Clinical relevance of positive voltage-gated potassium channel (VGKC)-complex antibodies: experience from a tertiary referral centre

Ross W Paterson, Michael S Zandi, Richard Armstrong, Angela Vincent, Jonathan M Schott

ABSTRACT

Background Voltage-gated potassium channel (VGKC)-complex antibodies can be associated with a range of immunotherapy-responsive clinical presentations including limbic encephalitis, Morvan’s syndrome and acquired neuromyotonia. However, there are patients with positive levels in whom the significance is uncertain.

Objective To evaluate the clinical significance associated with positive (>100 pM) VGKC-complex antibodies.

Methods Over a 4-year period, 1053 samples were sent for testing of which 55 were positive. The clinical presentations, final diagnoses and responses to immunotherapies, when given, were assessed retrospectively and the likelihood of autoimmunity was categorised as definite, possible, unlikely or undetermined (modified from Zuliani et al 2012).

Results Only 4 of the 32 patients with low-positive (100–400 pM) levels were considered definitely autoimmune, 3 with peripheral nerve hyperexcitability and 1 with a thymoma; 3 were given immunotherapies. Of the remaining 28 with low-positive levels, 13 (3 of whom had tumours) were considered possibly autoimmune, and 15 were unlikely or undetermined; 1 was given immunotherapy unsuccessfully. Of the 23 patients with high-positive (>400 pM) levels, 12 were given immunotherapies, 11 of whom showed a good response. 11 were considered definitely autoimmune, 10 with limbic encephalitis (antibody specificity: 5 LG1, 1 contactin2, 2 negative, 2 untested) and 1 with a tumour. In the remaining 12, autoimmunity was considered possible (n=9; most had not received immunotherapies), or unlikely (n=3).

Conclusions As antibody testing becomes more widely available, and many samples are referred from patients with less clear-cut diagnoses, it is important to assess the utility of the results. VGKC-complex antibodies in the range of 100–400 pM (0.1–0.4 nM) were considered clinically relevant in rare conditions with peripheral nerve hyperexcitability and appeared to associate with tumours (12.5%). By contrast high-positive (>400 pM; >0.4 nM) levels were considered definitely (38%) or possibly (49%) clinically relevant, but not all patients had a ‘classical’ limbic encephalitis and some did not receive immunotherapies.

INTRODUCTION

Autoantibodies against the voltage-gated potassium channel (VGKC) complex measured by radioimmuno precipitation have been reported in a broad spectrum of immunotherapy-responsive neurological illnesses in children and adults. Initially, low levels (<400 pM) were associated with peripheral nerve hyperexcitability syndromes (mainly acquired neuromyotonia), but higher levels were subsequently found in Morvan’s syndrome, and in patients with reversible limbic encephalitis. In the first series, 10 patients with limbic encephalitis had VGKC-complex values from 450 to 5128 pM, but some controls were reported to have low positive titres. Many subsequent reports have confirmed that VGKC-complex antibodies are associated with limbic encephalitis and a broader range of phenotypes, and in some patients, VGKC-complex antibodies <400 pM did appear to be clinically relevant.

Latterly it has become clear that VGKC antibodies are not directed against the VGKC itself, but against other cell surface antigens that form part of the VGKC complex. Antigenic targets include leucine-rich glioma-inactivated protein 1 (LG1), typically associated with limbic encephalitis (LE) and a distinctive seizure disorder, faciobrachial dysautonomia. Antibodies to Contactin2 are much less frequent but there are some reports of antibodies to LG1 in patients with different clinical presentations.

Methods To evaluate the potential of VGKC-complex antibodies to LE and other autoimmunity, we reviewed all cases of VGKC-complex antibodies from a tertiary referral centre in London, UK, and performed a retrospective analysis of a large case series from a single institution.

RESULTS

Clinical relevance of positive VGKC-complex antibodies

DEFINITION OF AUTOIMMUNITY

Autoimmunity was considered possible (n=9; most had not received immunotherapies), or unlikely (n=3).

METHODS

Following an initial brief audit of 44 VGKC-complex antibody-positive subjects, we formed a comprehensive retrospective review of all patients seen at the National Hospital for Neurology and Neurosurgery, Queen Square, who

http://dx.doi.org/10.1136/jnnp-2013-305811
had VGKC antibodies requested between January 2008 and January 2012. The electronic records of clinic correspondence, neuropsychology, imaging and other blood results were reviewed. Subjects were classified as having ‘high positive’ ($\geq$400 pM) or ‘low positive’ (100 to 400 pM) VGKC-complex antibodies. Duration of follow-up was defined as the time from the first to the most recent neurological assessment. The treating neurologist’s final diagnosis was recorded. The likelihood of each patient having an autoimmune VGKC-complex antibody-related syndrome was retrospectively determined using a modified version of previously published guidelines (10 tables 1 and 2).

**SEROLOGY**

Serum VGKC-complex antibodies were tested using a radioimmunoprecipitation assay. Cell-based assays for CASPR2, LGI1 and contactin 2 antibodies were performed using human embryonic kidney cells transfected with cDNAs encoding the relevant proteins.  

**RESULTS**

An initial audit of 44 VGKC-complex antibody-positive patients demonstrated that 32 had values $<400$ pM, of whom 90% were not considered to be autoimmune, whereas 9 of the 12 with higher levels were considered to have an immunotherapy-responsive autoimmune disease. To study this more formally we collected all results from 2008 to January 2012 inclusive.

During this time, the serum of 1053 subjects was tested for VGKC-complex antibodies of whom 55 subjects (5.5%) had one or more positive levels (>100 pM). Thirty-two subjects were only ever low positive; and the remaining 23 had high-positive levels at presentation or on subsequent sampling. A summary of clinical presentation, VGKC-complex, LGI1 and CASPR2 antibodies and treatment responses (where available) in the low- and high-positive groups are given in tables 1 and 2.

**Low-positive patients**

Thirty-two subjects, assessed in general neurology, specialist cognitive disorders, neuromuscular and movement disorder services, only ever had low positive levels (100–400 pM; mean age 52 (SD 18.8), male:female ratio 0.9:1). Many of these subjects had symptoms for many months (median 24, range 1–132 months) at the time the antibodies were first measured. They were followed up for a median of 23.5 (0–48) months. Four had more than one VGKC-complex antibody measured, and in three the subsequent samples were within the normal range ($<100$ pM).

Only four of these patients (13%) were considered to have a definite or probable autoimmune disease (one with a paraneoplastic cerebellar syndrome, one with acquired neuromyotonia and two with Morvan’s syndrome). The latter two patients were given immunotherapies, although only one (a patient with Morvan’s and a prior thymoma resection) showed a significant improvement (reduction in modified Rankin score from 5 to 3). The remaining 28 patients had a variety of peripheral ($n=3$), central ($n=17$) or psychiatric ($n=4$) disorders; no definite diagnosis was made in four patients (see table 1). Although 13 individuals were retrospectively categorised on the basis of clinical features and investigations as possibly having an autoimmune disorder, none was given immunotherapy. Antibodies directed against LGI1 or CASPR2 antibodies were present in only 3 of the 32 patients (table 1). Two of these had peripheral nerve hyperexcitability, but they were not given immunotherapy.

Three of the 32 patients had a malignancy first detected around the time the antibodies were tested (endometrial cancer in one, breast cancer in two); a further three subjects were screened for malignancy with whole body fluorodeoxyglucose (FDG) PET scanning which was negative in each case.

**High-positive patients**

Seventeen patients had high levels of VGKC-complex antibodies ($>400$ pM) ranging from 401 to 6846 pM when first tested, and a further six individuals initially had low values which rose to high on subsequent sampling. The clinical features are summarised in table 2.

The mean age was 59 (SD 19.4) with a male:female ratio of 1.3:1, and median time from symptom onset to VGKC-complex measurement of 30 months (range 0.5–206). On the basis of the clinical syndrome and/or response to immunotherapies, 11 (including one individual fulfilling criteria for paraneoplastic disease) were retrospectively classified as having a definite autoimmune condition. Ten individuals had limbic encephalitis, of whom six of eight tested had LGI1 (5) or contactin-2 (1) antibodies. These 10 patients were treated with immunotherapies and showed a good response to treatment. Nine of the remainder were retrospectively assessed as possibly having an autoimmune condition but were not given immunotherapies. Two other individuals had a neurodegenerative condition, and one had hepatic toxicity.

Overall, the suggested autoimmune categories (figure 1) were related to the antibody titre (figure 2A; $p=0.013$, Fisher’s exact test). It was noted that the median time from symptom onset to antibody testing was six months (range 0.5–120) in those patients considered to have a definite VGKC-complex-related disease compared with 28 months (range 0.5–206) in those classified as possible or unlikely (figure 2B; $p=0.04$, Kruskal–Wallis test).

**DISCUSSION**

In this unselected retrospective series of adults seen in a tertiary neurology setting, 5.5% of patients showed a positive VGKC-complex antibody result. While the range of conditions in which antibody testing was requested, was inevitably subject to referral bias and may not be entirely representative of the conditions well described in the context of VGKC-complex antibodies, such as limbic encephalitis, whereas low positive values were more likely to have an alternative, non-autoimmune or unclear diagnosis. The results confirm that high titres of VGKC antibodies are very likely to be clinically relevant, particularly when tested within a few months of onset, whereas values $<400$ pM should be interpreted with care. Nevertheless, the relatively high number (4/32, 13%) of low-positive cases with tumours suggests that low-positive VGKC-complex antibody levels may be an onconeural marker in some situations.  

Many of the ‘high-positive’ patients had limbic encephalitis or related syndromes (relapsing encephalopathy, seizures), although one subject had an alternative autoantibody (anti-glutamate decarboxylase (GAD)) that was consistent with their neurological presentation of Stiff Person Syndrome. Although the patients with limbic encephalitis were treated with good responses, neurologists had exercised their clinical judgement in not trying immunotherapy for most of the related syndromes, which, therefore, were defined as ‘possible’ autoimmune. By contrast, the ‘low-positive’ patients had many more neurodegenerative diagnoses, atypical presentations or uncertain diagnoses,
Table 1 Clinical features in patients with VGKC-complex antibodies <400 pM

<table>
<thead>
<tr>
<th>Subject</th>
<th>Highest VGKC levels; other specific antibody if found*</th>
<th>Physician’s diagnosis/duration of follow-up</th>
<th>CSF (cerebrospinal fluid)</th>
<th>Response to immunotherapies</th>
<th>VGKC-complex related syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>M, 57</td>
<td>398</td>
<td>Morvan’s syndrome/34 months</td>
<td>2 WC, Pro N</td>
<td>Yes</td>
<td>Definite</td>
</tr>
<tr>
<td>M, 72</td>
<td>168</td>
<td>Morvan’s syndrome and CIDP/36 months; Thymoma</td>
<td>NT</td>
<td>Yes</td>
<td>Definite</td>
</tr>
<tr>
<td>F, 71</td>
<td>124, NT</td>
<td>Autoimmune/paraneoplastic cerebellar syndrome; Endometrial carcinoma</td>
<td>1 WC, Pro 0.64, cytology, culture negative</td>
<td>No</td>
<td>Paraneoplastic</td>
</tr>
<tr>
<td>F, 67</td>
<td>301/LGI1</td>
<td>Neuromyotonia/9 months</td>
<td>NT</td>
<td>Not tried</td>
<td>Definite</td>
</tr>
<tr>
<td>F, 69</td>
<td>144, NMDAR low, GlyR</td>
<td>Amnesia/23 months</td>
<td>Acellular, Pro N, cytology –ve</td>
<td>Not tried</td>
<td>Possible</td>
</tr>
<tr>
<td>F, 64</td>
<td>170</td>
<td>None made/28 months</td>
<td>Acellular, Pro 0.7, OCB +ve serum and CSF</td>
<td>Not tried</td>
<td>Possible</td>
</tr>
<tr>
<td>M, 25</td>
<td>173</td>
<td>Brainstem migraine/6 months</td>
<td>&lt;1 WC, 16 RC, Pro 0.43, Culture –ve</td>
<td>Not tried</td>
<td>Possible</td>
</tr>
<tr>
<td>F, 22</td>
<td>316</td>
<td>Idiopathic generalised epilepsy/9 months</td>
<td>46 WC, (lymph); 4 RC, Pro 0.46, Culture –ve; OCB +ve serum only</td>
<td>Not tried</td>
<td>Possible</td>
</tr>
<tr>
<td>F, 86</td>
<td>358/LGI1 and CASPR2</td>
<td>Idiopathic Parkinson’s disease/3 months</td>
<td>NT</td>
<td>Not tried</td>
<td>Possible</td>
</tr>
<tr>
<td>F, 40</td>
<td>391</td>
<td>Myoclonic jerks of undetermined aetiology/7 months</td>
<td>NT</td>
<td>Not tried</td>
<td>Possible</td>
</tr>
<tr>
<td>M, 48</td>
<td>201</td>
<td>Infective encephalopathy improvement with antibiotics/24 months</td>
<td>7 WC (lymph); 0 RC, Pro N, Culture –ve</td>
<td>Not tried</td>
<td>Possible</td>
</tr>
<tr>
<td>M, 18</td>
<td>117/LGI1 and CASPR2</td>
<td>Nerve hyperekctability syndrome/18 months</td>
<td>NT</td>
<td>Not tried</td>
<td>Possible</td>
</tr>
<tr>
<td>M, 55</td>
<td>100</td>
<td>Length dependent sensory axonal neuropathy/13 months</td>
<td>NT</td>
<td>Not tried</td>
<td>Possible</td>
</tr>
<tr>
<td>F, 38</td>
<td>149</td>
<td>Suspected muscle channel disease/44 months</td>
<td>NT</td>
<td>Not tried</td>
<td>Possible</td>
</tr>
<tr>
<td>F, 43</td>
<td>212</td>
<td>Dystonia of undetermined aetiology/40 months; Breast cancer</td>
<td>WC&lt;1, RC 1200, OCB +ve in CSF and serum, Pro 0.7</td>
<td>Not tried</td>
<td>Possible paraneoplastic</td>
</tr>
<tr>
<td>F, 42</td>
<td>138</td>
<td>Functional movement disorder/51 months, Breast cancer</td>
<td>Acellular, Pro N, OCB –ve</td>
<td>Not tried</td>
<td>Possible Paraneoplastic</td>
</tr>
<tr>
<td>F, 23</td>
<td>186</td>
<td>None made, spontaneous improvement/6 months</td>
<td>Acellular; Pro N, OCB –ve</td>
<td>Not tried</td>
<td>Possible</td>
</tr>
<tr>
<td>M, 64</td>
<td>143</td>
<td>Multi-system atrophy/60 months</td>
<td>&lt;1 WC, 8 RC, OCB –ve, cytology –ve</td>
<td>IVIG, no improvement</td>
<td>Unlikely</td>
</tr>
<tr>
<td>F, 61</td>
<td>183</td>
<td>Alzheimer’s disease/34 months</td>
<td>NT</td>
<td>Not tried</td>
<td>Unlikely</td>
</tr>
<tr>
<td>F, 75</td>
<td>221</td>
<td>Lewy Body Dementia/8 months</td>
<td>Acellular, Pro N</td>
<td>Not tried</td>
<td>Unlikely</td>
</tr>
<tr>
<td>M, 75</td>
<td>130</td>
<td>Embolic stroke secondary to atrial fibrillation/6 months</td>
<td>32 RC, &lt;1 WC, Pro N</td>
<td>Not tried</td>
<td>Unlikely</td>
</tr>
<tr>
<td>F, 74</td>
<td>106</td>
<td>Alzheimer’s disease/12 months</td>
<td>Acellular, Pro N</td>
<td>Not tried</td>
<td>Unlikely</td>
</tr>
<tr>
<td>F, 54</td>
<td>118</td>
<td>Motor Neurone disease/24 months</td>
<td>NT</td>
<td>Not tried</td>
<td>Unlikely</td>
</tr>
<tr>
<td>F, 57</td>
<td>119</td>
<td>Lewy Body dementia/vascular dementia/24 months</td>
<td>NT</td>
<td>Not tried</td>
<td>Unlikely</td>
</tr>
</tbody>
</table>

*All available samples were tested for LGI1 and CASPR2; most were not tested for contactin-2. Likelihood of VGKC-complex antibody being related to autoimmunity was determined based on previously published guidelines, modified as follows: definite: recognised neurological syndrome and treatment with immunotherapy successful; possible: recognised or possible neurological syndrome and immunotherapy unsuccessful or unretried; unlikely: unrelated clinical syndrome and more likely alternative diagnosis; undetermined: unrelated clinical syndrome and no likely relevance. Some samples were not available for retesting (NA).

CIDP, chronic idiopathic demyelinating polyneuropathy; LGI1, leucine-rich glioma-inactivated 1; NMDAR, methyl-D-aspartate; N, normal; NT, not tested; OCB, oligoclonal bands; Lymph, lymphocytes; Pro, protein (normal range < 0.65 g/l); RC, red cells; VGKC, voltage-gated potassium channel; WC, white cells.

and only 12% were considered to have a definite autoimmune condition, and only four were given immunotherapy. Importantly, no patient with a VGKC-complex level <400 pM had limbic encephalitis or epilepsy of unknown aetiology, although such patients have been identified elsewhere, and low-positive VGKC levels have been associated with classical autoimmune syndromes in a few children. As many of the low-positive cohorts had unrelated or alternative diagnoses, these data provide strong evidence that considerably less weight should be put on a low- rather than a
high-positive antibody level. An exception to this is in the context of peripheral nerve hyperexcitability consistent with neuromyotonia, where VGKC-complex antibodies at low levels are common and do not necessarily show binding to LGI1 or CASPR2 (Vincent, M Kiernan, O Watanabe, unpublished data).

Thus, we suggest that low-positive VGKC-complex antibodies may be secondary to other disease pathologies, and it is possible that they are part of a normal repertoire of autoantibodies found in otherwise healthy individuals, which can, under certain circumstances, rise to detectable levels. It is notable that a significant proportion of the low-positive cohorts was diagnosed with a neuroidegenerative disorder and in one case with a genetically defined disease, Allgrove syndrome.16 In keeping with recent reports that VGKC levels can occasionally be elevated in cases of Creutzfeld Jacob disease,17 these cases demonstrate that elevated antibodies can be found in degenerative neurological diseases, when their presence is unlikely to be clinically relevant.

The number of subjects (3/32, 9.4%) who were positive for LGI1 or CASPR2 in the low-positive VGKC cohort was significantly lower than that (6/20, 30%) of the unselected high-positive subjects (χ² 4.5, p=0.034) and that (6/8, 75%) of the limbic encephalitis subjects tested. However, two patients with a definite clinical diagnosis of autoimmune limbic encephalitis were not positive for LGI1 or CASPR2 antibodies, suggesting that there are other antibody targets within the VGKC complex as suggested previously9 and by others.18 Those with high-positive VGKC-complex and LGI1 antibodies all had limbic encephalitis, confirming that LGI1 antibodies are common in this condition.7

A relatively high proportion (three cases, 10%) of patients with low-positive antibodies had tumours (three with breast cancer, one with endometrial carcinoma) and one further subject had a thymoma that was resected before he presented. This is significant higher than the expected incidence within the general
population (0.004% per annum age in the 40–59 age range; 0.014% per annum in the 60–74 age range, http://www.cancerresearchuk.org/cancer-info/cancerstats) and very similar to another recent study.18 While the long duration of neurological symptoms without overt evidence of malignancy in a significant proportion of cases is reassuring, and screening for malignancy may well have been prompted by other factors, the prevalence of underlying malignancy in the low-positive group as a whole is unknown and should be the focus of further study.13

Recently, Klein et al18 reported the clinical presentations of a larger group of VGKC-complex positive patients showing that a small proportion of low-positive VGKC-complex patients are also positive for LGI1 or CASPR2. Although clinical follow-up of these cases was limited, they suggest that these are more likely to be of clinical relevance. Our findings are broadly compatible with their findings; patients with lower antibody titres were less likely to have a specific antigenic target or to have a probable autoimmune condition, except those with LGI1 or CASPR2 antibodies and/or with hyperexcitability syndromes. However, one patient with idiopathic Parkinson’s disease and VGKC antibodies at 358 pM was positive for LGI1 and CASPR2 antibodies but was not considered by the treating neurologist to have a definite autoimmune diagnosis and was not treated as such.

It is not clear why high antibody titres are more associated with central nervous system disorders, often without peripheral dysfunction, while low antibody titres cluster more with peripheral hyperexcitability. Certainly very high VGKC/LGI1 antibodies are associated with limbic encephalitis,7 9 as shown here, but lower levels of both can be found in peripheral nerve hyperexcitability. Such examples serve to illustrate that antibody results need always to be interpreted in the clinical context, and that our understanding of the pathophysiological underpinnings of many of these syndromes is still incomplete. Nevertheless, the broad rule of thumb that low-positive titres—particularly without a specific antigenic target—are less likely to be relevant is of potential clinical utility.

In conclusion, in this retrospective series, high-positive VGKC antibodies strongly predicted an autoimmune or possibly autoimmune aetiology, while low antibody titres cluster more with peripheral hyperexcitability. Certainly very high VGKC/LGI1 antibodies are associated with limbic encephalitis,7 9 as shown here, but lower levels of both can be found in peripheral nerve hyperexcitability. Such examples serve to illustrate that antibody results need always to be interpreted in the clinical context, and that our understanding of the pathophysiological underpinnings of many of these syndromes is still incomplete. Nevertheless, the broad rule of thumb that low-positive titres—particularly without a specific antigenic target—are less likely to be relevant is of potential clinical utility.

In conclusion, in this retrospective series, high-positive VGKC antibodies strongly predicted an autoimmune or possibly autoimmune aetiology, suggesting that the cut-off that we use for a high-positive antibody level (>400 pM) has clinical utility, at least for adults. By contrast, the majority of patients with low-positive VGKC-complex antibodies did not have an identifiable antigenic target, or a syndrome thought to be related to the presence of the antibodies; in many patients, a definite alternative diagnosis was reached, and the result rarely altered management. In some cases, however, low-positive VGKC-complex antibodies may reflect a broader autoimmune tendency. The possibility of an occult—perhaps unrelated—neoplasm should always be considered in individuals with a definite or possible autoimmune syndrome. While our data suggest a possible increased risk of an occult tumour in individuals with isolated low-positive antibodies, irrespective of the clinical syndrome, in most patients, these antibodies are not markers of paraneoplasia and further studies are required to demonstrate whether and which occult tumours should be actively sought. These data suggest that, particularly in the absence of a specific antigenic target or a phenotype associated with VGKC-complex antibodies (eg, limbic encephalitis, fasciobrachial dystonic seizures, neuromyotonia), low positive antibodies should be interpreted with care. In case of doubt antibody levels should be rechecked after an interval to ensure they are not rising.

**Figure 1** Summary of all patients tested for voltage-gated potassium channel antibodies, their diagnostic classification and autoimmune probability.

**Figure 2** (A) The number of patients in each of the given categories divided between high and low voltage-gated potassium channel-complex antibodies (p=0.004, χ², analysed on GraphPad Prism). (B) The duration of symptoms at sampling in patients divided between definite, possible and unlikely or undetermined categories. One-way ANOVA, p=0.04.
Neuro-inflammation

Acknowledgements We are very grateful to Dr Leslie Jacobson and Ms Rosie Pettingill for helping with some of the antibody tests. This study was supported by the NIHR Queen Square Dementia BRU and the Oxford NIHR Biomedical Research Centre.

Contributors The study was initiated by AV with RWP, MSZ and RA. The medical records were searched and data were compiled by RWP, MSZ and RA. JMS and AV reviewed the findings and the data presentation. The final manuscript was written by RWP, AV and JMS.

Funding No specific funding was received for this work. The Oxford Neuroimmunology Service receives support from the NIHR Oxford Biomedical Research Centre.

Competing interests AV and the University of Oxford hold patents and receive royalties and payments for antibody tests. JMS is a HEFCE Clinical Senior Lecturer and receives funding from Alzheimer’s Research UK and the Alzheimer’s Society. RWP, MSZ and RA have no competing interests.

Ethics approval AV received approval from Oxfordshire REC A (07/Q1604/28 Immune factors in neurological diseases) for the study of any patients whose samples were referred for testing. This work was submitted to the UCL/UCLH Joint Research Office who confirmed its classification as Service Evaluation rather than research according to NHS Health Research Authority guidance, and accordingly that review by a Research Ethics Committee was not required (http://www.nres.nhs.uk/applications/is-your-project-research/).

Provenance and peer review Not commissioned; externally peer reviewed.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 3.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/3.0/

REFERENCES