did not detect an association of HLA-A*31:01 with CBZ-SJS/TEN, which is in contrast to two previous studies that reported a stronger association with SJS/TEN as compared with CBZ-HSS or MPE, with ORs of 26 and 34, respectively.30,31 The number of SJS cases in our study was limited, particularly when taking into consideration that three SJS cases carried HLA-B*15:02, reducing our power to detect an association. However, besides the two studies reporting a strong association,30,31 evidence from other adult-based studies with respect to the association of HLA-A*31:01 with CBZ-SJS/TEN is also conflicting. For example, no significant association was observed in another study in Japanese patients39 or in a Korean study.32

Figure 1 Origin of HLA-A*31:01 risk variant and proxy SNP rs1061235T carriers. (a) Self-reported origins of HLA-A*31:01-positive patients’ grandparents (n = 12 patients) and of (b) rs1061235T carriers’ grandparents (n = 21 patients) are shown. Aboriginal, Aboriginal Canadian, including First Nations, Inuit, Métis; HLA, human leukocyte antigen; SE/E Asia, southeast or east Asia; S/W Asia, south or western Asia; SNP, single-nucleotide polymorphism.
Random sampling effects due to the small numbers of cases and differences in the clinical characterization of SJS/TEN may partly explain these discrepant findings. Nevertheless, assuming an effect size as reported previously (OR ≥26), the probability of, by chance, observing no risk variant carriers among six CBZ-SJS cases in our study was <2.3%. Random sampling error alone is thus unlikely to explain the absence of HLA-A*31:01 among SJS cases. Therefore, although no conclusions can be drawn regarding an association of HLA-A*31:01 with CBZ-SJS/TEN or lack thereof, our findings combined with others suggest that this association may not be as strong as initially reported. Further studies investigating HLA-A*31:01 in the context of CBZ-SJS/TEN are thus needed in both pediatric and adult patients.

By contrast, the observed association of HLA-B^*15:02 with CBZ-SJS/TEN is in full agreement with previous findings, with HLA-B^*15:02 being observed in all children with CBZ-SJS/TEN who reported Asian origins. Of particular interest, we report here for the first time an HLA-B^*15:02-positive patient of Sri Lankan origin, suggesting the presence and clinical relevance of HLA-B^*15:02 in Sri Lanka. Data on the population frequency of HLA-B^*15:02 in Sri Lanka are, to our knowledge, not available. However, HLA-B^*15:02 has been associated with CBZ-SJS/TEN in patients from India. Given the geographical proximity, the presence of HLA-B^*15:02 in patients from Sri Lanka would not be surprising.

Similarly, we report here for the first time the presence of HLA-A^*31:01 in CBZ hypersensitivity patients of Aboriginal Canadian and Latin American origin, demonstrating the relevance of this risk variant across a broader range of ancestries. This finding is in agreement with high population frequencies reported for HLA-A^*31:01 in many ethnic groups. In particular, high HLA-A^*31:01 population frequencies (up to 48% carrier frequency) have been reported in Aboriginal Americans. This is in agreement with our observation that Aboriginal Canadian ancestry was common (19%) in HLA-A^*31:01 carriers and suggests a high relevance of HLA-A^*31:01 for Aboriginal American patients receiving CBZ.

A larger proportion of patients were of European origin in the CBZ-tolerant group as compared with the hypersensitivity cases in our study. Because the frequencies of HLA alleles differ between populations, this difference in ancestry between cases and controls could potentially affect our results. In particular, the overrepresentation of patients carrying rs1061235T but not HLA-A^*31:01 may be partly attributed to the different frequencies of non-European origins between cases and controls. Many of these patients carried HLA-A^*33:03 and were of Asian origin, in whom HLA-A^*33:03 is more common than it is in European populations. On the other hand, given the consistency of our findings between the overall cohort and a subgroup analysis in European patients, as well as the overall agreement of our findings with other studies, any effect of ancestral differences between cases and controls is unlikely to affect the overall conclusions of this study regarding the associations of HLA-A^*31:01 and HLA-B^*15:02 with CBZ hypersensitivity. Nevertheless, future studies in pediatric patients that include CBZ-tolerant children with non-European ancestries should be conducted.

Finally, although HLA-B^*15:02 and HLA-A^*31:01 are important risk factors in the context of CBZ hypersensitivity, not all patients carrying these variants developed a hypersensitivity reaction. In fact, based on the combined evaluation of both risk markers in our population of ancestrally diverse Canadian children and an estimated frequency of hypersensitivity reactions of 5%, approximately three of four patients carrying a risk variant are expected to tolerate CBZ. Similarly, HLA-A^*31:01 or HLA-B^*15:02 was not present in all CBZ hypersensitivity cases. The two risk variants therefore appear to be neither sufficient nor necessary to trigger CBZ hypersensitivity. Further studies are needed to identify additional risk factors in order to improve the prediction of hypersensitivity reactions and avoid unnecessary withholding of CBZ from patients in whom it would be safe. Promising findings have recently been reported in the context of HLA-B^*15:02, where certain T-cell receptor clonotypes were found to be expressed in combination with HLA-B^*15:02 to elicit an in vitro reaction to CBZ.

Nevertheless, in spite of the relatively low positive predictive power of these genetic tests, the severity of SJS/TEN and HSS combined with the availability of equally effective alternative therapies for many indications justifies genetic testing for HLA-B^*15:02 and HLA-A^*31:01 to identify children and adults at higher risk for CBZ hypersensitivity. Even though HLA-B^*15:02 is rare in some populations, carriers of the risk variant can still occur. Furthermore, the frequency of HLA-B^*15:02 is unknown for many populations. We therefore suggest that predictive testing for both variants in all patients, irrespective of their ancestry, is the safest approach. In particular, a combined test for both markers is likely to be beneficial in ethnically diverse populations, in which the full genetic ancestry of patients may not be known and many patients are of mixed origins.

In conclusion, these findings demonstrate that HLA-A^*31:01 and HLA-B^*15:02 are predictive of CBZ-induced hypersensitivity reactions in children and that HLA-A^*31:01 is a relevant marker in patients of various ancestries. Further investigation is needed to elucidate the magnitude of the association of HLA-A^*31:01 with CBZ-SJS/TEN and AGEP, but these pharmacogenetic markers have great potential to reduce the occurrence of severe and life-threatening hypersensitivity reactions and improve the safety of CBZ therapy.

METHODS

Patients. All study participants were recruited through the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) in pediatric hospitals across Canada. Blood or saliva samples were obtained for genetic analyses. Clinical data were obtained through detailed review of medical records, performed blinded to genotype data by a clinical pharmacologist, a dermatologist, and an adverse drug reaction surveillance clinician. Self-reported ancestry was obtained from all patients with respect to country of origin of patients, parents, and grandparents. No genetic analyses were conducted to confirm reported ancestries; however, a previous investigation of 524 CPNDS samples revealed good concordance between self-reported and genetic ancestry.

SJS/TEN (including SJS, SJS/TEN overlap, and TEN), HSS, and AGEP were defined according to the criteria suggested by the Phenotype Standardization Project for immune-mediated drug-induced skin injury. Patients with SJS/TEN or HSS were further characterized using an in-house scoring tool (available from the authors on request) to assess the likelihood of diagnosis, differentiating between possible, probable, and
highly likely cases on the basis of the available clinical data (e.g., presented symptoms, temporal relationship between drug and reaction onset, and likelihood of other etiologies) and incorporating elements from the ALDEN algorithm to assess drug causality. All SJS/TEN and HSS cases included in this study were classified as probable or highly likely.

MPE were defined as any cutaneous reaction occurring within the first 8 weeks of CBZ treatment and resulting in CBZ discontinuation, with or without fever but without other systemic symptoms. One patient developed a rash, fever, and conjunctival injection after initiation of CBZ and phenytoin, and developed a rash with fever upon rechallenge with CBZ only. This patient was classified as having MPE. One patient developed mucosal lesions and bilateral conjunctivitis but no skin lesions. Because not all diagnostic criteria for SJS were fulfilled, the patient was afebrile, and no clear infectious etiology was identified, this patient was classified as possibly having erythema multiforme and grouped with those having MPE for statistical analyses.

CBZ-tolerant controls were defined as patients taking CBZ for at least 8 weeks without any adverse reaction. Patients tolerating CBZ who experienced a cutaneous adverse reaction to other drugs (n = 13) were excluded from the control population.

Five patients with OXC-induced hypersensitivity reactions were genotyped for an exploratory evaluation of indications for an association of the same HLA variants with hypersensitivity reactions to this structurally similar drug. Characterization of OXC-induced hypersensitivity reactions was performed using the same criteria as for CBZ. No OXC-tolerant controls were included because the number of available control patients (n = 13) was too small to enable any meaningful statistical analyses.

Written informed consent or assent was obtained from all study participants or their parents or legal guardians. This study was approved by the ethics committees of all participating universities and hospitals.

Genotyping. Genomic DNA was extracted using the QIAamp or the QIAasympohony DNA purification systems (Qiagen, Toronto, Canada). Genotyping was performed using the PG1502 DNA Detection Kit (Pharmigene, Taipei, Taiwan) for direct typing of HLA-B*15:02. For HLA-A*31:01, a Custom Taqman SNP Genotyping Assay (Applied Biosystems, Foster City, CA) was used for rs1061235, a proxy SNP showing an absolute correlation (R^2 = 1) with HLA-A*31:01 in Europeans. Genotyping was performed according to standard protocols on a 7500 Fast Real-Time PCR System (Applied Biosystems). In addition, direct sequence-based typing for HLA-A*31:01 was also performed as described previously, using primers 11–240 and 13–249 for allele-specific amplification of exons 2 and 3 for HLA-A*31 and *33.

Statistical analysis. All statistical analyses were conducted using the statistical software R. Associations of genetic variants with CBZ hypersensitivity were assessed using Fisher’s exact test and a dominant genetic model. For evaluation of clinical variables in patients with and without CBZ hypersensitivity, the Wilcoxon–Mann–Whitney test was used for continuous variables and Fisher’s exact test for categorical variables. Two-sided P values <0.05 were considered statistically significant.

SUPPLEMENTAL MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

ACKNOWLEDGMENTS

We thank all the children and their families for their participation in the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) and this study. We also acknowledge all members of the CPNDS active adverse drug reaction surveillance consortium, who were instrumental in identifying and enrolling study participants and who helped in the detailed clinical characterization of hypersensitivity reactions. The consortium members are listed below:

Vancouver, British Columbia: British Columbia Children’s Hospital, Child and Family Research Institute, Pharmaceutical Outcomes Programme and Centre for Molecular Medicine and Therapeutics: Bruce C. Carleton, Michael R. Hayden, Colin J.D. Ross, Stuart MacLeod, Anne Smith, Claudette Hildebrand, Lucia I. Castro-Pastrana, Reza Ghannadan, Ursala Amstutz, Catherine Carter, Michelle Higginson, Linhua Zhang, Naim Massah, Fudan Miao, and Adrienne Borrie; Calgary, Alberta: Alberta Children’s Hospital: Cheri Niissen-Jordan, David Johnson, Linda Verbeek, Rick Kaczowka, Andrea Hurton, and Patti Stevenson; London, Ontario: London Health Sciences Centre: Michael J. Rieder and Becky Malkin; Montreal, Quebec: Hôpital Sainte-Justine: Jean-Francois Bussières, Denis Lebel, Pierre Barret, Aurélie Closon, and Eve Courbon; Ottawa, Ontario: Children’s Hospital of Eastern Ontario: Régis Vaillancourt, Pat Elliott-Miller, Elaine Wong, Herpreet Mankoo, Brenda Wilson, and Lauren O’Connor; Health Canada: Maurica Maher; Toronto, Ontario: Hospital for Sick Children: Gideon Koren, Shinya Ito, Paul Nathan, Mark Greenberg, Miho Inoue, Facundo Garcia Bournissen, Toshihiro Tanaka, Sachi Sakaguchi, Hisaki Fujii, Mina Ogawa, Ryoko Ingrain, Taro Kamiya, and Smita Karande; Sunnybrook Health Sciences Centre: Neil Shear; Winnipeg, Manitoba: Winnipeg Children’s Hospital: Kevin Hall, Nick Honcharik, Shanna Chan, and Michelle Staub. This study was funded by the Canadian Institutes of Health Research and the Canada Foundation for Innovation. Additional support was received from Genome Canada, the Canadian Dermatology Foundation, the University of British Columbia, and the Child and Family Research Institute.

AUTHOR CONTRIBUTIONS

B.C.C., U.A., C.J.D.R., L.I.C.-P., M.J.R., N.H.S., and M.R.H. wrote the manuscript; B.C.C., U.A., C.J.D.R., and M.R.H. designed the research; B.C.C., U.A., L.I.C.-P., M.J.R., and N.H.S. performed the research; U.A. analyzed data.

CONFLICT OF INTEREST

M.J.R. holds the Canadian Institute of Health Research–GlaxoSmithKline chair in Pediatric Clinical Pharmacology at the University of Western Ontario. N.H.S. has been a paid consultant for Novartis. The other authors declared no conflict of interest.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THIS TOPIC?

✓ Up to 10% of patients initiating CBZ therapy experience hypersensitivity reactions, including life-threatening SJS and hypersensitivity syndrome. HLA-B*15:02 is a well-known genetic marker for CBZ-induced SJS in Asian patients. HLA-A*31:01 was recently identified as a risk factor for CBZ hypersensitivity in adult patients from Europe and Asia.

WHAT QUESTION DID THIS STUDY ADDRESS?

✓ The association of HLA-A*31:01 with CBZ hypersensitivity required further replication, particularly in children and patients of other ancestries. To address this gap, we investigated HLA-A*31:01 together with HLA-B*15:02 in ancestrally diverse children with CBZ hypersensitivity from across Canada.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

✓ HLA-A*31:01 was associated with CBZ-induced hypersensitivity syndrome and MPE in children with diverse ancestries. HLA-B*15:02, but not HLA-A*31:01, was associated with CBZ-induced SJS.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS

✓ These results demonstrate the clinical relevance of predictive HLA genotyping in children to identify patients at risk for CBZ hypersensitivity, as well as the importance of HLA-A*31:01 as a risk factor across ancestries.
1. Landmark, C.J., Fosmark, H., Larsson, P.G., Ryttner, E. & Johnnenssen, S.J. Prescription patterns of antiepileptic drugs in patients with epilepsy in a nation-wide population. *Epilepsy Res.* 95, 51–59 (2011).

2. van de Vrie-Hoekstra, N.W., de Vries, T.W., van den Berg, P.B., Brouwer, O.F. & de Jong-van den Berg, L.T. Antiepileptic drug utilization in children from 1997-2005—a study from the Netherlands. *Eur. J. Clin. Pharmacol.* 64, 1013–1020 (2008).

3. Wheelless, J.W., Clarke, D.F., Arzinamoglu, A. & Carpenter, D. Treatment of pediatric epilepsy: European expert opinion, 2007. *Epileptic Disord.* 9, 353–412 (2007).

4. Wheelless, J.W., Clarke, D.F. & Carpenter, D. Treatment of pediatric epilepsy: expert opinion, 2005. *J. Child Neurol.* 20, suppl. 1, S1–S6; quiz S59 (2005).

5. Segal, A.R., Doherty, K.M., Leggott, J. & Zlotoff, B. Cutaneous reactions to drugs in children. *Pediatrics* 120, e1082–e1096 (2007).

6. Castro-Pastrana, L.I., Ghannadan, R., Rieder, M.J., Dahlke, E., Hayden, M. & Carleton, B. Cutaneous adverse drug reactions in children: an analysis of reports from the Canadian Pharmacogenomics Network for Drug Safety (CPNDS). *J. Popul. Ther. Clin. Pharmacol.* 18, e106–e120 (2011).

7. Pellock, J.M. Carbamazepine side effects in children and adults. *Epilepsia* 28 (suppl. 3), S64–S70 (1987).

8. Roujeau, J.C. Clinical heterogeneity of drug hypersensitivity. *Toxicology* 209, 123–129 (2005).

9. Chang, D.K. & Shear, N.H. Cutaneous reactions to anticonvulsants. *Semin. Neurol.* 12, 329–337 (1992).

10. Marson, A.G. et al. SANAD Study Group. The SANAD study of effectiveness of carbamazepine, gabapentin, lamotrigine, oxcarbazepine, or topiramate for treatment of partial epilepsy: an unblinded randomised controlled trial. *Lancet* 369, 1000–1015 (2007).

11. Aref, H. et al. Comparison and predictors of rash associated with 15 antiepileptic drugs. *Neurology* 68, 1701–1709 (2007).

12. Wolkentin, K.G., Phillips, K.A. & Post, R.M. Rash complicating carbamazepine treatment. *J. Clin. Psychopharmacol.* 14, 408–413 (1994).

13. Konishi, T., Naganuma, Y., Hongo, K., Murakami, M., Yamatani, M. & Okada, T. Carbamazepine-induced skin rash in children with epilepsy. *Eur. J. Pediatr.* 152, 605–608 (1993).

14. Roujeau, J.C. et al. Medication use and the risk of Stevens-Johnson syndrome or toxic epidermal necrolysis. *N. Engl. J. Med.* 333, 1600–1607 (1995).

15. Carleton, B. et al. Rash complicating carbamazepine-induced cutaneous adverse reactions. *Clin. Pharmacol. Ther.* 126, 372–377 (2019).

16. Roujeau, J.C., Guillaume, J.C., Fabre, J.P., Penso, D., Fléchét, M.L. & Girre, J.P. Toxic epidermal necrolysis (Lyell syndrome). Incidence and drug etiology in France, 1981-1985. *Arch. Dermatol.* 126, 372–377 (1990).

17. Schlienger, R.G. & Shear, N.H. Antiepileptic drug hypersensitivity syndrome. *Epilepsia* 39 (suppl. 7), S25–S27 (1996).

18. Pirmohamed, M. et al. Phenotype standardization for immune-mediated drug-induced skin injury. *Clin. Pharmacol. Ther.* 89, 896–901 (2011).

19. Mockenhaupt, M. The current understanding of Stevens-Johnson syndrome and toxic epidermal necrolysis. *Expert Rev. Clin. Immunol.* 7, 803–813; quiz 814 (2011).

20. Wolf, R., Orion, E., Marcos, B. & Matz, H. Life-threatening acute adverse cutaneous drug reactions. *Clin. Dermatol.* 23, 171–181 (2005).

21. Roujeau, J.C. & Stern, R.S. Severe adverse cutaneous reactions to drugs. *N. Engl. J. Med.* 331, 1272–1285 (1994).

22. Finkelstein, Y. et al. Recurrence and outcomes of Stevens-Johnson syndrome and toxic epidermal necrolysis in children. *Pediatrics* 128, 723–728 (2011).

23. Chung, W.H. et al. Medical genetics: a marker for Stevens-Johnson syndrome. *Nature* 288, 486 (2004).

24. Hung, S.L. et al. Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions. *Pharmacogenet. Genomics* 16, 297–306 (2006).

25. Tasaneeyakul, W. et al. Association between HLA-B*1502 and carbamazepine-induced severe cutaneous adverse drug reactions in a Thai population. *Epilepsia* 51, 926–930 (2010).

26. Chen, P. et al. Taiwan 5S5 Consortium. Carbamazepine-induced toxic effects and HLA-B*1502 screening in Taiwan. *N. Engl. J. Med.* 364, 1126–1133 (2011).

27. Lonjou, C. et al. A Registry Group on Stevens-Johnson syndrome…early and late aspects. *Pharmacogenomics* 6, 265–268 (2006).

28. Kaniwa, N. et al. SJS Research Group. HLA-B*1511 is a risk factor for carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrosis in Japanese patients. *Epilepsia* 51, 2461–2465 (2010).

29. US Food and Drug Administration. Information for Healthcare Professionals: Drug Safety Information for Healthcare Professionals: Dangerous or Even Fatal Skin Reactions—Carbamazepine (marketed as Carbatrol, Equetro, Tergetol, and generics) <http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm124718.htm> (2007) Accessed 12 December 2012.

30. McCormack, M. et al. HLA-A*3101 and carbamazepine-induced hypersensitivity reactions in Europeans. *N. Engl. J. Med.* 364, 1134–1143 (2011).

31. Ozeki, T. et al. Genome-wide association study identifies HLA-A*3101 allele as a genetic risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population. *Hum. Mol. Genet.* 20, 1034–1041 (2011).

32. Kim, S.H. et al. Adverse Drug Reaction Research Group in Korea. Carbamazepine-induced severe cutaneous adverse reactions and HLA genotypes in Koreans. *Epilepsia* 97, 190–197 (2011).

33. Then, S.M., Rani, Z.Z., Raymond, A.A., Jamal, R. Frequency of the HLA-B*1502 allele contributing to carbamazepine-induced hypersensitivity reactions in a cohort of Malaysian epilepsy patients. *Asian Pac. J. Allergy Immunol.* 29, 290–293 (2011).

34. Kears, G.L., Abdel-Rahman, S.M., Alander, S.W., Blowey, D.L., Leeder, J.S. & Kauffman, R.E. Developmental pharmacology—drug disposition, action, and therapy in infants and children. *N. Engl. J. Med.* 349, 1157–1167 (2003).

35. Becker, M.L. & Leeder, J.S. Identifying genomic and developmental causes of adverse drug reactions in children. *Pharmacogenomics* 11, 1591–1602 (2010).

36. Meyer Sauteur, P.M., Goetschel, P. & Lautenschlager, S. *Mycoptiasis* and mucositis—part of the Stevens-Johnson syndrome spectrum. *J. Dtsch. Dermatol. Ges.* 10, 740–746 (2012).

37. Levi, N. et al. Medications as risk factors of Stevens-Johnson syndrome and toxic epidermal necrolysis in children: a pooled analysis. *Pediatrics* 123, e297–e304 (2009).

38. Gonzalez-Galarza, F.F., Christmas, S., Middleton, D. & Jones, A.R. Allele frequency net: a database and online repository for immune gene frequencies in worldwide populations. *Nucleic Acids Res.* 39, D913–D919 (2011).