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Validation of a multigenic model to predict seizure control in newly treated epilepsy

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Summary A multigenic classifier based on five single nucleotide polymorphisms (SNPs) was previously reported to predict treatment response in an Australian newly-diagnosed epilepsy cohort using a *k*-nearest neighbour (*k*NN) algorithm. We assessed the validity of this classifier in predicting response to initial antiepileptic drug (AED) treatment in two UK cohorts of newly-diagnosed epilepsy and investigated the utility of these five SNPs in predicting seizure control in general. The original Australian cohort constituted the training set for the classifier and was used to predict response to the first well-tolerated AED monotherapy in independently recruited UK cohorts (Glasgow, $n = 281$; SANAD, $n = 491$). A ''leave-one-out'' cross-validation was also employed, with training sets derived internally from the UK datasets. The multigenic classifier

Abbreviations: AED, antiepileptic drug; ANOVA, analysis of variance; CBZ, carbamazepine; CI, confidence interval; DNA, deoxyribonucleic acid; FN, false negative; FP, false positive; GGE, genetic generalised epilepsy; *k*NN, *k*-nearest neighbour; LTG, lamotrigine; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value; SANAD, standard and new antiepileptic drugs (trial); SNP, single nucleotide polymorphism; TN, true negative; TP, true positive; UK, United Kingdom; UNC, unclassified epilepsy; VPA, valproate.

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using the Australian cohort as the training set was unable to predict treatment response in either UK cohort. In the ''leave-one-out'' analysis, the five SNPs collectively predicted treatment response in both Glasgow and SANAD patients prescribed either carbamazepine or valproate (Glasgow OR = 3.1, 95% CI = 1.4—6.6, $p = 0.018$; SANAD OR = 2.8, 95% CI = 1.3—6.1, $p = 0.048$), but not those receiving lamotrigine (Glasgow OR = 1.3, 95% CI = 0.6—2.8, $p = 1.0$; SANAD OR = 2.2, 95% CI = 0.9—5.4, $p = 0.36$) or other AEDs (Glasgow OR = 0.6, 95% CI = 0.2—2.0, $p = 1.0$; SANAD OR = 1.9, 95% CI = 0.9—4.2, $p = 0.36$). The Australian-based multigenic *k*NN model is not predictive of initial treatment response in UK cohorts of newly-diagnosed epilepsy. However, the five SNPs identified in the original Australian study appear to collectively have a predictive influence in UK patients prescribed either carbamazepine or valproate.

Introduction

Genetic influences on antiepileptic drug (AED) responsiveness have been the subject of increasingly intensive investigation in the past decade. The majority of these studies have focused on single nucleotide polymorphisms (SNPs) in candidate genes, such as those encoding drug receptor and drug transporter proteins (Haerian et al., 2012; Hilger et al., 2012; Leschziner et al., 2006; Manna et al., 2011; Siddiqui et al., 2003; Szoeke et al., 2009). With relatively few positive associations, and even fewer successful replications, there has been a growing acceptance that the complexities of treatment response are unlikely to be explained by a single genetic variant. Pharmacogenetic investigations in the epilepsy field now routinely include multiple genes implicated in both the pathogenesis of epilepsy and the pharmacokinetic and pharmacodynamic pathways of AEDs (Cavalleri et al., 2011; Grover et al., 2010; Johnson et al., 2011; Petrovski et al., 2009; Sánchez et al., 2010). Such studies generate a large volume of data and have necessarily precipitated an interest in analytical approaches, such as the development of machine learning algorithms, to efficiently identify patterns of genetic variants that associate with phenotypes of interest in high-dimensional data (Nicodemus and Malley, 2009).

A recent ''proof of principle'' study reported a multi-SNP classification model that predicted response to initial AED treatment in Australian patients with newly-diagnosed epilepsy. A total of 4041 SNPs from 279 candidate genes were genotyped in 115 patients and a *k*-nearest neighbour (*k*NN) machine learning algorithm was adopted to develop a classification model based on the genotype of five of these SNPs (Petrovski et al., 2009). The predictive value of the model was subsequently confirmed in two small, independent Australian cohorts.

Before this classifier can be considered for clinical use, it requires validation in larger cohorts and its reliability across populations and health care systems confirmed. The aim of the current study was to assess the wider clinical utility of the Australian multigenic *k*NN model. This was achieved by applying it to the prediction of drug responsiveness in two independent cohorts of newly-diagnosed epilepsy from the UK. The analysis also permitted exploration of the influence of the five SNPs that comprise the Australian *k*NN model in predicting treatment outcome within those UK cohorts.

Methods

Study populations

The Australian cohort in which the multigenic *k*NN model was originally derived consisted of 115 patients with newly-diagnosed epilepsy, as described previously (Petrovski et al., 2009).

The 'Glasgow' cohort consisted of 285 new-onset epilepsy patients who were diagnosed and started on their first ever AED treatment at the Epilepsy Unit, Western Infirmary, Glasgow, UK (Szoeke et al., 2009). Although ostensibly a cross-sectional out-patient clinic population, a large proportion of these individuals had participated in randomised trials of AED monotherapy. Drug response phenotypes in the Glasgow cohort were identified by retrospectively reviewing prospectively-collected clinical trial and/or hospital notes. All patients were of self-reported European ancestry and all provided informed consent for the collection and pharmacogenetic analysis of DNA. The study was approved by the West Ethics Committee, North Glasgow University Hospitals NHS Trust in September 2002 (ref: 02/119(2)).

The 'SANAD' cohort was drawn from patients who had participated in the standard and new antiepileptic drugs (SANAD) study, an un-blinded, multicentre, randomised trial comparing the clinical and cost effectiveness of established and newer antiepileptic medications in patients with newly-diagnosed epilepsy from across the UK (Marson et al., 2007a, 2007b). More than 2400 patients were enrolled in the trial and followed-up prospectively for a minimum period of one year from initiation of the first ever AED, with DNA collected from a subset of the trial population (Leschziner et al., 2006; Marson et al., 2007a, 2007b). A total of 520 SANAD participants of self-reported European ancestry were included in this analysis, all of whom provided informed consent for the collection and analysis of DNA. SANAD DNA collection was approved by the North—West Multicentre Research Ethics Committee in August 2002 (ref: MREC 02/8/45).

Phenotyping

Response to AED treatment in the Glasgow and SANAD cohorts was determined in accordance with the definitions employed in the original Australian study (Petrovski et al., 2009); these were consistent with those proposed by Kwan et al. (2010) in their seminal paper on the

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definitions of drug resistant epilepsy. Patients were considered to be ''responders'' if they remained free from seizures throughout the first 12 months of AED treatment. In contrast, patients who continued to experience unprovoked seizures during the first year of therapy despite AED treatment were considered to be ''non-responders''. In cases where the first AED was discontinued within 3 months as a result of intolerable adverse effects, the second AED was considered initial therapy for the purposes of this analysis.

Genotyping

Samples from the Glasgow cohort were genotyped for the five SNPs that comprised the Australian multigenic classifier (i.e. *SCN4B* rs658624, *SCN4B* rs678262, *GABBR2* rs2808526, *SLC1A3* rs4869682, *KCNQ1* rs2283170) at the Australian Genome Research Facility using an iPLEX Gold assay on the Sequenom MassARRAY compact analyser (Sequenom Inc., San Diego, California, USA). Of 285 Glasgow samples, four failed genotyping at one or more of the five SNPs, leaving 281 available for analysis. Sufficient clinical information and DNA was available for 520 of the SANAD cohort, with 491 successfully genotyped for each of the five SNPs in the Department of Molecular and Clinical Pharmacology, University of Liverpool on a Sequenom MassARRAY iPLEX platform (Sequenom, Hamburg, Germany), in accordance with the manufacturer's instructions (Gabriel et al., 2009).

Data analysis—Cohort comparison

Differences between the cohorts in terms of age at enrolment, sex, initial AED, epilepsy type and treatment response were assessed using ANOVA and Chi-square tests, as appropriate. Each of the five SNPs was also assessed for independent association with AED response in each of the two UK cohorts using the Cochran–Armitage test for trend.

Data analysis—kNN model

Methods for development of the Australian multigenic *k*NN classifier model have been described in detail elsewhere (Petrovski et al., 2009). Briefly, the classifier uses a training dataset of patients with known treatment responses and SNP genotypes to predict responses in an independent cohort of patients (test dataset) for whom genotypes alone are known. Each patient in the test dataset is positioned in an *N*-dimensional space (in this case $N = 5$, representing the number of SNP genotypes in the model) defined by the training dataset, with response predicted by simple majority of known treatment responses amongst its *k*-nearest neighbours (i.e. the individuals with the most similar genetic profiles at this combination of five SNPs). The number of nearest neighbours found to give optimal prediction of drug response in the original Australian cohort was $k = 9$.

The Australian multigenic *k*NN classifier model was evaluated in the UK cohorts in 3 ways: (1) using Australian patients to predict response in UK cohorts. The *k*NN model was applied using the original Australian cohort ($n = 115$) as the training dataset and each of the two UK cohorts as the test datasets; (2) re-deriving the *k*NN classifier using the SANAD cohort as both training and test datasets. The SANAD cohort was randomly stratified into training (70% of patients, $n = 343$) and test (30% of patients, $n = 148$) datasets. The *k*NN parameters were identical to those employed in the original Australian study (i.e. five SNPs [*N*] and nine nearest neighbours [*k*]) and investigators running the model were blinded to treatment responses in the SANAD test dataset; (3) testing performance of the *k*NN model using a cross-validation approach in UK cohorts. Individual patients within each of the respective UK cohorts was classified using a *k*NN model built on a ''leave-one-out'' training set comprising the remaining samples ($n - 1$) in that cohort to determine the overall performance of the Australian five-SNP model. In the Glasgow cohort, for example, which comprised a total of 281 patients, the predicted response for each individual (i.e. $n = 1$) was determined by the majority response amongst the remaining individuals (i.e. $n = 280$) who shared the most similar genotype profile and who were exposed to the same AED. Analyses were performed using both the *k*NN model and a fully-fitted logistic regression model (SAS Institute Inc., Cary, NC).

For each *k*NN model, the likelihood of predicting successful treatment outcome was determined by calculating the odds ratio (with 95% confidence intervals), positive and negative predictive values (with 95% confidence intervals), and a Fisher's exact 2-tail *p*-value. Where appropriate, *p*-values were adjusted using a Bonferroni correction to account for multiple testing.

Results

Cohort comparison

Comparison of the demographics and clinical characteristics of Glasgow and SANAD cohorts with those of the original Australian cohort revealed significant differences in the choice of initial AED (both $p < 0.0001$) and in the frequency of drug responsiveness (both $p < 0.0001$) (Table 1). There were no significant differences in epilepsy type between the Australian and Glasgow cohort but the SANAD cohort had a significantly higher frequency of unclassified epilepsy than either Australian and Glasgow cohorts ($p < 0.001$). This likely reflects the fact that epilepsy type was determined at initial presentation only in the SANAD cohort, whereas the Australian and Glasgow cohorts benefitted from the opportunity to review the diagnosis at follow-up visits. None of the five SNP genotypes employed in the multigenic model was found to be independently associated with AED response in the UK cohorts (Table 2). The significant association ($p = 0.004$ for trend) between genotype of rs2283170 and response to ''other AEDs'' in the Glasgow cohort did not survive correction for multiple testing and was derived from a sub-group of just 52 patients and was therefore considered spurious.

Using Australian patients to predict response in UK cohorts

A *k*NN model built on five SNP genotypes and drug response phenotypes from the original Australian cohort ($n = 115$) failed to predict treatment response on the basis of

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Table 1 Comparison of cohort characteristics.

			Australian ($n = 115$)	Glasgow ($n = 285$)	SANAD ($n = 491$)	p-Value
Age	Mean (±SD)		43.7 (±19)	41.7 (±14)	39.3 (±18)	ns
Sex	N (%)	Male	61 (53.0%)	157 (55.1%)	269 (54.8%)	ns
		Female	54 (47.0%)	128 (44.9%)	222 (45.2%)	
Initial AED	N (%)	CBZ	66 (57.4%)	26 (9.1%)	123 (25%)*	<0.0001
		VPA	44 (38.3%)	92 (32.3%)	50 (10.2%)	
		LTG	2 (1.7%)	115 (40.4%)	139 (28.3%)	
		Other	3 (2.6%)	52 (18.2%)	179 (36.5%)	
Epilepsy type	N	GGE	27 (23.5%)	92 (32.3%)	80 (16.3%)	<0.001†
	(%)	Focal	84 (73.0%)	185 (64.9%)	332 (67.6%)	
		UNC	4 (3.5%)	8 (2.8%)	79 (16.1%)	
Outcome at 12 months	N	Responder	128 (71.9%)	152 (53.3%)	234 (47.7%)	<0.001
	(%)	Non-responder	50 (28.1%)	133 (46.7%)	257 (52.3%)	

AED = antiepileptic drug, CBZ = carbamazepine, GGE = genetic generalised epilepsy, LTG = lamotrigine, N = number, ns = non-significant, SD = standard deviation, UNC = unclassified epilepsy, VPA = valproate.

* Includes 35 patients initially treated with oxcarbazepine.

† Statistical difference between SANAD cohort and both Australian and Glasgow cohorts. p-values have not been adjusted for multiple testing.

genotype alone in either the Glasgow ($n = 281$) or SANAD ($n = 491$) cohorts (both $p > 0.05$).

Re-deriving the *k*NN classifier using the SANAD cohort

An attempt was made to predict treatment response, in a blinded manner, in a test dataset ($n = 148$) of patients from the SANAD cohort using a re-derived *k*NN classifier based on SNP genotypes and drug responses in the SANAD un-blinded training dataset ($n = 343$). The classifier correctly identified 26 responders and 52 non-responders but incorrectly identified 26 non-responders as responders (false positives) and 44 responders as non-responders (false negatives) (Table 3). When applied only to those patients in the test dataset treated with either carbamazepine or valproate ($n = 50$), the re-derived *k*NN classifier correctly identified 10 responders and 14 non-responders but incorrectly identified 10 non-responders as responders and 16 responders as non-responders (Table 3).

Table 2 Uncorrected association of individual SNP genotypes with treatment outcome.

SNP	AED	Glasgow cohort		SANAD cohort*	
		p-Value for trend	Odds ratio (95% CI)	p-Value for trend	Odds ratio (95% CI)
rs2283170	CBZ/VPA	0.8	1.0 (0.6–1.9)	0.6	1.2 (0.7–1.8)
	LTG	0.6	1.1 (0.7–2.0)	0.2	0.7 (0.4–1.2)
	Other AED	0.004	0.3 (0.1–0.7)	0.8	1.1 (0.7–1.7)
rs2808526	CBZ/VPA	0.3	0.8 (0.4–1.3)	0.8	1.0 (0.7–1.6)
	LTG	0.4	1.3 (0.7–2.2)	0.7	0.8 (0.6–1.5)
	Other AED	0.9	1.1 (0.4–2.2)	0.3	0.8 (0.5–1.3)
rs4869628	CBZ/VPA	1.0	1.0 (0.6–1.7)	0.1	1.4 (0.9–2.1)
	LTG	0.3	0.8 (0.5–1.3)	0.3	0.8 (0.5–1.3)
	Other AED	0.8	0.9 (0.4–2.0)	0.5	1.2 (0.8–1.8)
rs658624	CBZ/VPA	0.2	1.4 (0.8–2.4)	0.5	1.1 (0.7–1.8)
	LTG	0.3	1.4 (0.8–2.3)	0.6	0.9 (0.6–1.4)
	Other AED	0.5	1.1 (0.6–2.8)	0.8	0.8 (0.5–1.3)
rs678262	CBZ/VPA	0.4	0.8 (0.5–1.4)	0.1	0.7 (0.5–1.1)
	LTG	0.07	0.6 (0.4–1.0)	0.5	1.2 (0.7–1.9)
	Other AED	0.2	0.6 (0.3–1.4)	0.3	1.3 (0.8–1.9)

AED = antiepileptic drug, CBZ = carbamazepine, CI = confidence interval, LTG = lamotrigine, SNP = single nucleotide polymorphism, VPA = valproate.

* Thirty five patients initially treated with oxcarbazepine were included in the carbamazepine group. p-Value for trend calculated by Cochran-Armitage test. Glasgow cohort numbers: CBZ/VPA = 118, LTG = 112, Other AED = 51. SANAD cohort numbers: CBZ/VPA = 173, LTG = 139, Other AED = 179. p-Values have not been adjusted for multiple testing.

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Table 3 Predictive performance of the 5-SNP *k*NN model using training ($n = 343$) and test ($n = 148$) datasets in the SANAD cohort.

AED	*n*	TP	FP	TN	FN	PPV (95% CI)	NPV (95% CI)	Odds ratio (95% CI)	*p*-Value
CBZ/VPA*	50	10	10	14	16	50% (23.7—76.3)	47% (25.2—69.4)	0.9 (0.28—2.72)	0.7
Other AED	98	16	16	38	28	50% (28.5—71.5)	58% (41.6—72.1)	1.4 (0.58—3.17)	0.3
Combined	148	26	26	52	44	50% (32.8—67.2)	54% (41.1—66.7)	1.2 (0.60—2.32)	0.4

AED = antiepileptic drug, CBZ = carbamazepine, CI = confidence interval, *k*NN = *k*-nearest neighbour, NPV = negative predictive value, PPV = positive predictive value, SNP = single nucleotide polymorphism, VPA = valproate. TP = true positive (responders correctly classified as responders), FP = false positive (non-responders incorrectly classified as responders), TN = true negative (non-responders correctly classified as non-responders), FN = false negative (responders incorrectly classified as non-responders).

* Six patients initially treated with oxcarbazepine were included in the carbamazepine group. *p*-values have not been adjusted for multiple testing.

Cross-validation analysis in UK cohorts

A ''leave-one-out'' cross validation analysis was performed in each of the two UK cohorts by predicting treatment response for each individual patient using five SNP genotypes in the remainder ($n - 1$) of the respective cohort. In the Glasgow cohort, the five SNP combination was significantly predictive of treatment response in those patients initially prescribed either carbamazepine or valproate (positive and negative predictive values of 67% and 60%, respectively, corrected $p = 0.018$) but not those prescribed lamotrigine (corrected $p = 1.0$) or other AEDs (corrected $p = 1.0$) (Table 4). This observation extended to the SANAD cohort, where the five SNP combination was again predictive of treatment response in those patients initially prescribed either carbamazepine or valproate (positive and negative predictive values of 69% and 56%, respectively, corrected $p = 0.048$) but not those prescribed lamotrigine (corrected $p = 0.36$) or other AEDs (corrected $p = 0.36$) (Table 4).

Logistic regression analysis of ''leave-one-out'' data

A fully-fitted logistic regression model supported observations from the ''leave-one-out'' analysis that these five SNPs have predictive value for treatment response in patients initially prescribed carbamazepine or valproate. A regression model incorporating all five SNPs and developed on patients ($n = 123$) within the SANAD un-blinded training dataset that initially received carbamazepine or valproate predicted successful treatment outcome with positive and negative predictive values of 69% each (corrected $p = 7.5 \times 10^{-5}$). The regression model was less powerful when developed on patients ($n = 220$) prescribed other AEDs, with positive and negative predictive values of 63% and 59%, respectively (corrected $p = 0.021$) (Table 5). A permutation test was performed to identify the likelihood of over-estimation caused by limited patient numbers ($n = 123$) treated with either carbamazepine or valproate, with results showing that randomised logistic regression models, using the same number of carbamazepine/valproate responders and non-responders, are unlikely to achieve a *p*-value $< 7.5 \times 10^{-5}$ based on these five SNP profiles by chance ($p < 0.05$).

Discussion

A substantial proportion of people with epilepsy continue to have seizures despite treatment with appropriate AEDs (Duncan et al., 2006; Kwan and Brodie, 2000). Failure to control seizures is often associated with increased physical and psychosocial morbidity, as well as an elevated risk of death (Duncan et al., 2006). Prescribing practice in epilepsy considers patient age, gender, and clinical features of the seizure disorder but is otherwise largely empirical. The identification of biological markers that provide improved prediction of treatment response in an individual patient would be of significant clinical value (Chan et al., 2011).

The majority of complex biological traits, including response to treatment in epilepsy, are most likely explained by a large number of genetic variants, each of small effect size (Goldstein, 2009; Hirschhorn, 2009; Kraft and Hunter, 2009). As a result, predictive models for complex traits require the capacity to incorporate a multitude of weak effects and to model potentially complex interactions amongst them. This is achieved by the *k*NN approach, which was originally described by Fix and Hodges (1989) and has since become an important classification and clustering tool with diagnostic applications in a number of medical research fields, including cancer, immunoassay based anti-nuclear antibody tests, microarray experiments, drug toxicity, and rheumatoid arthritis (Binder et al., 2005; Crimins et al., 2005; Kim et al., 2004; Liu et al., 2009; Martin et al., 2006).

We assessed the broader utility of the Australian multi-SNP model by applying it to two independently collected cohorts of patients with newly treated epilepsy from the UK. In both Glasgow and SANAD patients, the multigenic classifier failed to predict response to the first well-tolerated AED when the original Australian cohort was used as the training dataset. The classifier also failed when the training dataset was partially re-defined using the SANAD cohort. Thus in terms of the primary aim of this study, direct validation of the classifier was unsuccessful—its predictive capacity did not directly translate from one population to another. Possible explanations for this include population differences, differences in phenotypic definitions and methods of ascertainment, failure to re-calibrate the *k*NN parameters, or a false positive signal in the original study.

To explore potential population differences, we interrogated both Glasgow and SANAD cohorts using a cross-validation ''leave-one-out'' approach. Each of the UK

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Table 4 Predictive performance of the 'leave-one-out' *k*NN approach in UK cohorts.

Cohort	AED	*n*	TP	FP	TN	FN	PPV (95% CI)	NPV (95% CI)	Odds ratio (95% CI)	*p*-Value[†]
Glasgow	CBZ/VPA	118	49	24	27	18	67% (51.8–79.6)	60% (40.6–76.8)	3.1 (1.4–6.6)	0.018
	LTG	112	35	30	25	22	54% (38.0–69.0)	53% (34.8–70.8)	1.3 (0.6–2.8)	1.0
	Other AED	51	15	17	8	11	47% (25.9–68.9)	42% (17.6–70.8)	0.6 (0.2–2.0)	1.0
SANAD	CBZ/VPA[*]	123	29	13	45	36	69% (48.6–84.3)	56% (41.3–69.0)	2.8 (1.3–6.1)	0.048
	LTG	97	18	12	40	27	60% (36.4–80.0)	60% (43.8–73.9)	2.2 (0.9–5.3)	0.36
	Other AED	123	21	17	52	33	55% (34.6–74.3)	61% (47.1–73.7)	1.9 (0.9–4.2)	0.36

AED = antiepileptic drug, CBZ = carbamazepine, CI = confidence interval, *k*NN = *k*-nearest neighbour, LTG = lamotrigine, NPV = negative predictive value, PPV = positive predictive value, VPA = valproate. TP = true positive (responders correctly classified as responders), FP = false positive (non-responders incorrectly classified as responders), TN = true negative (non-responders correctly classified as non-responders), FN = false negative (responders incorrectly classified as non-responders).

[*] Twenty nine patients initially treated with oxcarbazepine were included in the carbamazepine group.

[†] *p*-Values have been adjusted using a Bonferroni correction to account for multiple testing.

cohorts acted as their own training dataset and the analysis was stratified by initial AED to take account of differences in drug use between UK and Australian patients. The ''leave-one-out'' analysis indicated that the five SNPs had a collective predictive capacity for both Glasgow and SANAD patients treated with either carbamazepine or valproate but not other AEDs. This is consistent with the Australian patients in whom the classifier was originally derived having been predominantly treated with carbamazepine or valproate (>95% of patients) and, unlike the UK cohorts, had very little lamotrigine use (<2% of patients). A subsequent permutation test confirmed that randomised fully fitted logistic regression models developed on the SANAD training dataset would have been unlikely to achieve a *p*-value $<7.5 \times 10^{-5}$ based on the five SNP profiles, further suggesting that the association with carbamazepine or valproate treatment response was unlikely to have occurred by chance. On that basis, it is reasonable to conclude that although the classifier does not directly translate from one population to another, the five SNPs originally identified in an Australian population of newly treated epilepsy might be genetic markers of response to treatment with either carbamazepine or valproate in the UK populations. Further studies on newly treated cohorts will help to better understand these associations; those studies should be stratified by drug to ensure that differences in AED use are accounted for.

The results of the ''leave-one-out'' cross validation supports a biological significance of these five SNPs. They comprise two SNPs in the *SCN4B* gene (encoding the β_4 subunit of the voltage-gated sodium channel) and one each in the *GABBR2* gene (encoding $GABA_B$ receptor subunit 2), *KCNQ1* gene (encoding the $K_v7.1$ subunit of the delayed rectifier potassium channel), and *SLC1A3* gene (encoding excitatory amino acid transporter 1). None of the SNPs is exonic or in strong ($r^2 \geq 0.8$) linkage disequilibrium with any known protein-coding variant and while two of the genes (*SCN4B* and *KCNQ1*) have only limited expression in normal brain tissue (Waldegger et al., 1999; Yu et al., 2003), we cannot rule out the possibility that they are over-expressed in epileptic brain. Further bioinformatic analysis using online tools (Kent et al., 2002; Yuan et al., 2006) revealed that all SNPs except rs678262 are in transcription factor binding domains but only rs2283170 was predicted to influence transcription factor binding characteristics. Thus, the functional significance of these SNPs remains unclear. Other than the fact that they exist within epilepsy candidate genes, we do not have a satisfactory explanation for their possible association with treatment response in newly-diagnosed epilepsy. Likewise, why they appear to preferentially predict response to carbamazepine and valproate but not other AEDs, such as lamotrigine, remains unexplained. There is nothing in our current understanding of the mechanisms of action of these drugs that would be helpful in understanding this association.

The failure to observe a direct replication of the predictive capacity of the *k*NN classifier may have been the result of differences in phenotype, inclusion criteria, ethnicity (15% of the Australian cohort was of non-Caucasian ancestry), and methods of ascertainment. Although all patients included in this analysis had new-onset epilepsy, the environments in which they were recruited and followed-up were different. These differences may have introduced

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Table 5 Predictive performance of the fully fitted logistic regression model in the SANAD training dataset.

AED	*n*	TP	FP	TN	FN	PPV (95% CI)	NPV (95% CI)	Odds ratio (95% CI)	*p*-Value[†]
CBZ/VPA[*]	123	49	22	36	16	69% (53.5–81.3)	69% (50.9–83.2)	5.0 (2.3–10.9)	7.5×10^{-5}
Other AED	220	26	15	106	73	63% (42.9–80.2)	59% (49.6–68.2)	2.5 (1.2–5.1)	0.021
Combined	343	66	42	137	98	61% (48.6–72.3)	58% (49.9–66.2)	2.2 (1.4–3.5)	0.0018

AED = antiepileptic drug, CBZ = carbamazepine, CI = confidence interval, NPV = negative predictive value, PPV = positive predictive value, VPA = valproate. TP = true positive (responders correctly classified as responders), FP = false positive (non-responders incorrectly classified as responders), TN = true negative (non-responders correctly classified as non-responders), FN = false negative (responders incorrectly classified as non-responders).

[*] Twenty nine patients initially treated with oxcarbazepine were included in the carbamazepine group.

[†] *p*-Values have been adjusted using a Bonferroni correction to account for multiple testing.

inconsistencies in the classification of responder and non-responder status and might also explain the significantly greater frequency of non-response in the Glasgow and SANAD cohorts. For example, a sizeable proportion of the Glasgow cohort and all of the SANAD cohort were participants in randomised trials, which are associated with greater attrition and typically report lower rates of responders than observational studies. Nonetheless, significant differences in response rates between training and tests datasets was arguably the most significant confounder in efforts to directly validate the classifier, particularly when coupled with failure to re-calibrate the *k*NN parameters to accommodate it.

Other explanations for the differences in response frequencies between the Australian and UK cohorts include diagnostic rigour, local policies on treatment initiation, and heterogeneity in drug use and dosing. These issues apply to all pharmacogenetic investigations in newly-diagnosed epilepsy and are not restricted to this analysis. Accordingly, the senior authors of this article are closely involved in several international epilepsy genetics efforts (i.e. EpiPGX (http://www.epipgx.eu/), EPI4K (http://www.epgp.org/epi4k/), and the ILAE Consortium on Complex Epilepsies), all of which recognise the importance of standardising inclusion criteria and phenotypic definitions for epilepsy pharmacogenetic studies.

Another potential confounder was the use of *k*NN parameters reported in the original Australian study and derived from a population of patients that was significantly smaller than either of the two UK cohorts. Failure to re-calibrate the *k*NN parameters to accommodate larger training datasets with differing response frequencies may have impacted on the accuracy of prediction. In the *k*NN classifier, the number of nearest neighbours (*k*) is dependent on the size and phenotype frequency of the training dataset and prediction is based on a simple majority phenotype amongst those nearest neighbours. In a larger training dataset, it is possible that fewer nearest neighbours would be required for accurate prediction. A future strategy which incorporates re-calibration of the *k*NN parameters, preferably coupled with genome-wide SNP and next generation sequencing data, might prove to have greater predictive utility from one population to another. Adjusting for seizure type, syndrome and aetiology (should numbers allow) might also improve likelihood of success.

Finally, the possibility that the original report was a false positive seems unlikely, given the association between 5-SNP genotype and response to treatment with either carbamazepine or valproate in UK cohorts using both ''leave-one-out'' and fully-fitted logistic regression approaches. This finding, together with validation in two independent Australian cohorts reported in the original study, lends weight to the significance of these SNPs as biomarkers of response to initial drug therapy in newly-diagnosed epilepsy. Whether the classifier is truly specific for carbamazepine and valproate alone or indicative of treatment responsiveness in general remains to be determined.

Conclusions

Our results indicate that the ''proof of concept'' *k*NN model, developed in an Australian cohort of newly-diagnosed epilepsy, is not directly applicable to other epilepsy populations. Nevertheless, the five SNPs reported in the original study do appear to have a collective influence in predicting response to treatment with either carbamazepine or valproate in the UK patients tested here. This observation requires additional replication attempts with larger cohort sizes to better understand the reported relationship between this multi-SNP combination and early seizure control in new-onset epilepsy patients.

Conflict of interest statement

MJB is a consultant for Eisai Ltd., has received research support from Eisai Ltd., GSK and UCB Pharma, has received honoraria for speaking engagements on behalf of Eisai Ltd., GSK, Medtronic, Novartis, Sanofi and UCB Pharma, and has received support for conference attendance from UCB Pharma. MRJ has received honoraria for speaking engagements on behalf of Eisai Ltd., GSK and UCB Pharma and has received support for conference attendance from GSK. AGM has received research support from Pfizer, has received honoraria for speaking engagements on behalf of GSK and Sanofi, and has received support for conference attendance from Eisai Ltd., GSK, Janseen-Cilag, Pfizer, and UCB Pharma. TJO has received honoraria for speaking engagements on behalf of Sanofi, SciGen, and UCB Pharma. GJS has received honoraria for speaking engagements on behalf of Eisai Ltd., GSK and UCB Pharma and has received support for conference attendance from GSK and UCB Pharma. The remaining authors report no conflicts of interest.

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