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ORIGINAL ARTICLE

Nicotinic receptor abnormalities as a biomarker in idiopathic generalized epilepsy

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Abstract

Purpose Mutations of cholinergic neuronal nicotinic receptors have been identified in the autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), associated with changes on PET images using [^{18}F]-F-85380-A (F-A-85380), an α4β2 nicotinic receptor ligand. The aim of the present study was to evaluate potential changes in nicotinic receptor availability in other types of epilepsy.

Methods We included 34 male participants, 12 patients with idiopathic generalized epilepsy (IGE), 10 with non-lesional diurnal focal epilepsy, and 12 age-matched healthy controls. All patients underwent PET/CT using F-A-85380 and [^{18}F]-fluorodeoxyglucose (FDG), 3D T1 MRI and diffusion tensor imaging (DTI). F-A-85380 and FDG images were compared with the control group using a voxel-wise (SPM12) and a volumes of interest (VOI) analysis.

Results In the group of patients with IGE, the voxel-wise and VOI analyses showed a significant increase of F-A-85380 ratio index of binding potential (BP_{RI}, corresponding to the receptor availability) in the anterior cingulate cortex (ACC), without structural changes on MRI. At an individual level, F-A-85380 BP_{RI} increase in the ACC could distinguish IGE patients from controls and from patients with focal epilepsy with good accuracy.

Conclusions We observed focal changes of density/availability of nicotinic receptors in IGE, namely an increase in the ACC. These data suggest that the modulation of α4β2 nicotinic receptors plays a role not only in ADNFLE, but also in other genetic epileptic syndromes such as IGE and could serve as a biomarker of epilepsy syndromes with a genetic background.

Keywords Nicotinic receptors · Focal epilepsy · Idiopathic generalized epilepsy · PET · F-A-85380

Introduction

Changes in neuronal nicotinic acetylcholine receptors (nAChRs) have been identified in a form of familial focal epilepsy, the autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (now called sleep-related hypermotor epilepsy), characterized by the occurrence of hypermotor seizures during sleep [1]. The nAChRs participate in many physiological functions, namely neuronal excitability and release of neurotransmitters. Nine different nAChR subunits exist in the mammalian brain, building homo- or hetero-pentameric receptors, and are functionally diverse. In the brain, the predominant functional subtypes are composed of either α7 subunits or both α and β subunits, including the α4β2 and α3β4 subtypes [2]. The α4β2 nAChRs are widely distributed throughout the brain, particularly in the thalamus and brainstem/cerebellum, in the whole cortex and basal ganglia, and play major roles in nicotine addiction and cognition, in addition to their known involvement in congenital epilepsy

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[2–5]. The α7 nAChR is the other isoform widely expressed in the brain, associated with many neuropsychiatric diseases including schizophrenia [2, 5]. Most of the α4β2 receptors are presynaptic autoreceptors or heteroreceptors playing a neuromodulator role by increasing the release of various neurotransmitters, while others are postsynaptic and mediate fast excitatory synaptic transmission [6]. Despite in vitro electrophysiological data demonstrating a gain of function of the mutated receptors, the mechanisms leading to ADNFLE are still unknown. The brain distribution of nAChRs has been studied by positron emission tomography (PET) using [^{18}F]-fluoro-A-85380 (F-A-85380), a high affinity tracer for α4β2 nAChRs, in a group of eight ADNFLE patients who carried a nAChR mutation, whether in the α4 or the β2 subunit [7]. It showed a significant increase of nAChR volume of distribution (Vt) in the midbrain and the cerebellum in ADNFLE patients versus a group of control subjects, confirmed by statistical parametric mapping (SPM) analysis. Moreover, a decreased density in the right dorsolateral prefrontal region was also observed in ADNFLE patients [7]. These results suggested that the increase in nAChR density in the midbrain could be involved in the pathophysiology of ADNFLE through the role of brainstem cholinergic systems in the ascending arousal system.

The aim of the present study was i) to verify whether the increase of nAChRs observed in ADNFLE patients was specific to this form of epilepsy and not a common feature to all epilepsies and ii) to evaluate potential changes in nAChR cerebral distribution in other epilepsy syndromes, given the neuromodulatory role of these receptors. Indeed, some polymorphisms in nAChR subunit genes have been associated with other forms of epilepsy. For instance, polymorphisms in the *CHRNA4* gene coding for the nAChR α4 subunit were reported in idiopathic (genetic) generalized epilepsies (IGE) [8]. Therefore, a genetic molecular nAChR defect may also contribute to IGE pathogenesis. To test the hypothesis that alteration of nAChR cerebral distribution is common to all forms of epilepsy or, alternatively, directly related to a genetic molecular defect, we studied the cerebral distribution of nAChRs (i) in a group of patients with IGE and (ii) in a group of patients with non lesional focal epilepsy. We compared these two groups to age-matched healthy volunteers ("control group").

Methods

Patient population

We studied three groups of non-smoking male subjects: 12 patients with idiopathic generalized epilepsy (IGE group or "genetic generalized epilepsy" according to the new classification [9]) (mean age ± SD: 34.1 ± 8.7 years; range: 18-51), 10 patients with non-lesional diurnal focal epilepsy (focal epilepsy group or "focal epilepsy with unknown cause" according to the new classification [9]) (mean age ± SD: 37.9 ± 10.5; range: 24-56), and 20 age-matched healthy volunteers (control group) (mean age ± SD: 35.9 ± 9.1, range: 18-51), among whom 12 were studied with F-A-85380 (mean age ± SD: 34.2 ± 9.9, range: 18-51) and eight with [^{18}F]-fluorodeoxyglucose (FDG) (mean age ± SD: 38.5 ± 7.6, range: 27-51) (Table 1). Exclusion criteria were the consumption of tobacco or of any drug of abuse during the last 12 months, a neurological disorder other than epilepsy, a psychiatric disorder, a lesion on brain MRI, a sleep disorder, high blood pressure, heart or arterial disorder, asthma, renal or hepatic failure, hyperthyroidism, type 1 diabetes or severe dyslipidemia. The IGE group included only patients with genetic generalized epilepsies of adolescence-adult onset: five patients with juvenile absence epilepsy (JAE), two patients with juvenile myoclonic epilepsy (JME) and five patients with epilepsy with generalized tonic-clonic seizures alone ("GTCS only"). All five patients with JAE had GTCS in addition to absence seizures. The clinical characteristics of the patients with IGE and with focal epilepsy are presented in Table 1.

All 22 patients had at least one interictal EEG in their medical file. All the patients underwent PET/CT imaging using F-A-85380, PET/CT using FDG, and 3D T1 MRI for volumetric analyses. The healthy volunteers underwent 3D T1 MRI, and a PET/CT imaging using either F-A-85380 or FDG.

No seizures were reported in the patients within the week before the F-A-85380 or the FDG PET examination.

All procedures performed in this study were in accordance with the Swiss ethical standards and with the 1964 Helsinki Declaration and its later amendments; the study protocol was approved by the ethics committee of the Geneva University Hospitals (CER 10-041) and by the Swiss agency for medications (Swissmedic: study n°2011DR1031). The study was recorded in ClinicalTrials.gov (n° NCT03268369). Written informed consent was obtained from all participants.

Imaging studies

MRI acquisition, processing and analysis

MR images were obtained using a 3 T Siemens Prisma MRI scanner (Erlangen, Germany) in all the individuals. The essential imaging parameters are as follows: 3D T1 MPRAGE: sagittal acquisition, 176 slices, voxel size 1x1x1 mm3, TE 1.94 ms, TR 2300 ms, 1 average. DTI acquisition: 30 diffusion directions, b = 1000 s/mm^2 isotropically distributed on a sphere, 1 reference b = 0 s/mm^2, 64 slices, voxel size 2x2x2 mm3, TE 84 ms, TR 8800 ms, 1 average.

A grey matter voxel-based morphometry (VBM) analysis [10] was carried out using the FSL software package (http://www.fmrib.ox.ac.uk/fsl/), according to the standard procedure described in details before [11]. We performed a whole brain

Table 1 Clinical data of the patients in the group of patients with non lesional focal epilepsy and in the group of patients with idiopathic generalized epilepsy (IGE). L, left; R, right. AED, antiepileptic drug; d, day; GTCS, generalized tonic-clonic seizures; JAE, juvenile absence epilepsy; JME, juvenile myoclonic epilepsy; szs, seizures; y, years. LTG, lamotrigine; CBZ, carbamazepine; LVT, levetiracetam; OXC, oxcarbazepine; VPA, valproic acid; LCS, lacosamide; TPM, topiramate; PB, phenobarbital; PHT, phenytoin

Patient (age)	Age at onset	Type of epilepsy	GTCS occurrence	Current AED treatment	Previous AEDs	Seizure outcome
Focal epilepsy						
201 (39 y)	34 y	Temporal (L)	Yes	LTG 200 mg/d	VPA	No more seizures
202 (42 y)	30 y	Lateral temporal	Yes	CBZ 400 mg/d	VPA, LVT	No more seizures
203 (57 y)	14 y	Temporal (R)	Yes	LTG 200 mg/d	VPA	1 nocturnal GTCS /week
204 (29 y)	22 y	Frontal (R)	Yes	LVT 1500 mg/d	CBZ, LVT	1 GTCS/year
206 (28 y)	25 y	Temporal	Yes	OXC 1800 mg/d	VPA, LTG, LCS	1-2 nocturnal GTCS/month Rare focal diurnal szs
207 (32 y)	28 y	Lateral temporal (L)	Yes	CBZ 400 mg/d	LVT	No more seizures
208 (33 y)	5 y	Temporal	Yes	None	CBZ, TPM, LVT	No more seizures
209 (49 y)	40 y	Frontotemporal (L)	Yes	VPA 1000 mg/d	–	No more seizures
210 (24 y)	20 y	Occipital?	Yes	VPA 1000 mg/d (bad compliance)	–	1 focal secondarily generalized/year
212 (46 y)	43 y	Temporoinsular (ecstatic szs)	Yes (only one)	None	–	1 focal seizure/month
IGE						
301 (28 y)	17 y	GTCS only	Yes	LTG 300 mg/d	–	4 GTCS/year
302 (29 y)	13 y	JAE	Yes	VPA 1000 mg/d	–	1 GTCS/year
303 (31 y)	15 y	JME	Yes	LVT 750 mg/d	VPA	No more seizures
305 (29 y)	15 y	JAE	Yes	VPA 1500 mg/d	LVT, LTG	3 GTCS/year
306 (31 y)	23 y	JME	Yes	VPA 1000 mg/d	–	1 GTCS/year
307 (40 y)	33 y	GTCS only	Yes	VPA 1000 mg/d	–	No more seizures
308 (38 y)	12 y	JAE	Yes	CBZ 600 mg/d	PB	1 GTCS/year; absences
309 (19 y)	15 y	JAE	Yes	LTG 200 mg/d	PHT	1 GTCS/year
310 (52 y)	14 y	GTCS only	Yes	VPA 1000 mg/d	–	No more seizures
311 (36 y)	14 y	JAE	Yes	VPA 1000 mg/d	–	No more seizures
312 (33 y)	27 y	GTCS only	Yes	VPA 1500 mg/d + LVT 1000 mg/d	–	1 GTCS/year
313 (45 y)	26 y	GTCS only	Yes	VPA 1000 mg/d + LVT 2000 mg/d	–	1 GTCS/year

analysis, as well as an analysis of our volumes of interest (VOI), based on the results of the PET analysis, i.e. the anterior cingulate cortex, using the Harvard-Oxford Cortical Structural Atlas implemented in FSL. This second analysis was performed to increase the sensitivity to detect VBM changes in the regions with significant F-A-85380 changes, in order to exclude any impact of volumetric changes on the changes we observed on PET imaging. Voxel-wise GLM was applied using permutation-based non-parametric testing, with threshold-free cluster enhancement (TFCE) correction for multiple comparisons, following standard procedures [12], considering fully corrected p-values <0.05 as significant.

The DTI data were analysed with the Brainance software package (Advantis Medical Imaging, Eindhoven, the Netherlands) using a fractional anisotropy (FA) threshold of 0.15 and an angle threshold of 27° for the fiber tracking process. We analyzed the regions showing significant changes in the group analyses of F-A-85380 (see the following), namely the ACC and the insula, given the relevance of anatomic cingulo-insular connections. In short, the pre-defined anatomic VOI of the insula and ACC, as defined in the Automated Anatomic Labelling (AAL) atlas, were back-projected into the individual space through non linear coregistration to each subject's T1 weighted scan using MNI as an intermediate space. Then, average summary statistics were acquired into the VOI including mean and standard deviation (SD) of FA, RD (radial diffusivity), MD (mean diffusivity). In a second step, tractography was performed between ACC and insula VOI for each hemisphere, and parameters including fiber length and FA were calculated along the fiber tracts. All VOI and fiber tracking results were compared using un-paired parametric t-tests implementing Bonferroni correction for multiple comparison.

PET acquisition and processing

All PET scans were performed on a Siemens Biograph PET/CT tomograph, using an acquisition protocol previously validated against full quantification in a subsample of subjects [7, 13].

Each subject received 200 MBq (mean: 205 ± 9 MBq, range: 187-219) of F-A-85380, as established in previous studies [7, 13]. Prior to the tracer injection, a sample of venous blood was taken, and the ratio of parent compound free and protein-bound was measured for each subject. In order to minimize differences in the activation state of the cholinergic system, all subjects were kept in a comparable resting awake condition between the injection and the PET acquisition that started 3 h later and lasted 1 h. During PET acquisition, six venous plasma samples were drawn and radioactivity counted in a cross-calibrated gamma-counter. Unchanged radiotracer fraction was measured in the plasma using solid-phase extraction. The radioactivity due to unchanged F-A-85380 was expressed as a fraction of the total radioactivity found in the eluted samples. We calculated volume of distribution (Vt)

parametric images at 210-240 min post injection with respect to the free fraction of unmetabolized F-A-85380.

We obtained parametric images of the specific uptake ratio of different regions with respect to the corpus callosum, coined "ratio index of binding potential (BP_{RI})" as in a previous paper and computed with the established formula: [BP_{RI} brain region = (Vt brain region/Vt corpus callosum) - 1] [14]. This simplified approach to calculate BP_{RI} as outcome has been adopted in many clinical studies previously [14–17].

FDG PET dynamic images were acquired over 60 min starting with the administration of 200 MBq of FDG and two venous samples were collected at 35 and 45 min post-injection. Vt parametric images were calculated over the last 30 min, as previously described [7]. Regional metabolism proportionally scaled to global activity was compared as described in the following.

PET image processing was done using statistical parametric mapping software SPM12, implemented on a MATLAB platform (Mathworks, Inc).

Parametric PET images were coregistered to individual T1 MR images and then normalized applying the normalization parameters calculated by the unified segmentation/normalization of MRI into the Montreal Neurological Institute (MNI) space. Spatially normalized images were smoothed with an 8-mm full-width-at-half-maximum Gaussian filter.

F-A-85380 and FDG PET statistical analyses

Voxel-wise analysis The groups of patients were compared with the group of controls using unpaired two-sample t-tests at the whole brain level, using a non-parametric permutation/randomisation approach as implemented in the Statistical nonParametric Mapping toolbox of SPM (SnPM13) [18]. We set the threshold for significance at the voxel-wise p value at peak level of 0.05, applying family-wise error (FWE) correction. A small volume correction (SVC) was also performed within the volumes of interest (VOI) showing significant group differences, as detailed in the following paragraph.

Volumes of interest (VOI) analysis We analyzed parametric PET images (F-A-85380 BP_{RI} and FDG Vt) in standardized VOI, as implemented in the WFU pickatlas (v.2.4) toolbox of SPM, originally developed for functional MRI data and extensively used for regional analyses of PET data [19]. Specifically, we included 45 cortical and subcortical regions of the AAL atlas in each hemisphere (the complete list of regions included is provided at http://neuro.imm.dtu.dk/wiki/Automated_Anatomical_Labeling), plus the pons, the midbrain, the medulla, the anterior and posterior cerebellum, and the corpus callosum. We compared regional values averaged between the two hemispheres (given the significant correlation between homologous left and right regions for F-A-85380 data) among the three clinical groups by a Kruskal-Wallis test, reporting significant ($p < 0.05$) increases or decreases in IGE patients and focal epilepsy patients, compared pairwise to the control group, applying Dunn-Bonferroni correction for the post hoc pairwise analyses (IBM SPSS Statistics, v.22). We also computed a global cortical BP_{RI}, as the mean value weighted for the size of the VOI, to test the association of the global cortical BP_{RI} with age across the whole population and with disease duration and medication type in the patient population.

When mean F-A-85380 BP_{RI} values differed between groups, we also tested the ability of regional changes, to discriminate one group of patients from the other group of patients and from the group of controls, in order to estimate the validity of these measurements as biomarker, using a receiver operating curve (ROC) approach (IBM SPSS Statistics, v.22).

When mean F-A-85380 BP_{RI} values were significantly different from controls in a group of patients, we tested its association with clinical parameters (disease duration, type of medication and age).

Finally, in the group of healthy controls, we tested the differential distribution of nAChRs across cortical regions to replicate previously published findings of a richer cortical density in the cingulo-insular network, using the same median polish approach [20].

Results

MRI

The VBM analyses showed no TFCE-corrected supra-threshold group differences.

The DTI analysis showed no difference neither in the VOI analysis (bilateral ACC and insula) nor in the tractography (number or length of fibers between ACC and insula or FA) between the IGE group and the control group.

F-A-85380 PET analyses

Voxel-wise analysis

In the group of IGE patients, the voxel-wise SnPM analysis revealed a significant ($p < 0.05$, FWE corrected) increase of F-A-85380 BP_{RI} (corresponding to the receptor density) in the left anterior cingulate cortex (ACC), which, when applying SVC, became bilateral (Fig. 1 and Table 2). Importantly, volumetric analysis did not show any significant change in the ACC volume in the group of IGE patients compared with the control group (as previously mentioned).

In the group of patients with focal epilepsy, no significant changes of F-A-85380 tracer uptake were observed at whole brain level.

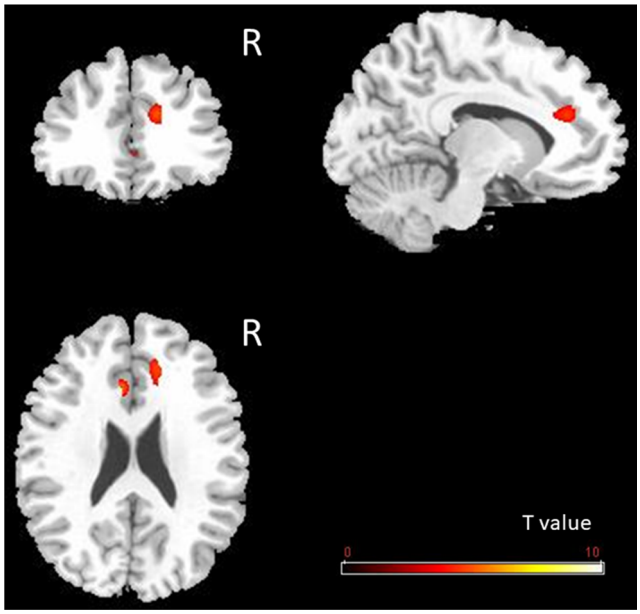

Fig. 1 Nicotinic receptor (nAChR) density increase in idiopathic generalized epilepsy (IGE). Voxel-wise analysis of F-A-85380 hyperfixation in IGE patients (patients $n = 12$, controls n = 12), showing a significant increase in the group of IGE patients in the anterior cingulate cortex (ACC), bilaterally, overlaid on a standard MR template (see text for details). The color bar indicates the T-value, values shown are above 3.73 ($p < 0.05$, FWE corrected). R indicates the right hemisphere

Volumes of interest (VOI) analysis

The regional analysis confirmed a significant increase in F-A-85380 BP_{RI} in the ACC (average of the two hemispheres) and to a lesser degree an increase in the putamen in the IGE group compared with the control group. No significant changes were observed in the group of patients with focal epilepsy (Table 3).

Within the IGE group, the different subgroups (JAE, JME and GTCS only) all showed the tendency to a higher F-A-85380 BP_{RI} value in the ACC (Fig. 2).

The ROC analysis showed that F-A-85380 BP_{RI} in the ACC could discriminate patients with IGE from controls and from patients with focal epilepsy with an area under the curve (AUC) of 0.823 ($p = 0.007$, 95% CI: 0.651-0.995) and of 0.825 ($p = 0.01$, 95% CI: 0.636-1), respectively. A cut-off value of 0.32 had a sensitivity of 92% and a specificity of 67% to discriminate IGE from controls and a sensitivity of 92% and a specificity of 70% to discriminate IGE from patients with focal epilepsy (Fig. 3).

There was no association between global cortical F-A-85380 BP_{RI} or BP_{RI} in the ACC and age ($p = 0.121$ and $p = 0.226$, respectively).

There were no associations between global cortical F-A-85380 BP_{RI} or BP_{RI} in the ACC and disease duration or type of medication in the patient population (all $p > 0.5$).

The analysis of the cortical distribution of nAChRs in healthy controls confirmed that the regions showing the highest cortical BP_{RI} were the insula (median BP_{RI}: 0.28) and the anterior and middle cingulate cortex (median BP_{RI}: 0.31), the posterior cingulate cortex (median BP: 0.16), the Heschl gyrus (median BP_{RI}: 0.22) and the hippocampus (median BP_{RI}: 0.32), significantly higher than the median cortical BP_{RI} (0.11), as previously reported [20].

FDG PET analyses

Voxel-wise analysis

No significant changes were observed at $p < 0.05$, FWE corrected.

Volumes of interest (VOI) analysis

The regional analysis showed no significant region-wise changes in the IGE group. In the group with focal epilepsy, a significant increase in glucose metabolism was detected in the lateral superior frontal cortex, in the medial frontal cortex and in the supplementary motor area, compared with the control group.

Discussion

Our present study revealed a statistically significant higher nAChR density in the ACC in IGE patients when compared with healthy age-matched individuals or with patients with non lesional diurnal focal epilepsy. This pattern was different from what we reported previously in ADNFLE patients [7]. The present data, therefore, suggest that the pattern observed in ADNFLE was specific and not related to a mechanism shared by all forms of epilepsy. In addition, regions of decreased nAChR density (and parallel hypometabolism) in ADNFLE

Table 2 Results of the whole brain voxel-wise analysis of F-A-85380 ratio index of binding potential (BP_{RI}) in the group of patients with idiopathic generalized epilepsy (IGE patients), compared with controls, at family wise error (FWE) correction at $p < 0.05$ (see text for details)

Cluster	x	y	z	T	*p* value	Cluster extent
Left anterior cingulate gyrus	−8	24	22	6.02	0.003	45
Right anterior cingulate gyrus	14	36	22	5	0.008	99

Table 3 VOI analysis. Mean ratio index of F-A-85380 binding potential (BP_{RI}) with standard deviations for the regions differing significantly in idiopathic generalized epilepsy (IGE) and focal epilepsy, compared with controls. *significant increase, compared with the control group at post hoc Kruskal-Wallis analysis, corrected for multiple pairwise comparisons

Region	Controls	IGE	Focal epilepsy	*p*
Anterior cingulate cortex	0.31 ± 0.06	0.38 ± 0.06*	0.30 ± 0.07	0.009
Putamen	0.50 ± 0.09	0.60 ± 0.109*	0.51 ± 0.10	0.020

patients were not found in the two groups of patients in the present study.

A possible role of the ACC in the generation of generalized seizures?

Accumulated evidence during the last 15 years has favored a "cortical hypothesis" of IGE, where the thalamus is rather secondarily involved and plays a role of rhythm generator in the thalamocortical network [21]. The concept that spike and wave (SW) discharges are initiated in a cortical onset zone has been increasingly accepted in animal models and was particularly studied in two genetic models of absence epilepsy in the rat, the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and the WAG-Rij [22, 23]. In both models, electrophysiological and fMRI data demonstrated that SW discharges are initiated in the perioral region of the somatosensory primary (S1) cortex [24–28]. In the GAERS, intracellular electrophysiological recordings of neurons in the different layers of S1 suggested that pyramidal cells of the deep layers trigger SW discharges [22, 27]. From the S1 cortex, SW discharges rapidly spread to the motor cortex and the ventrobasal thalamus within less than 1 s while a sustained interplay between the cortex and the thalamus may participate in the maintenance of the SW discharges [22, 27].

In line with these experimental data, EEG discharges in patients with absence seizures or JME were shown to first occur in mesial frontal and orbital frontal cortical regions and to secondarily diffuse to the rest of the cortex and the thalamus [29, 30]. The mesiofrontal regions, including the ACC, are known to play a role in the spreading of epileptic discharges [31]. A frontal cortical onset was also suggested in childhood absence epilepsy by a magnetoencephalographical and an fMRI study, respectively [32, 33]. Neuroimaging volumetric studies have also supported focal alterations in IGE. Controversial results of morphometric analyses are reported in the literature, with either a reduction in gray matter volume in the supplementary motor area and posterior cingulate cortex [34], in the ACC alone [35], or an increased gray matter volume in mesiofrontal cortical structures [36, 37] including the ACC [38], associated to a bilateral reduction in thalamic volume [37, 38].

Functional MRI studies in IGE have also shown changes in the connectivity of the ACC, yet also contradictory. A reduced

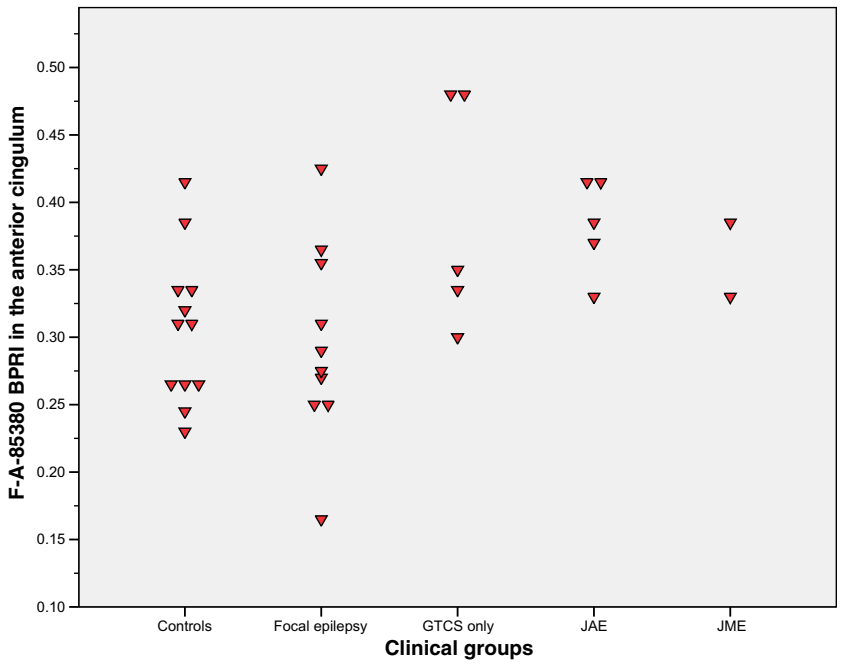

Fig. 2 F-A-85380 binding potential in clinical groups. Scatterplot of the F-A-85380 ratio index of binding potential (BP_{RI}) in the anterior cingulate cortex in controls, patients with focal epilepsy and in the three subgroups of idiopathic generalized epilepsy (IGE), namely patients with epilepsy with generalized tonic-clonic seizures alone (GTCS only, five patients), with juvenile absence epilepsy (JAE, five patients), and with juvenile myoclonic epilepsy (JME, two patients). The dotted line represents the BP_{RI} value (0.32) best discriminating patients with IGE from controls or patients with focal epilepsy (see Results for details)

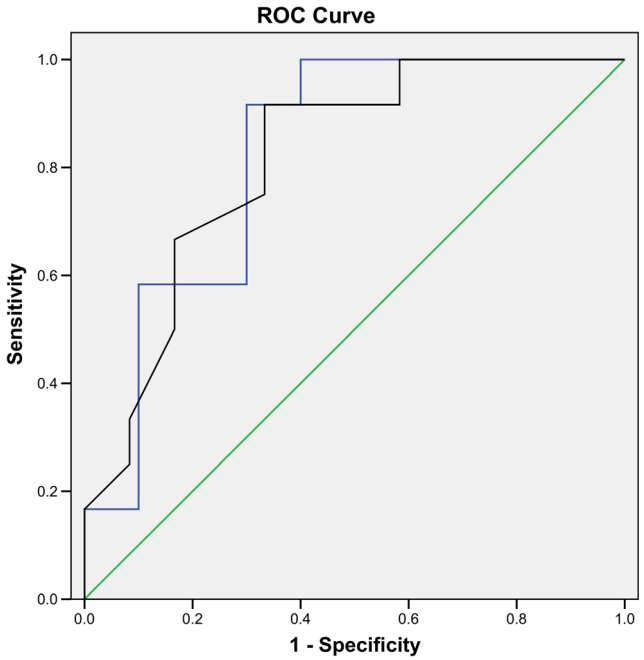

Fig. 3 Discriminating ability of nicotinic receptor (nAChR) density in the ACC for the different clinical groups. Receiver operating curve (ROC) analysis of F-A-85380 binding potential (BP_{RI}) in the anterior cingulate cortex (ACC) to discriminate patients with idiopathic generalized epilepsy (IGE) from control individuals [black line, area under the curve (AUC) 0.823] and from patients with focal epilepsy (blue line, AUC 0.825), differing significantly from the diagonal line of no-discrimination (green)

functional connectivity was shown between the ACC and the primary and secondary somatosensory cortex in JME patients [39]. On the contrary, an increased connectivity was reported between the bilateral ACC and other cortical regions and the thalamus and putamen in another study of patients with IGE including GTCS, suggesting a functional reorganization [31]. An increased structural and functional connectivity between the mesiofrontal pre-SMA and the motor cortex was reported by others in JME [40].

Previous FDG-PET studies performed in JME patients reported either no significant change compared to healthy controls [21], or a decreased metabolism in the ventral premotor cortex, dorsolateral prefrontal cortex and left premotor area [41]. Our investigation did not observe significant differences in glucose metabolism between patients and controls.

Finally, in our IGE group the most significant increase was in the left ACC. Importantly, there is a large literature showing that lateralized abnormalities are possible in IGE, underlying that structural and electrophysiological changes can be prominent on one hemisphere even in generalized epilepsies that are considered related to bihemispheric widespread networks rather than to generalized abnormalities [67, 68, 21]. Our findings are in line with these observations.

A potential contribution of nAChRs in the process of ictogenesis in IGE?

Several data in animal models suggest a contribution of cortical nAChRs in the pathogenesis of IGE. Lesions of the cholinergic nucleus basalis in GAERS suppressed SW discharges [44], as well as the systemic administration of nicotine [42]. There were, however, contradictory results reported in WAG-Rij rats, with an increase of SW discharges after the selective removal of the cholinergic input from the nucleus basalis [43], and an inhibition of the corticothalamocortical synchronous activity related to cholinergic activation of the nucleus basalis, but mostly mediated via muscarinic receptors [45].

An increase in nAChR density in the ACC could modify neuronal excitability and favor the hyperactivity and/or hyperexcitability of ictogenic neurons [46]. This neuromodulation could be through the direct activation of postsynaptic nAChRs on pyramidal neurons in deep layers [47] and/or the activation of presynaptic α4β2 nAChRs on glutamatergic neurons, leading to local depolarization and activation of voltage-gated Ca2+ channels (VGCC) [48]. An alternative hypothesis is a direct effect on GABAergic neurons. The presence of nAChRs on GABAergic neurons was demonstrated in animal models [49]. A study using chronic nicotine exposure in adolescent rats showed that the effects of nicotine in the ACC appear to involve GABA interneurons [50]. The activation of nAChRs on low-threshold spiking and regular spiking GABAergic interneurons in the prefrontal cortex layer V would increase inhibitory GABAergic inputs to the layer V pyramidal cells [51]. Both types of interneurons express mRNA for α4 and β2 subunits and showed inward currents upon direct nicotine application [52]. The contribution of nAChRs in the pathogenesis of IGE was also supported by a recent study investigating the effects of a specific microRNA (miR) (non-coding RNA regulating the expression level of genes) in a transgenic mice model. The absence of this miR changed the expression of some nAChR subunits (α5, α7) and induced a cortical hypersynchronization with electrophysiological similarities to SWs [53].

The mechanism leading to a local increased nAChR density in IGE is also unknown. Genetic polymorphisms in nAChR subunits could induce a defective program of axonal pruning and of synapse elimination. The pruning in mediofrontal regions is known to be late in humans (starting at around 3-4 years of age and continuing up to adolescence). A defect in receptor density would be concordant with an age-related neurological disorder particularly sensitive to the period of adolescence. Spine pruning alterations within excitatory synapses have already been implicated in developmental neurological disorders such as autism spectrum disorder (often associated with epilepsy) [54, 55]. Interestingly, previous reports have suggested a beneficial effect of smoking or nicotine patches on epileptic activity in patients with nAChR mutations and ADNFLE [56–58].

Endophenotypes observed in genetic epilepsies such as cognitive deficits could be related to a common underlying biochemical abnormality (such as a change in the cerebral distribution of some receptors). This is supported by the fact that in children with IGE, cognitive deficits are present at the time of the diagnosis, without clear worsening over time after the onset of epilepsy [59]. Moreover, impairment of cognitive functions was reported in unaffected (non epileptic) family members in families with IGE [60].

However, we cannot exclude the alternative that the increased nAChR density has no causal role on the epileptogenesis and is an epiphenomenon.

Nicotinic receptors in the ACC as supportive biomarker

The significant increase of F-A-85380 that we observed in the ACC in the group of IGE was also useful, at an individual level, to identify IGE subjects from controls or patients with focal epilepsy, providing an excellent discrimination (AUC above 0.8) [61]. This result suggests that F-A-85380 could have a diagnostic supportive value in epilepsy, particularly in patients with nocturnal GTCS only or in patients with diurnal GTCS without any clinical focal onset and without any typical generalized or focal EEG signature. The presence of an increased F-A-85380 uptake in the ACC in such patients could support a diagnosis of IGE.

Interestingly, this increased nAChR density appears specifically in a cortical area (ACC) with a normally higher BP_{RI} density, as compared with the global cortical density. A special richness in nAChRs was indeed shown in the cingulo-insular network in healthy volunteers [20]. The fact that the availability of nAChRs is even higher in part of this network in patients with IGE could be a marker of changes in network activation. Further studies are needed to demonstrate a change in the abilities of detection of salient stimuli or in other tasks related to this network in patients with IGE.

Methodological limitations of the study

Several limitations may be considered in our study. Firstly, the major intrinsic limitation of the tracer F-A-85380 is its slow brain kinetics and the long time after injection required in humans to reach equilibrium [62, 63]. We adopted a static late imaging protocol, without arterial sampling, as previously validated [7] and used in other clinical studies, e.g. [64]. We have chosen the corpus callosum as the reference region, as previously suggested [14–17], although this region has a low specific binding.

Secondly, our IGE group of 12 patients included patients with different syndromes (five JAE, two JME and five GTCS only). These forms of epilepsy have similar triggering factors, are sensitive to the same antiepileptic drugs and have a similar electrophysiological signature, the generalized SW discharges. Therefore, we postulate a common pathophysiological basis and pooled the data in the same "IGE" group. Interestingly, our results showed a shared tendency to a higher nAChR density in the ACC in the three IGE subgroups (JAE, JME and GTCS only) when each was compared with the control group. Thirdly, we cannot exclude an interaction with the antiepileptic drugs taken by the patients that were somewhat different between IGE patients and patients with focal epilepsy: valproate was mainly used in IGE patients (8/12 in IGE versus 2/10 in focal epilepsy), whereas carbamazepine/oxcarbazepine were more used in patients with focal epilepsy; lamotrigine was used in two patients with IGE and two patients with focal epilepsy (Table 1). Valproate does not interfere with the $\alpha4\beta2$ nAChRs while carbamazepine was shown to inhibit these receptors [65], as well as lamotrigine [66], but a focal cortical effect of the drugs on neurochemical pathways seems unlikely. Fourth, the sample size for each of the three clinical groups was small. For this reason the power of our study was modest and we may have missed smaller differences between the groups, yet we observed statistically significant differences between the groups in some cortical areas. Finally, the potential use of F-A-85380 as diagnostic supportive tool in clinical routine is limited by the availability of nicotinic PET tracers only in selected centers and, as any PET investigation, by the need of a clinical justification for a procedure associated with exposure to ionizing radiations.

Conclusion

The present PET study is the first study showing a neurochemical structural difference in ACC in patients with IGE. Its absence in other forms of epilepsy (ADNFLE or non lesional diurnal focal epilepsy) argues against an epiphenomenon related to the epileptic seizures. This neurochemical difference affects a cortical region already shown to have possible structural changes and functional alterations in this form of epilepsy. Further studies are needed to confirm our result, and help to understand the role of this local cortical receptor increase and support its direct involvement in the pathogenesis of IGE.

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Author contributions FP, MW, MS, OR, YS, VG and SH contributed to the conception and design of the study, VG, SH, GZ, RG, YS, FP and MW performed acquisition and analysis of data, FP, VG, MS, OR and SH drafted the manuscript.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study involving human participants were conducted in accordance with the Swiss ethical standards and with the 1964 Helsinki declaration and its later amendments. The study protocol was approved by the Ethics Committee of the Geneva University Hospitals (CER 10-041) and by the Swiss agency for medications (Swissmedic: study n°2011DR1031). The study was recorded in ClinicalTrials.gov (n° NCT03268369).

Written informed consent was obtained from all participants.

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