

## SHORT REPORT

## Antibodies against glutamic acid decarboxylase: prevalence in neurological diseases

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### Abstract

**High prevalence of autoantibodies against glutamic acid decarboxylase (GAD-Ab) in stiff man syndrome (SMS) not only helps diagnosis, but also suggests immune mediated impairment of GABAergic functions. However, the presence of GAD-Ab has also been reported in other neurological syndromes. Therefore the prevalence of GAD-Ab was investigated in SMS, progressive encephalomyelitis with rigidity and myoclonus (PERM), and in other neurological diseases (OND).**

**Serum antibodies against the GAD isoforms, GAD65 and GAD67, were investigated with radioimmunoassays in 13 patients with SMS, nine with PERM, 279 consecutive patients with OND, and in 100 normal controls.**

**Results—Prevalence of GAD65Ab was around 80% in patients with SMS/PERM compared with 5% in patients with OND and 1% in normal controls. Prevalence of GAD67Ab was 60% in SMS/PERM, 2% in patients with OND, and 1% in normal controls. Raised GAD-Ab clustered in an OND subgroup with sporadic progressive ataxia, but not in OND subgroups with recognised neuroimmunological diseases. In conclusion, increased GAD-Ab is neither a non-specific epiphenomenon of neuronal damage nor a common feature of recognised neuroimmunological disorders. In neurological diseases, GAD-Ab may be a pathogenetic agent or a marker for an ongoing autoimmune process, or both.**

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Glutamic acid decarboxylase (GAD) is the rate limiting enzyme in the synthesis of the widespread inhibitory transmitter  $\gamma$ -amino butyric acid (GABA).<sup>1</sup> Autoantibodies directed against GAD (GAD-Ab) are present in many patients with stiff man syndrome (SMS) and its variants,<sup>2–4</sup> but also in patients with other neurological disorders (OND)<sup>5–11</sup> suggesting that an immune mediated attack against GAD or GABAergic neurons might have a pathogenetic role. This study aimed at defining the

prevalence of GAD-Ab among neurological patients.

### Patients and methods

We investigated serum samples from a total of 301 neurological patients. Among these, 13 had the clinical diagnosis of SMS, and nine of its “plus” variant, progressive encephalomyelitis with rigidity and myoclonus (PERM).<sup>3,4</sup> Patients with paraneoplastic SMS or the stiff limb syndrome were not included. Two hundred and seventy nine patients with OND were consecutively admitted to the inpatient and outpatient units of the Department of Neurology, Heidelberg University within a predetermined 6 week time span. Their 290 neurological diagnoses comprised cerebrovascular accidents (n=68), neuromuscular diseases (n=39), or basal ganglia disorders (n=30) (fig 1). All patients gave their informed consent for blood testing. One hundred healthy blood donors without individual or family history for type I diabetes mellitus or autoimmune disease (which themselves are recognised risk factors for neurological disease) served as normal controls. Serum samples of the control subjects and patients with OND were stored at  $-20^{\circ}\text{C}$  until used for this study. Serum specimens from the 22 patients with SMS/PERM were collected over a period of 5 years and stored at  $-20^{\circ}\text{C}$ . All serum samples concomitantly underwent radioimmunoassay.

### DETECTION OF GAD-Ab

Autoantibodies to GAD65 and GAD67 were determined as described previously using recombinant human antigens.<sup>12</sup> Antibody concentrations were expressed in units (U). As commercial kits are available only for the detection of GAD65Ab, assays established in our laboratory were used. Moreover, commercially available assays so far are not validated in workshop programmes. Our GAD65Ab assay was evaluated in the Second GAD Antibody Proficiency Program with values of 100% for sensitivity, specificity, validity, and consistency.<sup>13</sup> Because of different assay formats and the lack of international standard serum samples, it is difficult to compare different assays. Our cut off points may correspond to about 1 U in the commercial GAD65Ab radioimmunoassays.

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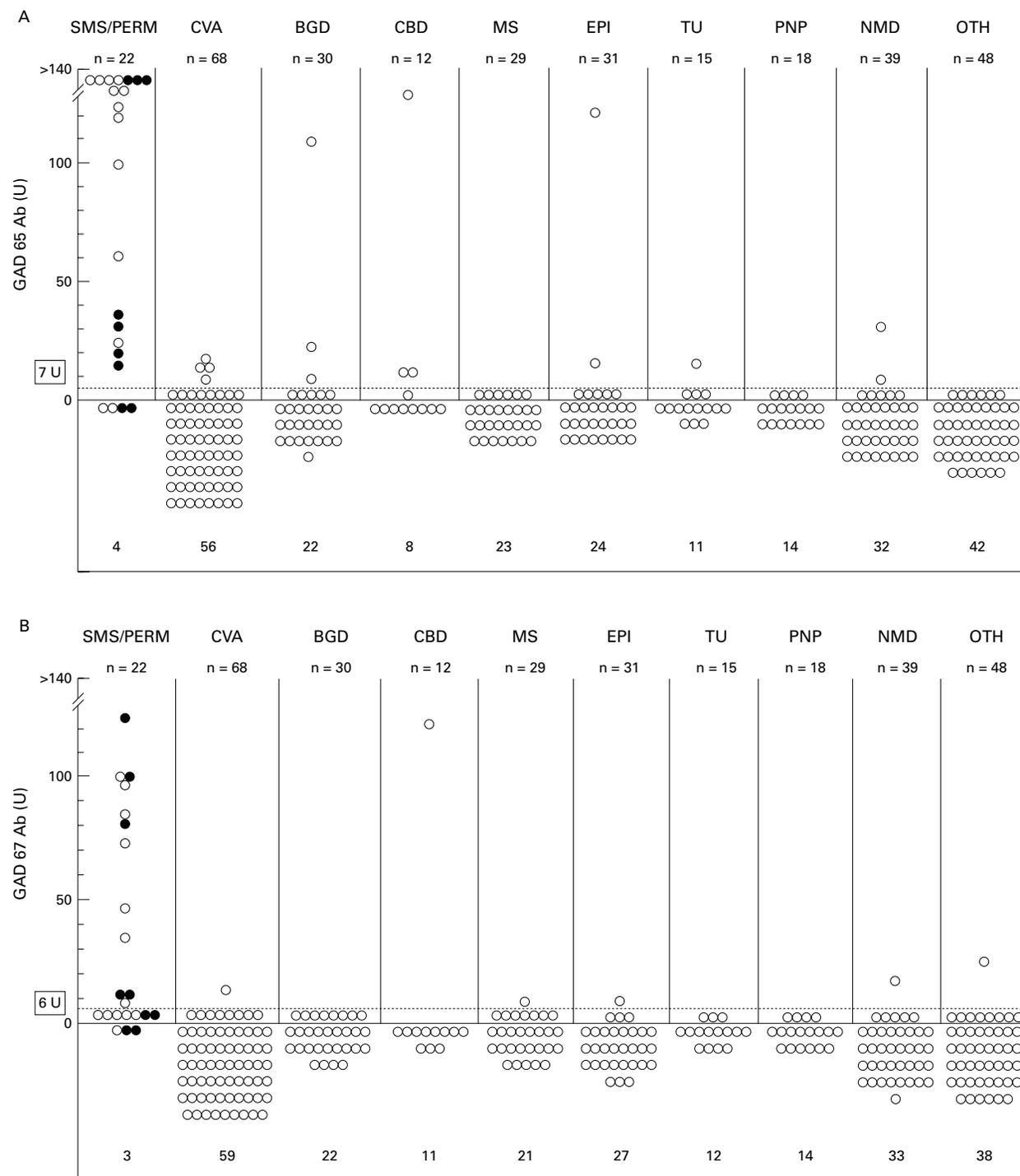


Figure 1 Distribution of GAD65Ab (A) and GAD67Ab (B) (U) as shown by radioimmunoassay. Twenty two patients with SMS/PERM are compared with 279 patients with 290 other neurological diseases. SMS/PERM=Stiff man syndrome/progressive encephalomyelopathy with rigidity and myoclonus; CVA=cerebrovascular accident; BGD=basal ganglia disease; CBD=sporadic progressive ataxia; MS=multiple sclerosis; EPI=epilepsy; TU=tumour; PNP=polyneuropathy; NMD=neuromuscular disease; OTH=others. Data obtained from patients with PERM are labelled by filled circles. Figures in the second top lines of diagrams give number of diagnoses. Figures in the bottom lines of diagrams give number of zero values. Horizontal broken line represents cut off concentrations for normality (7 U for GAD65Ab, 6 U for GAD67Ab).

**Results**

**NORMAL LIMITS AND PREVALENCE OF RAISED GAD-Ab**

In the control group, GAD65Ab ranged between 0 and 11 U (median 1.1), and GAD67Ab between 0 and 11 U (median 0.8). The upper normal limit calculated from the normal controls (M+4SD) was 7 U for GAD65Ab and 6 U for GAD67Ab, respectively. With either

antibody, one normal subject had raised GAD-Ab. In the SMS/PERM group, by contrast, 18/22 patients had raised GAD65Ab, and 12/22 patients had raised GAD67Ab. This corresponds to a prevalence of 81.8 % and 54.5 %, respectively (table 1). In the OND group, a total of 18 patients (19 diagnoses) were identified with values for GAD65Ab or GAD67Ab above normal (total prevalence=6.5%). For the two

Table 1 Group data and prevalence of GAD autoantibodies

Group	n	Age (y)		GAD65Ab		GAD67Ab	
		Mean	Range	Raised (n)	Prevalence (%)	Raised (n)	Prevalence (%)
NC	100*	32.1	18–46	1	1.0	1	1.0
OND	279	53.3	5–87	14	5.0	6	2.2
SMS	13	58.1	28–74	11	84.6	7	54.0
PERM	9	50.1	31–68	7	78.0	5	56.0

\*No familial history for type 1 diabetes mellitus or autoimmune disease. NC=Normal controls; OND=other nervous diseases; SMS=stiff man syndrome; PERM=progressive encephalomyelitis with rigidity and myoclonus.

GAD isoforms, the corresponding figures were 14 of 279 (GAD65Ab; prevalence=5.0%) and six of 279 (GAD67Ab; prevalence =2.2%; table 1).

#### QUANTIFICATION OF GAD-Ab

Values for raised GAD65Ab ranged between 14 and 295 U (median 120) in patients with SMS/PERM, and in patients with OND between 8 and 130 U (median 31). Raised GAD67Ab was scattered between 6 U and 123 U (median 34) in the patients with SMS/PERM, and between 7 U and 121 U (median 26) in the OND group; 13 of 22 patients with SMS/PERM, but only three of 279 with OND had GAD65Ab above 40 U. The corresponding figures for GAD67Ab were eight of 22 patients with SMS/PERM and one of 279 patients with OND. Thus, patients with SMS/PERM had the highest prevalence and the highest values for both isoforms of GAD-Ab (table 1; fig 1).

#### CONCORDANCE

There was a high degree of concordance in the SMS/PERM group. Among 18 patients with SMS/PERM who had raised GAD65Ab, 14 also had raised GAD67Ab and, vice versa, all patients with raised GAD67Ab also had GAD65Ab values above normal. By contrast, among 14 patients with OND and GAD65Ab above the normal limit, only two also had raised GAD67Ab. Correspondingly, among six patients with OND with raised GAD67Ab, only two had concomitantly raised GAD65Ab. Moreover, in the SMS/PERM, but not in the OND groups, GAD65Ab and GAD67Ab showed a quantitative relation (GAD65Ab: GAD67Ab = 3.2).

#### CLINICAL CORRELATION

Neurological presentation of the patients with SMS/PERM did not differ with respect to the presence or absence of either GAD-Ab, nor to high or low GAD-Ab. However, nine patients with SMS/PERM—all with raised GAD-Ab—presented with systemic autoimmune diseases, five of them insulin dependent diabetes mellitus.

Among the patients with OND and GAD65Ab, four of 68 had cerebrovascular accidents of various degrees, three of 12 had sporadic progressive ataxia with adult onset, three of 30 had basal ganglia disorders, and two of 39 neuromuscular diseases. The corresponding figures for GAD67Ab were one of 68 (cerebrovascular accidents), one of 12 (sporadic progressive ataxia), none of 30 (basal

ganglia disorders), and one of 39 (neuromuscular diseases). A total of 49 patients had recognised neurological autoimmune diseases such as myasthenia gravis, polyneuritis, or multiple sclerosis. Two of them had raised GAD65Ab and three had raised GAD67Ab. Follow up of the patients with OND was unsystematic. However, two of them, both with raised GAD65Ab, developed autoimmune polyendocrine syndrome within 3 years.

#### Discussion

##### GAD-Ab AS A DIAGNOSTIC TOOL

Our data show that antibodies against GAD are increased, with high prevalence in patients with SMS/PERM, and with a much lower prevalence and lower values in patients with OND. This further substantiates the diagnostic value of GAD autoantibodies in the differential diagnosis of SMS/PERM.<sup>2,3</sup> Moreover, comparable prevalences and raised GAD-Ab in patients with SMS or PERM (table 1) further support the hypothesis of a close relation between both diseases. High prevalence of raised GAD-Ab has been reported also in patients with endocrine disorders, particularly in type 1 diabetes mellitus and autoimmune polyendocrine syndrome, and in their first degree relatives.<sup>12–15</sup> Indeed, comorbidity of SMS/PERM with autoimmune endocrine disorders is well recognised<sup>2–4</sup> and was present also in patients of this study. Thus both the absence and presence of GAD-Ab require cautious interpretation: About 20% of patients with the clinical diagnosis SMS/PERM tested negative for GAD-Ab with both assays. On the other hand, a source of positive GAD-Ab tests in neurological patients not having SMS or PERM is coexisting type 1 diabetes mellitus, prediabetic insulinitis, or autoimmune polyendocrine syndrome.<sup>14</sup> For diagnostic purposes, radioimmunoassay determination of GAD65Ab seems superior to GAD67Ab. Correlation of GAD65Ab and GAD67Ab in patients with SMS/PERM, but not in patients with OND suggests that the recognition of epitopes may differ between the two situations.<sup>16,17</sup>

##### IS THERE A ROLE FOR GAD-Ab IN THE PATHOGENESIS OF NEUROLOGICAL DISEASES?

We found GAD-Ab with an overall prevalence of 6.5% in consecutive patients with OND compared with 1% in normal controls. Three factors may contribute to such differences: (1) normal controls, but not patients with OND, were corrected for type 1 diabetes mellitus and other autoimmune diseases. However, the cut off (mean+4 SD) in our control group

corresponds to the 99th percentile of normal controls, which is widely accepted to discriminate between positive and negative antibody tests.<sup>12</sup> (2) Age may influence the prevalence of raised GAD-Ab,<sup>15</sup> and mean age in both OND and SMS/PERM groups exceeded the control group by 20 years. (3) Presence of GAD-Ab in the serum of a given patient with OND may hint at his risk for future development of type 1 diabetes mellitus and other autoimmune diseases<sup>12 15</sup> as risk factors, rather than imply neurological autoimmune disease.

GABAergic neurons and thus GAD65 and GAD67 are widely distributed in the CNS.<sup>1 18</sup> Therefore, a wide variety of lesions can be expected to cause presentation of the cytosolic antigen GAD. Low prevalence of GAD-Ab in the OND group suggests that autoimmunity directed against GAD is not simply a non-specific epiphenomenon of neuronal damage, nor a bystander reaction to a neurological autoimmune process. On the other hand each of the GAD isoforms is thought to be differentially involved in a range of normal and abnormal motor behaviours<sup>18</sup>; thus various motor symptoms might be attributed to an immune mediated disturbance of GABAergic function. Apart from SMS/PERM, the presence of GAD-Ab has been reported in patients with palatal myoclonus,<sup>8</sup> epilepsy,<sup>9 10</sup> sporadic progressive ataxia,<sup>5 7 11</sup> and retinopathy.<sup>19</sup> In most of these patients, however, the source of the antigen is not clear. Therefore, the pathogenetic role of GAD-Ab against one or both isoforms remains speculative. Nevertheless, the range of neurological diseases associated with autoimmunity against GAD apparently is wider than hitherto thought.

According to another hypothesis, the presence of GAD-Ab and high T cell responses to GAD in patients with type 1 diabetes mellitus or in their first degree relatives indicate a hyperimmune state.<sup>20</sup> This state may reveal itself in a wide range of organ specific autoimmune diseases. Presence of autoimmunity against GAD may serve as a marker for those at risk for autoimmune diseases not only of endocrine glands but also of the CNS.

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