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# Serum antibodies from epileptic patients react, at high prevalence, with Simian Virus 40 mimotopes

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**Running title: SV40 and epilepsy**

**Background and purpose:** It has been demonstrated that inflammation may contribute to epileptogenesis, and cause neuronal injury in epilepsy. In this study the prevalence of Simian Virus 40 (SV40) antibodies, a neurotropic polyomavirus, was investigated in serum samples from 88 epileptic children/adolescents/young adults.

**Methods**: Serum antibodies reacting with specific SV40 peptides were analysed by an indirect ELISA. Synthetic peptides corresponding to the epitopes of viral capsid proteins 1-3 used as SV40 antigens.

**Results:** A significant higher prevalence of antibodies against SV40 was detected in sera from epileptic patients compared to controls (41% vs 19%). Specifically, the highest significant difference was revealed in the cohort of patients of 1.1-10 year old (54% vs 21%), with a peak in the sub-cohort of 3.1-6 year old (65% vs 18%).

**Conclusion:** Our immunologic data suggest a strong association between the epilepsy and SV40 infection.

**Keywords**: epilepsy, children, Simian Virus 40, inflammation

# Introduction

An elegant study demonstrated that inflammation may contribute to epileptogenesis [1]. Indeed, systemic infections can induce immune and inflammatory reactions in the brain, despite the blood brain barrier. This pathogenetic mechanism could be responsible of the enhanced neuronal excitability.

It has been reported that several viruses may cause febrile and non-febrile seizures, independently form the severity of the viral infection [2]. Encephalitis is an inflammation and swelling of the brain, often caused by a viral infection with acute symptomatic seizures, followed by epilepsy [3]. The pathogen causing the encephalitis appears important in predicting the likelihood of later developing epilepsy [3]. Encephalopathies and epileptic seizures are usually distinct, but they are important components of neurologic illnesses [4].

The features of epilepsy associated with congenital cytomegalovirus (HCMV) infection have been reported [5], whereas HCMV congenital neuroinfection is known to increase the risk of postnatal seizures [5]. Herpesviruses, such as the human herpesvirus 8 (HHV-8) genome has been found in the brain tissue of patient with mesial temporal lobe epilepsy [6], while PCR analysis detected HHV-6 DNA in 50–69% of surgical resection specimens for mesial temporal lobe epilepsy, showing predominantly the variant HHV-6 B subtype [7].

Human metapneumovirus (hMPV) is a relatively recent addition to the multiplicity of viruses causing respiratory illness in infants and children. The association between hMPV infection and neurologic complications, such as status epilepticus or encephalitis, has been reported [8].

Focal cortical dysplasia type IIB (FCDIIB) is a sporadic developmental malformation of the cerebral cortex highly associated with pediatric epilepsy. A recent study reported a new association between human papillomavirus 16 E6 early protein (HPV16 E6) and FCDIIB suggesting a novel etiology for FCDIIB based on HPV16 E6 expression during fetal brain development [9].

Simian virus 40 (SV40) is a neurotropic viral agent of the Asian macaque (Macacus rhesus), which is its natural host. In SV40-positive monkeys, the virus may induce progressive multifocal leukoencephalopathy (PML) [10]. Previous reports indicated that SV40 is also a human virus, which may spread through saliva, urine and stools [19, 20] as it occurs for other viruses. In addition, SV40 sequences have been found in human brain tumors, in the central nervous system (CNS) disease cases and in tissues of normal children and adults [11-13]. SV40 early region encodes for the large T antigen (Tag), which is able to abolish the functions of p53 and pRB cellular proteins, and induces chromosomal aberrations in the host cell [14]. In vivo inactivation of pRb, detected in murine neuroprogenitor cells, leads to major CNS developmental defects and high seizure rates [15]. An amino (N)-terminal fragment of SV40 Tag, that binds and inactivates pRb, alters behavioral phenotypes, including ataxia and seizures. These data indicate that inactivation of pRb in radial glial cells, a population of neuroprogenitor cells, leads to specific disruptions in CNS patterning [15].

It has been reported that SV40 infection occurs in children, although at a low prevalence [13]. The detection of SV40 antibodies in children of different ages may suggest that distinct routes of transmission are responsible of SV40 infection [16-18]. Immunologic data reported that the SV40 infection is acquired early in life, as a possible consequence of transmission in the family and in community settings, such as schools [13, 19, 20]. SV40, because of its neurotropism may infect the brain and consequently could be responsible for some cases of epilepsy. Therefore, in this study the presence of anti SV40 antibodies in epileptic patients (EP) was investigated. To this purpose, serum samples from epileptic children, adolescents and young adults, together with controls, were analysed for exposure to SV40 infection with an indirect Enzyme-Linked Immunosorbent Assay (ELISA), employing synthetic peptides from the SV40 capsid viral protein 1-3 (VPs 1-2-3) epitopes or mimotopes.

# Material and methods

## Standard protocol approvals, ethics, and patient consents

The project was approved by the County Ethical Committee, Ferrara. Informed written consent was obtained by parents of the children and adolescents involved in this study.

## Patients and healthy subjects

Serum samples were from EP (n = 88; male = 49; female = 39) affected by different types of epilepsy (Tables 1, 2). The International League Against Epilepsy (ILAE) classification for epileptic seizures and epileptic syndromes as well as the guidelines for epidemiological studies in epilepsy were followed for diagnosis and classification. The etiology of epilepsy was grouped into three broad categories, i.e. idiopathic, symptomatic, and cryptogenic/probably symptomatic [22]. Serum samples from healthy subject (HS) (n = 214; male = 113; female = 101), with the same median age of EP, were collected in the 2004-2013 period (Tables 1, 2). Sera were collected after analysis, from discarded samples of the clinical laboratory of different Institutions in Italy. They were from the Clinical Laboratory Analysis, University Hospital, Ferrara; Clinical Laboratory Analysis, County Delta Hospital, Lagosanto; Sections of Neurology, and Pediatrics, University Hospital, Ferrara. Anonymously collected sera were coded with indications of age, gender and pathology, if any.



## SV40 mimotopes

Computer assisted analyses allowed us to select 2 specific SV40 peptides, from the late viral region by comparing the three capsid proteins, VP 1-2-3, with the amino acids of the human BK (BKV) and JC (JCV) polyomaviruses which are highly homologue to SV40, as well as with other, less homologue, polyomaviruses [12, 13]. Previous ELISA results indicated that the two SV40 peptides did not cross-react with the BKV and JCV hyperimmune sera employed as controls [12, 13]. The two peptides belong to the viral capsid proteins VP1/VP2/VP3 (web site,

http://www.ncbi.nlm.nih.gov/nuccore). The amino acid sequences of the two peptides, named VP1 B and VP2/3 C, respectively, are as follows:

VP1 B: NH2- NPDEHQKGLSKSLAAEKQFTDDSP- COOH

VP2/3 C: NH2- IQNDIPRLTSQELERRTQRYLRD- COOH

VP1 B and VP2/3 C mimotopes were selected as they react specifically in indirect ELISA with the rabbit hyperimmune serum, which had been experimentally immunized with SV40 (positive control serum). BKV and JCV hyperimmune sera did not react with VP1 B and VP2/3 C peptides (negative control sera). The amino acid residues of the two specific SV40 VP peptides show low homology with the VPs of the closely related BKV and JCV and their strains, and VPs of other less related human polyomaviruses [12, 13]. The synthetic peptides were synthesized by standard procedures and were purchased from UFPeptides s.r.l., Ferrara, Italy.

**Control immune sera**

Hyperimmune sera against SV40 and BKV were obtained in rabbits immunized with purified viral stocks as previously reported [12, 13]. The serum against JCV was kindly provided by Dr. Major, NIH, Bethesda (MD), U.S.A. The immune serum anti-BKV was titered by the hemagglutination inhibition (H.A.I.) test employing human erythrocytes from the 0, Rh+ group. Anti SV40 serum was titered by neutralization assay [12, 13].

**Indirect Enzyme-Linked Immunosorbent Assay (ELISA)**

Indirect ELISA was developed and standardized to detect specific antibodies against SV40 in human sera using synthetic peptides [12, 13]. *Peptide coating.* Plates were coated with 5 µg of the selected peptide for each well, diluted in 100 µl of Coating Buffer, pH 9.6 (Candor Bioscience, Germany). *Peptide blocking.* Blocking was made with 200 µl/well of the Blocking Solution (Candor Bioscience, Germany) at 37°C for 90 min. *Primary antibody adding.* Different wells were covered with 100 µl containing the following sera: positive-control,

represented by the immune rabbit serum containing anti-SV40 antibodies, negative controls represented by the immune sera anti-BKV and anti-JCV, and human serum samples under analysis diluted at 1:20 in Low Cross-Buffer pH 7.2 (Candor Bioscience, Germany). *Secondary antibody adding.* The solution contained a goat anti-human IgG heavy and light chain specific peroxidase-conjugate (Calbiochem-Merck, Germany) diluted 1:10,000 in Low Cross-Buffer. *Dye treatment and spectrophotometric reading.* Samples were treated with 100 µl of 2,2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid (ABTS) solution (Sigma-Aldrich, Milan) and then read at the spectrophotometer (Thermo Electron Corporation, model Multiskan EX, Finland) at a wavelength (λ) of 405 nm. This approach detects the color intensity in wells where the immunocomplexes were formed by optical density (OD). *Cut-off determination.* The cut-off was determined in each assay, by an OD reading of two negative controls, added to the standard deviation and multiplied three times (+3SD). Sera with antibodies against SV40 were considered VP-positive upon reacting to both peptides of the late region and when sera, which had been analyzed three times by indirect ELISA testing, gave the same positive result [12, 13].

**Statistical analysis**

The prevalences of SV40-positive sera from epileptic children adolescent were compared with that from healthy children and adolescent. To determine significances between two groups we used two-sided chi-square test with Yates' correction or Fisher's exact test, when required. Relative risk (RR) and 95% confidence intervals (95% CI) for the association between epilepsy and SV40-positive serum samples, were calculated. Serologic Profile of OD values were analyzed with one way Anova analysis, and Newman-Keuls Multiple Comparison Test (OD mean, 95% CI). All computational analyses were performed by Prism 4.0 (GraphPad software). We considered $P$ values $< 0.05$ to be statistically significant.

# Results

## Patients

In our investigation, indirect ELISA was employed to analyse serum samples from EP (n=88; median age 10 years; range 1.1-20 years), (Table 1) and HS (n=214; median age 9 years; range 1.1-17 years) (Table 2) [12]. EP were affected by idiopathic (I) (n=48), cryptogenic (C) (n=11), symptomatic (S) (n=28), and Not Classifiable (NC) (n=1), epilepsy type (Table 1).

EP were: n=49 affected by partial (P) epilepsy, n= 38 (G) generalized epilepsy, and n=1 not classifiable (NC) epilepsy (Table1). Among EP with idiopathic epilepsy, 19 were affected by partial epilepsy, whereas 29 by generalized epilepsy; all EP with cryptogenic epilepsy were affected by partial epilepsy; among EP with symptomatic epilepsy, 19 were affected by partial epilepsy, 8 by generalized epilepsy and 1 by not classifiable epilepsy (Table 2).

The median interval time from birth to the onset of epilepsy was 60 months (mo): specifically 72 mo on EP with idiopathic epilepsy, 84 mo on EP with cryptogenic epilepsy, 10 mo on EP affected by symptomatic epilepsy, 48 mo on 1 EP with not classifiable epilepsy type (Table 1).

The median interval time from the onset of epilepsy to sera collection was 45 mo: specifically 38 mo on EP with idiopathic epilepsy, 46 mo on EP with cryptogenic epilepsy, 94 mo on EP affected by symptomatic epilepsy, and 10 mo on 1 EP with not classifiable epilepsy type (Table 1).

The median interval time from birth to the onset of epilepsy, were 72, 48, 48, mo in EP with P, G, and NC epilepsy, respectively. The median interval time from the onset of epilepsy to sera collection, were 49, 39, 24, mo in EP with P, G, and NC epilepsy, respectively (Table 2).

## Indirect Enzyme-Linked Immunosorbent Assay (ELISA)

Serum samples had been diluted at 1/20, for reactivity to SV40 epitopes from VP1, peptide B. SV40-positive sera tested by indirect ELISA diluted at 1/20 had a general cut-off, by

spectrophotometric reading, in the range of 0.17-0.19 OD. This cut-off represents the value that discriminates SV40-negative (sample bellow OD 0.17-0.19) from SV40-positive samples (above OD 0.17-0.19). The positive control, represented by the SV40 hyperimmune serum, had an OD of up to 1.8, while the two JCV and BKV hyperimmune sera, which were employed as negative controls, had an OD of less than 0.1.

Serum samples from EP, which reacted with the SV40 VP1 B mimotope, reached an overall prevalence of 43% (38/88), (Table 3). Serum samples from HS, employed as controls, reacting with the SV40 VP1 B mimotope, had an overall prevalence of 25% (54/214), (Table 3). The same assay was then addressed to detect IgG class serum antibodies against SV40 VP2/3 epitopes, which are known as VP2/3 C peptide. It turned out that serum samples from EP reacted with the SV40 VP2/3 C peptide with the prevalence of 47% (41/88) which is similar to the prevalence detected previously for the VP1 B peptide in the respective cohorts of EP (Table 3). Serum samples from HS, employed as controls, reacting with the SV40 VP 2/3 C mimotope, had an overall prevalence of 22% (47/214), (Table 3). Conversely, seronegative samples for the SV40 VP1 B peptide failed to react with SV40 VP2/3 C epitopes. The exceptions were negligible and were represented by a few serum samples which were found to be negative for VP1 B, while testing positive for VP2/3 C peptide, and vice-versa. The difference was not statistically significant ($P > 0.05$) (Table 3).

Only the samples tested positive both for the VP1 B and VP2/3 C peptides were considered SV40 positive. The two indirect ELISAs, with two distinct VP peptides gave overlapping results, thus confirming the presence of anti-SV40 VPs antibodies in human sera from EP and controls (Table 3). The overall prevalence by combining SV40-positive sera, both for VP1 B and VP2/3 C peptides, was 41% (36/88) in EP (Table 3, Fig. 1) and 19% (41/214) in HS (Table 3). The sero-prevalence difference was statistically significant (**$P < 0.001$).

EP and HS, subdivided by age (Table 3, Fig. 1), exhibited a high significant SV40 antibody prevalence in the cohort of 1.1-10 years old, (54 % Vs 21%, ** $P < 0.001$), with the highest

difference in the sub-cohort of 3.1-6 years old (65% Vs 18%, *$P < 0.01$) (Table 2). No positive results were obtained with human peptide used as a control, which had an OD of less than 0.1 (0.088-0.098). This OD value is usually consistent with SV40-negative sera. Subsequently, with the age, the prevalence of SV40 antibodies declined in EP (11/42, 26% in the 11-20 ys age group) (Table 3, Fig. 1). The prevalence of 16%; 14/88, in HS (11-17 ys age group) [13] was similar to the prevalence revealed in EP with the same median age (Table 3, Fig. 1).

Relative risks (RR) were calculated and represented as RR value and 95% of confidence interval. RR in 3.1-6 years age group was 3.6 (1.7 to 7.3), 1-10 years age group was 2.5 (1.60 to 3.9) and overall RR in 1.1-20 years was 2.1 (1.4 to 3.1).

Moreover, the prevalence of SV40 antibodies was estimated in the different types of epilepsy (Table 1). Among SV40-positive EP 36/88 (41%), 14/48 (29%) were found to be affected by idiopathic epilepsy, 5/11 (45%) were found to be affected by cryptogenic epilepsy, and 17/28 (61%) were found to be affected by symptomatic epilepsy. The difference of SV40 antibodies prevalence between the cohort of patients with symptomatic epilepsy was statistically significant compared to the cohort of EP affected by idiopathic epilepsy (*$P < 0.05$), (Table 1).

Among SV40-positive EP, 23/49 (47%) were found to be affected by partial epilepsy, 12/38 (32%) by generalized epilepsy, and 1/1 (100%) affected by no classifiable epilepsy ($P > 0.05$), (Table 2). There were no differences in economic or social status between SV40-positive and SV40-negative EP.

**Serologic profiles**

Serologic profiles of serum antibody reactivity to SV40 mimotopes in EP and HS are presented in Fig. 2. Data are presented as values of OD readings at λ 405 nm, of serum samples diluted at 1:20 detected in indirect ELISA. In scatter dot plotting, each plot represents the dispersion of OD values to a mean level, indicated by the line inside the scatter with standard error of the

mean (SEM) for each age group of EP or HS analysed. The OD readings of serum samples were stratified by age: 1.1-3 ys, 3.1-6 ys, 7-10 ys, 11-20 ys. High levels of antibodies against SV40 VP1 B were observed in EP aged 3.1- 6 ys (0.36 OD, 95% CI = 0.29-0.44) vs. EP aged 7-10 ys (0.26 OD, 95% CI = 0.21-0.30, $P < 0.05$) and vs. EP aged 11-20 ys, (0.22 OD, 95% CI = 0.19-0.25, $P < 0.001$) (Fig. 2, Panel A). High levels of antibodies against SV40 VP1 C were observed in EP aged 3.1-6 ys (0.27 OD, 95% CI = 0.23-0.31) vs. EP aged 7-10 ys (0.21 OD, 95% CI = 0.17-0.24, $P < 0.05$) and vs. EP aged 11-20 ys (0.16 OD, 95% C I= 0.14-0.18, $P < 0.001$) (Fig. 2, Panel B). High levels of antibodies against SV40 VPs, both peptides VP B and VP C were observed in EP aged 3.1-6 ys (0.31 OD, 95% CI=0.27-0.36) vs. EP aged 7-10 ys (0.23 OD, 95% CI = 0.20-0.26, $P < 0.01$) and vs EP aged 11-20 ys (0.19 OD, 95% CI = 0.17-0.21, $P < 0.001$), (Fig. 2, Panel C).

## Discussion

Viral infections of the CNS can cause long-term neurological effects, including increased risk for seizures. Studies in animals have demonstrated that inflammatory reactions in the brain can contribute to epileptogenesis through an increase in the brain of the pro-inflammatory cytokine, interleukin (IL)-1beta [23].

The role of viral infections in the etiopathogenesis of human epilepsy, however, has not been established. SV40 is a polyomavirus that was inadvertently administered to humans worldwide by contaminated vaccines mainly anti-polio vaccines produced in the 1955-1963, in naturally SV40-infected monkey kidney cells [11]. However, it cannot be excluded that SV40 was already present in human before the administration of contaminated vaccines.

Several studies indicate that SV40 may be contagiously transmitted in humans by horizontal infection. The role of SV40 in causing human pathologies, including tumors of different types, is still debated [14].

In a previous study, serum samples from HS of young age, analyzed for exposure to SV40 infection with the same ELISA employed herein [13], suggested that SV40 infection is acquired early in life [13]. The Italian pattern appears to differ from that determined in studies conducted in Sweden [18] and U.S. [17] that showed a lower prevalence of antibodies against SV40 VP in children.

In our sample of EP the prevalence of antibodies against SV40 was significantly higher (41%) than in HS (19%) with the same median age ($P < 0.001$). Moreover, in EP the highest prevalence of SV40 antibodies was observed in the age groups of 3.1-6 ys (65%) ($P < 0.01$) and 1.1-10 ys (54%) ($P < 0.001$). SV40 prevalence declined in the cohort of EP of 11-20 ys (Fig. 1, Table 3). Indeed, in the cohort of EP of 11-20 ys the prevalence of antibodies against SV40 (26%) did not differ significantly compared to that of HS (19%) of the same median age.

Serologic profile reveals higher levels of antibodies against the SV40 mimotopes, VP B and VP C, in EP aged 3.1-6 ys than in those aged 7-10 ys and in EP aged 11-20 ys (Fig. 2).

Epilepsy is characterized by recurrent spontaneous seizures due to hyperexcitability and hypersynchrony of brain neurons. Nearly one-third of patients have seizures that are refractory to available medications. A deeper understanding of mechanisms may be required to conceive more effective therapies. Recent studies point to a significant contribution by the role of glia-induced hyperexcitability and inflammation in epilepsy [24]. Uncontrolled glial-mediated immunity can cause sustained inflammatory changes that facilitate epileptogenesis [24]. Astrocytes and microglia are activated by pathogens that leading to the release of proinflammatory mediators. Glia-mediated inflammation induced by various brain insults can promote seizures and epileptogenesis, especially when normal feedback mechanisms fail to limit and extinguish inflammation.

Recent studies conducted in animal models showed that SV40 has a broad tissue tropism, identified tissues that support viral DNA replication as the brain [25]. Reactivation of SV40 in immunosuppressed rhesus monkeys can cause a demyelinating progressive multifocal leukoencephalopathy (PML)-like illness, which is the prototypical disease of JCV in immunosuppressed patients [26]. SV40 can also cause a second CNS manifestation characterized by meningoencephalitis affecting cerebral gray matter without demyelination, which is histologically distinct from PML [27]. Recent investigation on polyomaviruses in autoptic brains from autistic children revealed the presence of SV40, BKV, and JCV polyomaviruses. The combined presence of the three polyomavirsues was significantly more frequent, 67%, than in controls, 23%. Moreover, higher prevalence of SV40 antibodies was detected by this specific immunologic assay in oncologic patients affected by glioblastoma multiforme (GBM) [29], whereas SV40 sequences and large T antigen expression were detected in human brain tumors [30-32].

A complex interaction between the CNS including the blood-brain barrier, multiple infections with various infectious agents occurring in the periphery or within the CNS, and the immune

response to those various infections should be understand before the etiology of epilepsy can be fully elucidated.

Our data suggest that SV40 infection could increase the risk of epilepsy in children together with other predisposing factors. SV40, because of its neurotropism may generate a persistent infection in the CNS, followed by the brain inflammation. One may speculate that SV40 acts as a cofactor in the onset/progression of epilepsy.

Our study has some weaknesses. It is retrospective and consequently we have no proof of the presence of SV40 before the onset of epilepsy, nor we know if the mothers of the patients with brain malformations or neonatal hypoxia had been infected during pregnancy.

The high prevalence of SV40 antibodies in epileptic patients has never been reported before. Further studies both in animal models and humans are needed to clarify the role of this neurotropic viral agent in the onset/progression of epilepsy.

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**Disclosure of conflicts of interest**

The authors declare no financial or other conflicts of interest.

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## FIGURES LEGENDS

**Figure 1** SV40 age-specific seroprevalence of antibodies in HS and EP. Comparison of SV40 age-specific seroprevalence of antibodies between EP, n= 88, (1.1-20 ys) age group with HS, n= 214 (1.1-17 ys) age group.

**Figure 2** Serologic profile of serum antibody reactivity to SV40 mimotopes. Immunologic data are from serum samples from EP. Data are presented as values of OD readings at λ 405 nm, of serum samples diluted at 1:20 detected in indirect ELISA testing. In scatter dot plotting, each plot represents the dispersion of OD values to a mean level, indicated by the line inside the scatter with standard error of the mean (SEM) for each age group of EP analyzed. The OD readings of serum samples stratified by age were:1.1-3 ys, 3.1-6 ys, 7-10 ys, and 11-20 ys, Data were analyzed with one way Anova analysis, and Newman-Keuls Multiple Comparison test (OD mean, 95% CI). (A) High levels of antibodies against SV40 VP1 B were observed in EP aged 3.1-6 ys (0.36 OD, 95% CI = 0.29-0.44) vs. EP aged 7-10 ys (0.26 OD, 95% CI = 0.21-0.30, $P < 0.05$) and vs. EP aged 11-20 ys, (0.22 OD, 95% CI=0.19-0.25, $P < 0.001$). (B) High levels of antibodies against SV40 VP1 C were observed in EP aged 3.1-6 ys (0.27 OD, 95% CI = 0.23-0.31) vs. EP aged 7-10 ys (0.21 OD, 95% CI = 0.17-0.24, $P < 0.05$) and vs. EP aged 11-20 ys (0.16 OD, 95% CI = 0.14-0.18, $P <0.001$). (C) High levels of antibodies against SV40 VPs, both peptides VP B and VP C were observed in EP aged 3.1-6 ys (0.31 OD, 95% CI = 0.27-0.36) vs. EP aged 7-10 ys (0.23 OD, 95% CI = 0.20-0.26, $P <0.01$) and vs EP aged 11-20 ys (0.19 OD, 95% CI = 0.17-0.21, $P <0.001$).

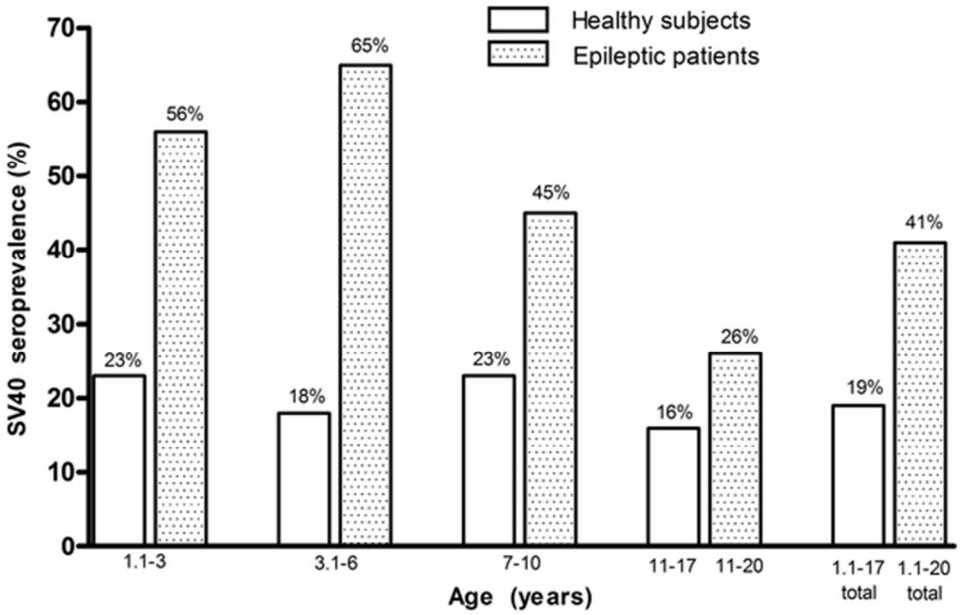


Figure 1 SV40 age-specific seroprevalence of antibodies in HS and EP. Comparison of SV40 age-specific seroprevalence of antibodies between EP, n= 88, (1.1-20 ys) age group with HS, n= 214 (1.1-17 ys) age group.
53x35mm (300 x 300 DPI)

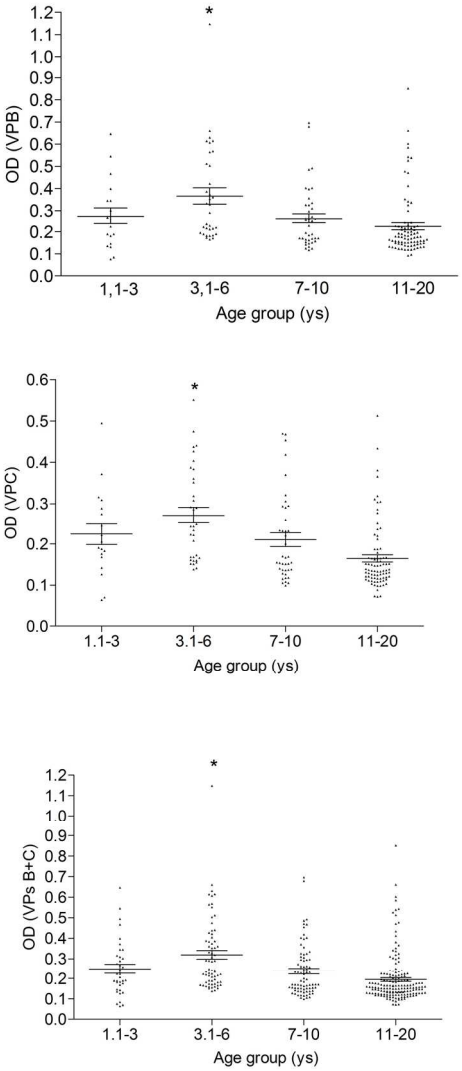


Figure 2 Serologic profile of serum antibody reactivity to SV40 mimotopes. Immunologic data are from serum samples from EP. Data are presented as values of OD readings at λ 405 nm, of serum samples diluted at 1:20 detected in indirect ELISA testing. In scatter dot plotting, each plot represents the dispersion of OD values to a mean level, indicated by the line inside the scatter with standard error of the mean (SEM) for each age group of EP analyzed. The OD readings of serum samples stratified by age were:1.1-3 ys, 3.1-6 ys, 7-10 ys, and 11-20 ys, Data were analyzed with one way Anova analysis, and Newman-Keuls Multiple Comparison test (OD mean, 95% CI). (A) High levels of antibodies against SV40 VP1 B were observed in EP aged 3.1-6 ys (0.36 OD, 95% CI = 0.29-0.44) vs. EP aged 7-10 ys (0.26 OD, 95% CI = 0.21-0.30, P < 0.05) and vs. EP aged 11-20 ys, (0.22 OD, 95% CI=0.19-0.25, P < 0.001). (B) High levels of antibodies against SV40 VP1 C were observed in EP aged 3.1-6 ys (0.27 OD, 95% CI = 0.23-0.31) vs. EP aged 7-10 ys (0.21 OD, 95% CI = 0.17-0.24, P < 0.05) and vs. EP aged 11-20 ys (0.16 OD, 95% CI = 0.14-0.18, P <0.001). (C) High levels of antibodies against SV40 VPs, both peptides VP B and VP C were observed in EP aged 3.1-6 ys (0.31 OD, 95% CI = 0.27-0.36) vs. EP aged 7-10 ys (0.23 OD, 95% CI = 0.20-0.26, P

<0.01) and vs EP aged 11-20 ys (0.19 OD, 95% CI = 0.17-0.21, P <0.001).
131x245mm (300 x 300 DPI)

**Table 1** Patients affected by epilepsy (EP): epilepsy type, median age, median time from birth to the onset of epilepsy, median time from onset of epilepsy to sera collection and relative SV40 positive sample

| Epilepsy type | Number of Sample | Median age (range age) ys | P:G:NC | Median Time from birth to the onset of epilepsy months | Median Time from onset of epilepsy to sera collection months | SV40 positive sample /sample analysed (%) |
|---|---|---|---|---|---|---|
| I | 48 | 10 (1.1-20) | 19:29:0 | 72 | 38 | 14/48 (29) |
| C | 11 | 16 (8-20) | 11:0:0 | 84 | 46 | 5/11 (45) |
| S | 28 | 9,5 (2-20) | 19:8:1 | 10 | 94 | 17/28 (61)* |
| NC | 1 | 5 | 0:1:0 | 48 | 10 | 0/1 |
| Total | 88 | 10 (1.1-20) | 49:38:1 | 60 | 45 | 36/88 (41) |

Types of epilepsy: I: Idiopathic, C:cryptogenic; S: symptomatic, NC: not classifiable. mo (months), ys (years). *The different prevalence of SV40 antibodies between the cohort of patients with symptomatic epilepsy was statistically significant compared to the cohort of patients affected by Idiopatic epilepsy ($P < 0.05$). Statistical analysis was performed with Fischer's exact test.

**Table 2** Pateints affected by epilepsy (EP): epilepsy type, median age, median time from birth to the onset of epilepsy, median time from onset of epilepsy to sera collection and relative SV40 positive sample.

| Epilepsy type | Number. of Sample | Median age (range age) ys | P:G:NC | Median Time from birth to the onset of epilepsy months | Median Time from onset of epilepsy to sera collection months | SV40 positive sample /sample analysed (%) |
|---|---|---|---|---|---|---|
| P | 49 | 12 (1-20) | - | 72 | 49 | 23/49 (47) |
| G | 38 | 10 (1-20) | - | 48 | 39 | 12/38 (32) |
| NC | 1 | 6 | - | 48 | 24 | 1/1 (100) |
| Total | 88 | 10 (1.1-20) | 49:38:1 | 60 | 45 | 36/88 (41) |

Types of epilepsy: P: partial; G: generalized. mo (months), ys (years). Statistical analyses revealed not significant differences in SV40 prevalence between EP affected by partial epilepsy with EP affected by generalized epilepsy (P>0.05). Statistical analysis was performed with Fischer's exact test.

**Table 3.** Prevalence of SV40 serum IgG antibodies in epileptic patients (EP) and healthy subjects (HS).

| Serum | Age (ys) | Number of subjects/patients | Male % | Number of positive samples (%) | | |
|---|---|---|---|---|---|---|
| | | | | VP B | VP C | VPs (B+C) |
| EP | 1.1-3 | 9 | 44 | 5 (56) | 7 (78) | 5 (56) |
| EP | 3.1-6 | 17 | 82 | 11 (65) | 12 (71) | 11 (65)* |
| EP | 7-10 | 20 | 45 | 9 (45) | 10 (50) | 9 (45) |
| EP | 1.1-10 | 46 | 59 | 25 (54) | 29 (63) | 25 (54)** |
| EP | 11-20 | 42 | 57 | 13 (31) | 12 (29) | 11 (26) |
| EP | 1.1-20 | total 88 | 56 | 38 (43) | 41 (47) | 36 (41)** |
| HS | 1.1-3 | 35 | 50 | 10 (29) | 9 (26) | 8 (23) |
| HS | 3.1-6 | 44 | 61 | 9 (20) | 9 (20) | 8 (18) |
| HS | 7-10 | 47 | 43 | 13 (28) | 14 (30) | 11 (23) |
| HS | 1.1-10 | 126 | 52 | 32 (25) | 32 (25) | 27 (21) |
| HS | 11-17 | 88 | 56 | 22 (25) | 15 (17) | 14 (16) |
| HS | 1.1-17 | total 214 | 53 | 54 (25) | 47 (22) | 41 (19) |

Human sera were from epileptic patients (EP) and healthy subjects (HS). The prevalence of SV40 antibodies in sera from EP was statistically significant higher than that of HS (* $P < 0.01$; ** $P < 0.001$). Statistical analysis was performed using the Chi square test with Yates' correction or Fischer's exact test, when required.

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