Cancer Risk in Long-term Users of Valproate: A Population-Based Case-Control Study

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Abstract

Background: **Inhibitors of histone deacetylases (HDAC) have shown promise as targeted cancer therapy. Valproate, an older anticonvulsant, has been shown to possess HDAC inhibitory activity. We undertook this case-control study to clarify whether long-term users of valproate had a reduced cancer incidence. If so, it would support HDAC inhibition as a pharmacologic principle in chemoprevention.**

Methods: **We identified 149,417 incident cancer cases in Denmark during the study period 2000 through 2005, and 597,668 age- and gender-matched controls. Data on history of cancer, past hospital admission diagnoses, and prescription history were obtained from the Danish Cancer Registry, the Danish National Patient Registry, and the Danish Prescription Registry. Primary exposure to valproate was defined as a cumulative dose of minimum 1,500 g within the past 5 years. Confounders were controlled by conditional logistic regression.**

Results: **Among the cases and controls, 81 (0.05%) and 260 (0.04%), respectively, were long-term users of valproate. For cancer overall, the crude and adjusted odds ratios were 1.25 [95% confidence interval (95% CI), 0.97-1.60] and 1.21 (95% CI, 0.95-1.56), respectively. Subgroup analyses revealed no dose or duration effect for overall cancer incidence, and no specific cancer site was found to be inversely associated with long-term use of valproate. For lung cancer, we found a positive but imprecise association (adjusted odds ratio, 2.32; 95% CI, 1.12-4.79).**

Conclusion: **Long-term valproate use is not associated with a reduced cancer risk. Our study does not support HDAC inhibition as a pharmacologic principle for general chemoprevention.** (Cancer Epidemiol Biomarkers Prev 2009;18(6):1714–9)

Introduction

Cancer develops through the accumulation of a wide variety of genetic and epigenetic alterations. Genetic alterations perturb the information transmitted by the DNA sequence and involve amplifications, translocations, deletions, and point mutations. Epigenetic modifications are concerned with the somatic heritability of information that modify gene expression without changing the DNA sequence, and the most studied epigenetic modifications involve DNA methylation and histone deacetylation. Deacetylation of histone leads to a more compact chromatin structure and has been found to play a crucial role in human carcinogenesis, and inhibitors of the enzyme histone deacetylases (HDACI) have been found to exert antiproliferative, antiapoptotic, and differentiation effects on several cancer cell lines (1).

The only drug with HDACI activity that has been used in large scale is the anticonvulsant valproate. This drug has been used for decades in the treatment of epilepsy, bipolar disorders, migraine, and neuropathic pain. Coincidentally, valproate is a potent inhibitor of class I and class II HDAC. *In vitro* studies have shown antineoplastic effects of valproate in tumor cell lines of a wide range of human cancers, including prostate cancer (2), breast cancer (3, 4), endometrial cancer (4, 5), teratocarcinoma (6), hematologic cancer (7), cervical cancer (8), neuroblastoma (9), and thoracic (lung, esophagus, and pleura) cancers (5). Of particular interest, and with potentially important clinical relevance, is the finding of strong antineoplastic activity of valproate in chemotherapy-resistant cancer cells (9).

The literature has numerous examples of the same pharmacologic principle being useful both in prevention and therapy. We did a population-based case-control study to investigate whether long-term exposure to valproate was associated with a reduced risk of cancer overall. If this could be shown, it would support the potential role of HDAC inhibition as a strategy for general chemoprevention.

Materials and Methods

The study was conducted as a population-based case-control study of incident cancers in Denmark (population, 5.4 million) during the period January 1, 2000 to December 31, 2005.

Data Sources. We used data from four different sources: the Danish Cancer Registry (DCR), the Danish

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National Registry of Patients (DNRP), the Prescription Database of the Danish Medicines Agency, and the Danish Person Registry.

The DCR has recorded incident cases of cancer on a nationwide basis during the period 1943 to 2003 and has been shown to have accurate and almost complete ascertainment of cancer cases (10). The cancer diagnoses in the DCR are coded according to a Danish version of the 7th revision of the International Classification of Diseases (ICD-7). At the time of study, the DCR was updated through 2003.

The DNRP contains data on all secondary care contacts in Denmark since 1977. From 1995, outpatient diagnoses have been included systematically. Discharge diagnoses are coded according to ICD-8 from 1977 to 1993 and ICD-10 since 1994. Virtually all medical care in Denmark is furnished by the public health authorities, whereby this data resource allows true population-based studies, covering all inhabitants of Denmark (11).

The Prescription Database of the Danish Medicines Agency contains data on all prescription drugs redeemed by Danish citizens since 1995. Prescription data include the Central Person Registry number, the date of dispensing, the substance, brand name, and quantity. The dosing instruction and the indication for prescribing are not recorded (12). Drugs are categorized according to the Anatomic Therapeutic Chemical code, a hierarchical classification system developed by the WHO for purposes of drug use statistics (13). The quantity for each prescription is expressed by the defined daily dose (DDD) measure, also developed by the WHO (13). The DDD for a drug is established by an expert panel as the typical maintenance dose when the drug is used by an adult for its main indication. Thereby, it is possible to provide aggregate statistics for related drugs with different potency. The DDD for valproate is 1,500 mg.

The Danish Person Registry contains data on vital status (date of death) and migrations in and out of Denmark, which allowed us to extract controls and to keep track of all subjects.

All data sources were linked by use of the Central Person Registry number, a unique identifier assigned to all Danish citizens since 1968 that encodes gender and date of birth. All linkage occurred within Statistics Denmark, a governmental institution that collects and maintains electronic records for a broad spectrum of statistical and scientific purposes.

Cases and Controls. Cases were all patients with a first time occurrence of a cancer diagnosis (the index date) in the period January 1, 2000 to December 31, 2005. For the period 2000 through 2003, we used the DCR. As DCR was not updated beyond 2003, we used the DNRP for 2004 and 2005. In DNRP, both inpatient and outpatient contacts were used to retrieve cases. Individuals who had a cancer diagnosis before 2000 were excluded, and we further excluded cases who were not inhabitants in Denmark at the index date or who immigrated to Denmark less than 5 y before index date.

Controls were extracted by use of a risk set sampling strategy. For each case, we selected four controls randomly among all Danish citizens with the same gender and birth year and who did not fulfill any of the exclusion criteria. Cases were eligible as controls until their first occurrence of cancer. The index date for the four matched controls was taken to be that of the corresponding case. Thereby, the estimated odds ratios (OR) are unbiased estimates of the incidence rate ratio (14). Because the DCR does not contain the exact diagnosis date, only month and year, we assigned the 15th of the particular month as the index date for cases identified in the period 2000 to 2003.

Analysis. Data were analyzed according to a conventional matched case-control design. Main exposure was use of valproate at a cumulated dose of at least 1,000 DDD, or 1,500 g, within a 5-y period before the index date. The reference for all analyses was never-use of valproate unless otherwise specified.

According to the literature, a potential antineoplastic effect of valproate may be expected for a broad variety of cancers, and in our primary analysis, we therefore used a composite end point defined by the occurrence of any type of cancer (DCR: ICD-7, 140-205; ICD10, C00-C97; ICD8, 140-207). Secondary analyses were done on specific cancer types: lung cancer, colon cancer, breast cancer, prostate cancer, hematologic cancers, tobacco-related cancer (cancers of the lung, larynx, buccal cavity, pharynx, esophagus, pancreas, bladder, renal pelvis, kidney, stomach, cervix, and acute myeloid leukemia), and non–tobacco-related cancer (all others).

Confounders were adjusted for by conditional logistic regression. The following potential confounders were included in the regression model: (*a*) a prior discharge diagnosis of chronic obstructive pulmonary disease (COPD) as a crude marker of heavy smoking; (*b*) a prior discharge diagnosis of inflammatory bowel disease; (*c*) a modified Charlson Index that contains 19 categories of comorbidity (Each category has an associated weight based on the adjusted risk of 1-y mortality. The higher the score, the more severe the burden of comorbidity. We disregarded the cancer diagnoses when computing the Charlson Index, as this could produce a nonsymmetrical comorbidity rating of cases and controls; ref. 15); and (*d*) a cumulated dose within the last 5 y before index date of nonsteroidal anti-inflammatory drug (NSAID) and high-dose aspirin (≥500 DDD), hormone replacement therapy (HRT; ≥500 DDD), oral contraceptives (≥500 tablets), finasteride (≥500 DDD), and statins (≥500 DDD).

None of the potential confounders changed the OR >5% when included in a bivariate model, and they may as such not qualify as important confounders (16). However, they were included on theoretical grounds as they were potential modifiers of the cancer risk. Both adjusted and unadjusted estimates are presented.

For individuals whose cumulative valproate dose was ≥200 DDD, we calculated the average daily dose as the cumulative dose between first and last prescription divided by the number of days between first and last prescription. We also explored if there was a cumulative dose-response relationship and/or a relation between the duration of exposure and the risk of cancer.

The specificity of the potential valproate effect was examined by running the same analyses for carbamazepine, oxcarbazepine, and lithium. The rationale was that any confounders at play in the valproate-cancer association would also be present for drugs with similar indication and use. Carbamazepine and oxcarbazepine are used as anticonvulsants and for neuropathic pain, and lithium is used as a "mood stabilizer" in bipolar affective disorder. We considered those exposed who had redeemed

Table 1. Characteristics of cancer cases and controls

	Cases (n = 149,417)	Controls (n = 597,668)
Men	71,248 (47.7%)	284,992 (47.7%)
Women	78,169 (52.3%)	312,676 (52.3%)
Age 0-39	7,391 (4.9%)	29,564 (4.9%)
Age 40-59	37,890 (25.4%)	151,560 (25.4%)
Age 60-79	77,628 (52.0%)	310,512 (52.0%)
Age ≥80	26,508 (17.7%)	106,032 (17.7%)
Ever a diagnosis of		
COPD	10,110 (6.8%)	33,348 (5.6%)
Inflammatory bowel disease	2,527 (1.7%)	7,424 (1.2%)
Diabetes	11,002 (7.4%)	41,074 (6.9%)
Long-term exposure to		
Valproate*	81 (0.05%)	260 (0.04%)
NSAIDs†	4,586 (3.1%)	15,549 (2.6%)
HRT†	9,409 (6.3%)	27,668 (4.6%)
Oral contraceptives†	2,399 (1.6%)	8,618 (1.4%)
Finasteride†	695 (0.5%)	2,081 (0.3%)
Statins†	5,259 (3.5%)	19,340 (3.2%)
Carbamazepine*	290 (0.2%)	1,049 (0.2%)
Oxcarbazepine*	227 (0.2%)	695 (0.1%)
Lithium*	229 (0.2%)	779 (0.1%)
Ever use of valproate	800 (0.5%)	2,412 (0.4%)
Charlson index 0	98,487 (65.9%)	411,586 (68.9%)
Charlson index 1	30,007 (20.1%)	107,943 (18.1%)
Charlson index ≥2	20,923 (14.0%)	78,139 (13.1%)
Cancer site		
Lung cancer	16,748 (11.2%)	NA
Colorectal cancer	17,322 (11.6%)	
Breast cancer	19,947 (13.3%)	
Prostate cancer	10,377 (6.9%)	
Tobacco-related cancer‡	37,721 (25.2%)	
Non–tobacco-related cancer	111,896 (74.9%)	
Hematologic cancer	9,079 (6.1%)	

*Defined by use of at least 1,000 DDD over the past 5 y.
†Defined by use of at least 500 DDD over the past 5 y.
‡Lung, larynx, buccal cavity, pharynx, esophagus, pancreas, bladder, renal pelvis, kidney, stomach, cervix, and acute myeloid leukemia.

prescriptions for at least 1,000 DDD within the past 5 y, and we had never-use of the particular drug as reference in all analyses.

Approval. The study was approved by Statistics Denmark's scientific board. Approval from an ethics committee was not required.

Power Calculation. The precision of our estimates depended largely on the number of cases exposed to valproate.

All other elements, unexposed cases, and exposed and unexposed controls were expected to contribute substantially less to the variance of the estimates. Based on age- and gender-specific exposure data in Odense Pharmacoepidemiologic Database covering ~10% of the Danish population (12) and national data on cancer incidence (17), we could calculate an expected 75 exposed cases in Denmark during a 6-y period under the null hypothesis. Assuming a Poisson distribution of exposed cases, this would allow a variation coefficient of 12% ($=1\sqrt{75}$) for the main estimate, if the OR was close to 1.0. The variation coefficient would increase to 17% if the OR was reduced to 0.5.

All statistical analyses were done by Stata version 8 (Stata Corp.).

Results

We identified 149,417 incident cancer cases in Denmark during the study period. Their median age was 67 years with an interquartile range of 57 to 77 years. Women and men accounted for 52.3% and 47.7% of the cases, respectively. The characteristics of the cases and the 597,668 controls are detailed in Table 1. As expected, the most prevalent cancer sites were breast (13.3%), colorectal (11.6%), lung (11.2%), and prostate (6.9%).

Among the cases, 800 (0.5%) had a record of valproate use, whereof 81 (0.05%) had used >1,000 DDD during the past 5 years. Corresponding figures for the controls were 2,412 (0.4%) and 260 (0.04%). The crude OR for cancer associated with long-term valproate use with cancer was 1.25 [95% confidence interval (95% CI), 0.97-1.60]. The adjusted OR was 1.21 (95% CI, 0.95-1.56). None of the specific cancer sites had estimates significantly below unity (Table 2). For lung cancer, the adjusted OR was significantly elevated (2.32; 95% CI, 1.12-4.79). Overall, adjustment for confounders had little effect on the estimates.

The explorative dose-response and duration-response analyses revealed no subsets with ORs below unity (Table 3). The highest cumulative doses also showed the highest ORs, 1.43 (95% CI, 0.82-2.50) and 1.59 (95% CI, 0.73-3.47), for 2,000 to 3,000 DDD and >3,000 DDD, respectively. All cancers were included in these analyses.

None of the subgroups defined by gender or age suggested any cancer-preventive effect (Table 4). Adjusted ORs varied between 1.06 and 1.47 with all CIs overlapping unity.

The adjusted estimates for carbamazepine, oxcarbazepine, and lithium were very similar to the main estimate, 1.09 (95% CI, 0.95-1.24), 1.27 (95% CI, 1.09-1.48), and 1.19 (95% CI, 1.03-1.39), respectively (Table 5).

Discussion

We found no indication of a cancer-preventive effect of long-term valproate exposure. This was found across a

Table 2. The association between long-term exposure to valproate and cancer risk according to cancer site

Cancer site	Cases exposed/nonexposed	Controls exposed/nonexposed	Crude OR (95% CI)	Adjusted* OR (95% CI)
All cancers	81/148,617	260/595,256	1.25 (0.97-1.60)	1.21 (0.95-1.56)
Colorectal	14/17,230	30/68,988	1.85 (0.98-3.50)	1.81 (0.96-3.42)
Hematologic	2/9,048	17/36,166	0.46 (0.11-2.00)	0.45 (0.10-1.94)
Lung	12/16,657	21/66,727	2.29 (1.12-4.65)	2.32 (1.12-4.79)
Breast	4/19,851	26/79,468	0.62 (0.21-1.76)	0.61 (0.21-1.75)
Tobacco related	21/37,534	65/150,243	1.29 (0.79-2.11)	1.23 (0.75-2.02)
Non–tobacco related	60/111,283	196/445,809	1.22 (0.92-1.63)	1.21 (0.91-1.62)
Prostate	6/10,334	18/41,331	1.30 (0.52-3.28)	1.34 (0.53-3.39)

*Adjusted for a previous diagnosis of inflammatory bowel disease, COPD, diabetes, Charlson index, or previous long-term exposure to HRT, oral contraceptives, finasteride, statins, and NSAIDs or high-dose aspirin.

Table 3. The association between valproate use and risk of cancer according to exposure pattern

Pattern	Cases exposed/nonexposed	Controls exposed/nonexposed	Crude OR (95% CI)	Adjusted* OR (95% CI)
Cumulative exposure (DDD)[†]				
200-499	121/148,617	436/595,256	1.11 (0.91-1.36)	1.05 (0.86-1.29)
500-999	94/148,617	285/595,256	1.33 (1.06-1.69)	1.30 (1.03-1.64)
1,000-1,999	55/148,617	196/595,256	1.12 (0.83-1.51)	1.10 (0.82-1.49)
2,000-2,999	17/148,617	46/595,256	1.48 (0.85-2.58)	1.43 (0.82-2.50)
≥3,000	9/148,617	21/595,256	1.71 (0.79-3.74)	1.59 (0.73-3.47)
Estimated daily dose (DDD/day)[‡]				
0-0.19	42/148,617	129/595,256	1.31 (0.92-1.85)	1.24 (0.87-1.75)
0.2-0.39	103/148,617	340/595,256	1.21 (0.97-1.51)	1.16 (0.93-1.45)
0.4-0.59	127/148,617	399/595,256	1.28 (1.05-1.56)	1.23 (1.01-1.51)
0.6-0.79	85/148,617	325/595,256	1.05 (0.82-1.33)	1.01 (0.79-1.28)
0.8-0.99	81/148,617	201/595,256	1.62 (1.25-2.10)	1.54 (1.19-2.00)
≥1.0	111/148,617	400/595,256	1.11 (0.90-1.37)	1.07 (0.86-1.32)
No. prescriptions[§]				
1-5	466/148,617	1293/595,256	1.44 (1.30-1.61)	1.38 (1.24-1.53)
6-11	124/148,617	399/595,256	1.24 (1.02-1.52)	1.19 (0.97-1.46)
12-23	111/148,617	388/595,256	1.14 (0.92-1.41)	1.09 (0.88-1.35)
≥24	99/148,617	332/595,256	1.21 (0.96-1.51)	1.17 (0.93-1.46)
Duration of valproate use (y)[‖]				
0-1.9	306/148,617	1022/595,256	1.20 (1.05-1.36)	1.14 (1.00-1.30)
2.0-2.9	60/148,617	207/595,256	1.18 (0.88-1.57)	1.12 (0.84-1.49)
3.0-3.9	53/148,617	164/595,256	1.29 (0.95-1.76)	1.24 (0.91-1.69)
≥4.0	130/148,617	401/595,256	1.30 (1.07-1.59)	1.27 (1.04-1.55)

NOTE: The reference for all analyses is never-use of valproate. All cancer sites are included. DDD = 1.5 g for valproate.
*Adjusted for a previous diagnosis of inflammatory bowel disease, COPD, diabetes, Charlson index, or previous long-term exposure to HRT, oral contraceptives, finasteride, statins, and NSAIDs or high-dose aspirin.
[†]Test for trend: $P = 0.25$.
[‡]Test for trend: $P = 0.83$.
[§]Test for trend: $P = 0.04$.
[‖]Test for trend: $P = 0.35$.

wide range of patient subgroups, different cancer sites, different assumptions about exposure, and different use patterns.

There are numerous studies showing effect of valproate in selected cancer cell lines (2-9, 18, 19) or patient groups, but to our knowledge, this is the first epidemiologic study of its chemopreventive potential.

For one cancer site, lung cancer, we found a significantly elevated risk (OR, 2.32; 95% CI, 1.12-4.79). The possible explanations include a genuine biological effect, confounding by smoking or a chance finding. We know of no factor related to cancer biology or pharmacology that would explain a specific promoting effect of valproate for lung cancer or a sensitizing effect toward tobacco. As for the possibility of confounding by smoking, surveys have indicated that the prevalence of smoking among epilepsy patients is 56% to 120% higher than for healthy subjects (20-22). Given that epilepsy is the main indication for long-term use of valproate and that the lower limit of the CI is 1.12, the estimate for lung cancer may well be explained by smoking as a confounder. We did, however, find very similar ORs for tobacco-related and unrelated cancers in general, 1.23 versus 1.21, and a chance finding is also possible. The link between valproate use and lung cancer was not a prespecified hypothesis in our protocol, and it would need further confirmation before any inferences can be made.

The strength of our study is a thorough and comprehensive registering of a large population base, the entire Danish population followed for at least 11 years. The validity of the diagnoses in the DCR is high (10), and the sensitivity and predictive value of cancer diagnoses in the DNRP are also high (23, 24). Finally, the prescription data in our data sources have a high accuracy (12, 25).

The valproate-cancer association might be sensitive to protopathic bias (26); that is, valproate could be used for an early manifestation of a cancer that becomes clinically apparent later. For example, valproate could be used to

Table 4. The association between long-term use of valproate and the risk of cancer according to patient subgroup

Subgroup	Cases exposed/nonexposed	Controls exposed/nonexposed	Crude OR (95% CI)	Adjusted* OR (95% CI)
Age (y)[†]				
0-39	6/7,385	23/29,541	1.04 (0.42-2.56)	1.06 (0.43-2.61)
40-59	28/37,862	95/151,465	1.18 (0.77-1.80)	1.10 (0.72-1.69)
60-79	40/77,588	123/310,389	1.30 (0.91-1.86)	1.24 (0.87-1.77)
≥80	7/26,501	19/106,013	1.47 (0.62-3.51)	1.47 (0.62-3.49)
Gender				
Men	46/71,202	135/284,857	1.36 (0.98-1.90)	1.32 (0.94-1.84)
Women	35/78,134	125/312,551	1.12 (0.77-1.63)	1.10 (0.76-1.60)

NOTE: All cancer sites are included.
*Adjusted for a previous diagnosis of inflammatory bowel disease, COPD, diabetes, Charlson index, or previous long-term exposure to HRT, oral contraceptives, finasteride, statins, and NSAIDs or high-dose aspirin.
[†]Test for trend: $P = 0.60$.

Table 5. The association between long-term use of carbamazepine, oxcarbazepine, or lithium and the risk of cancer

Drug	Cases exposed/nonexposed	Controls exposed/nonexposed	Crude OR (95% CI)	Adjusted* OR (95% CI)
Carbamazepine	290/147,698	1049/591,971	1.11 (0.98-1.27)	1.09 (0.95-1.24)
Oxcarbazepine	227/148,510	695/595,151	1.31 (1.13-1.52)	1.27 (1.09-1.48)
Lithium	229/148,777	779/595,397	1.18 (1.02-1.37)	1.19 (1.03-1.39)

NOTE: All cancer sites are included. The reference for all analyses is never-use of the drug in question.
*Adjusted for a previous diagnosis of inflammatory bowel disease, COPD, diabetes, Charlson index, or previous long-term exposure to HRT, oral contraceptives, finasteride, statins, and NSAIDs or high-dose aspirin.

treat symptomatic epilepsy from early brain metastases or to treat malignant neuropathy. This could potentially mask a genuine cancer-preventive effect of valproate. However, as our main exposure was defined by massive, long-term use, this is unlikely to be a problem. An underlying cancer would be evident long before a subject would qualify as exposed. Our main exposure defined by long-term use and repeated prescriptions also renders our study less sensitive to problems with exact timing of the cancer relative to valproate use and to issues of noncompliance or miscoded prescriptions.

Among the limitations are a relatively short study period, determined by the available data. However, the antiproliferative effects of valproate and new HDACIs shown in laboratory studies indicate that compounds with HDACI activity may also interrupt the development of cancer at later stages in carcinogenesis (5). In addition, we have done some analyses in the regional Odense Pharmacoepidemiologic Database prescription database that covers Funen County in Denmark as far back as 1990. For the 342 Funen County residents who fulfilled our main exposure criterion in 2006, the median cumulative exposure was 1,560 DDD (interquartile range, 1,280-2,300) and the median (known) duration was 11.1 years (interquartile range, 6.6-15.5). In other words, given that a person is classified as exposed, he is likely to have used valproate considerably longer and with higher cumulative dose than 5 years and 1,000 DDD. Our point estimate was above unity (i.e., a trend toward a carcinogenic effect). On these grounds, it is unlikely that a new study with even substantially longer duration would show a preventive effect.

Another limitation is the lack of data on lifestyle factors, particularly smoking, as discussed above. The register study frame within Statistics Denmark did not allow collection on data other than those held in the available registers. In addition, given the size of the material required to undertake our study, it would have been prohibitively resource demanding to collect data on lifestyle factors that would enable us to adjust for it. As a safeguard against other unknown confounders, we analyzed the association for oxcarbazepine, carbamazepine, and lithium that are all used for indications similar to valproate but do not posses HDACI properties. The OR estimates were very similar to those of valproate, albeit with better statistical precision, because there were more exposed subjects.

The valproate doses used in clinical practice could be too low to achieve a cancer-preventive effect. Valproate as HDACI has been tested *in vitro* in concentration ranges of 0.5 to 3.0 mmol/L (27-30), whereas the usual therapeutic interval is 0.35 to 0.70 mmol/L. However, there was nothing in our data to suggest a preventive effect within the highest dose range or with large cumulative doses. Finally, we cannot rule out that valproate could have carcinogenic potential. Observations from Milutinovic's group suggest that valproate might induce genes relating to cancer development and metastases by causing active DNA demethylation of oncogenes (31). However, the demethylation activity of valproate has not been confirmed by other groups. It has been reported that patients treated with valproate may develop overt acute leukemia (32, 33).

Our study does not refute the potential of general cancer chemoprevention by HDAC inhibition. But unfortunately, it cannot be shown by using existing data on subjects who coincidentally have taken a drug with HDACI activity on a long-term basis. There is also a possibility that valproate may be effective in particular sites without having "global" cancer-preventive effect. Our study did not have the statistical power to adequately exclude site-specific effects. Possibly, new generations of HDACI suitable for long-term administration could be tested in clinical trials in high-risk individuals.

Disclosure of Potential Conflicts of Interest

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