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# Liver enzymes and stroke risk in middle-aged German adults

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ABSTRACT

*Objective:* To investigate the association between the liver enzymes γ-glutamyltransferase (GGT) and (alanine aminotransferase) ALT and risk of stroke, its subtypes including TIA as well as fatal and non-fatal events.

*Methods:* A case-cohort study within the European Prospective Investigation into Cancer and Nutrition-Potsdam Study comprising 27548 middle-aged subjects was designed. GGT and ALT were measured in plasma of 353 individuals who developed a stroke and in 2110 individuals who remained free of cardiovascular events during a mean follow-up of 8.2 ± 2.2 years. Cox proportional-hazard models were applied to evaluate the association between liver enzymes and stroke risk.

*Results:* After adjustment for established clinical and lifestyle factors, a 1 unit change in naturally logged GGT was related to a 1.20 (95%CI: 1.03–1.40) increased stroke risk. Risk estimates did not significantly differ between fatal (Relative Risk (RR) = 1.35, 95%CI: 1.14–1.61) and non-fatal events (RR = 1.15; 95%CI: 0.97–1.36). ALT was not associated with overall stroke risk (RR = 0.95; 95%CI: 0.71–1.26). However, in subtype analyses we observed in multivariable adjusted models a significant increased risk of hemorrhagic stroke (RR = 2.00; 95% CI: 1.01–3.96), but decreased risk of ischemic stroke (RR = 0.66; 95%CI: 0.44–0.998).

*Conclusions:* Our data provide further evidence for a link between GGT, but not ALT and overall stroke suggesting that these biomarkers are involved in different pathways of disease development. Further studies are needed to clarify the putative relationships between ALT and subtypes of stroke.



## 1. Introduction

γ-glutamyltransferase (GGT) and alanine aminotransferase (ALT) are liver enzymes that are routinely used for the screening of liver disease [1]. In this context, for a long time GGT has been only known as a marker for alcohol related liver disease [2,3]. However, beginning in the last decade there is growing evidence about a linear association between GGT activity and risk of cardiovascular disease independent from alcohol intake [4,5]. More recently, both biomarkers and especially ALT have been discussed as marker for non-alcoholic fatty liver disease (NAFLD) [6], which itself is suggested as a risk factor for atherosclerosis and cardiovascular disease [7,8].

With respect to GGT, Fraser et al. demonstrated in a meta-analysis based on 7 prospective studies a strong association between GGT and stroke [4]. A recently published study on the association between GGT and stroke risk observed also a significant association, but in women only, based on a population of Japanese men and women [5]. Furthermore, the British Regional Heart Study, a prospective study of middle-aged men, observed a significantly increased risk of stroke, but was not able to differentiate types of stroke [9]. In contrast to the available evidence on associations between GGT and stroke risk, only very few prospective studies so far analyzed the association between ALT and the risk of stroke [4,10,11]. Therefore, we investigated the association between these liver enzymes and stroke risk in the EPIC-Potsdam study and provide additional data for subgroups of stroke including TIA and separate analyses for fatal and non-fatal events.

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## 2. Methods

### 2.1. Study population

The EPIC-Potsdam Study is part of the large-scale Europe-wide prospective cohort study EPIC and includes 27,548 individuals (16,644 women and 10,904 men). Participants were recruited between 1994 and 1998 from the general population of Potsdam and surroundings with the preferred ages 35–65 years in women and 40–65 years in men [12]. The baseline examination included standardized blood pressure measurements, anthropometric measurements, self-administered questionnaires on diet and lifestyle, PC-guided interviews including questions about prevalent diseases and blood sampling. Blood was collected from 95% of participants at the Potsdam center. All participants gave written informed consent and the Ethics Committee of the Federal State Brandenburg approved all study procedures. Information on incident diseases and changes in lifestyle is biennially assessed by self-administered questionnaires [13]. Response rates for all follow-up rounds so far have exceeded 90% at each occasion.

After exclusion of subjects with a history of stroke including TIA or myocardial infarction at baseline, we identified 179 ischemic strokes (IS), 173 TIA, 40 hemorrhagic strokes and 6 strokes with undefined etiology among 25150 participants during a mean follow-up of 8.2 ± 2.2 years. For the present analysis, 42 cases had to be excluded as blood specimens were not available, covariate data or biomarker measurements were missing. Of three individuals, who had both TIA and ischemic stroke, we considered only the first event, leaving 353 incident cases for analyses. Excluded cases did not differ from other cases in main baseline characteristics (data not shown).

The association of biomarker levels with risk of stroke was analyzed using a case-cohort design [14,15]. For these purposes we randomly selected a sub-cohort of 2500 individuals from the EPIC-Potsdam study population. Of the sub-cohort, 226 participants with a self-reported diagnosis of stroke or myocardial infarction at baseline or missing follow-up data were excluded from analyses. We further excluded 164 participants without blood samples or for whom not all biomarkers were available, leaving a final sub-cohort of 2110 subjects. No relevant differences in baseline characteristics were observed between excluded non-cases (other than prevalent cases) and non-cases used in our analyses (data not shown). In agreement with the case-cohort design, 44 incidents of stroke formed part of the subcohort. Thus the final case-cohort sample consisted of 353 cases (including 158 ischemic strokes, 36 hemorrhagic strokes with 25 intrarecerebral hemorrhage and 11 subarachnoidal hemorrhage, 155 TIA, and 4 undefined strokes) and 2066 non-cases (Fig. 1).

### 2.2. Ascertainment of stroke and TIA

All cases were verified by contacting the patients' attending physician or by review of death certificates [16]. In the follow-up questionnaire a validated, brief screening instrument for stroke and TIA was included. It consisted of one general question asking for physician-diagnosed stroke or TIA in the past. To increase sensitivity, the questionnaire included additional questions about typical stroke symptoms. Subsequent validation of stroke and TIA was based on medical records and followed an established protocol that included a standardized form. Information was collected about subtype of stroke with ICD-10 code, hospitalization, symptoms, duration, diagnostic procedures and outcome [16]. Stroke was defined as a focal neurologic deficit with a sudden onset and vascular mechanism lasting more 24 h, and a TIA was defined as a neurologic deficit lasting less than 24 h. For definition of TIA we did not consider pathological findings in brain imaging. 37.4% of TIA cases were diagnosed in a hospital. Furthermore, 73% of TIA cases had a MRI or CT scan indicating thorough work-up of suspicious symptoms in the majority of cases. Among the participants having had a MRI or CT, 18% had pathological findings confirming a cerebral ischemia. According to ICD-10 cases were classified as incident IS (ICD-10 I63.0-I63.9), intracerebral (ICD-10 I61.0-I61.9) or subarachnoidal hemorrhage (ICD-10 I60.0-I60.9), undetermined stroke (ICD-10 I64.0-I64.9) or TIA (ICD-10 G45.9).

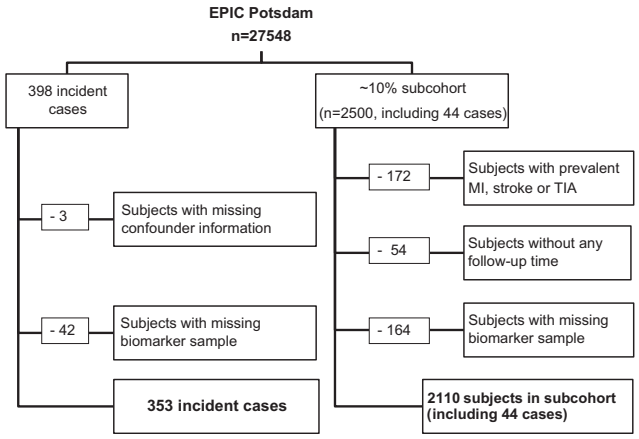


**Fig. 1.** Design of the case-cohort study based on the EPIC-Potsdam cohort.

### 2.3. Assessment of risk factors and covariates

Lifestyle characteristics, including regular physical exercise and smoking history, were documented at baseline by trained interviewers during a PC-guided interview. Sports activity was defined as the mean time spent on leisure time physical activities during the summer and winter seasons (hours/week). Prevalent hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or self-reporting of a diagnosis or of use of antihypertensive medication. The prevalence of diabetes at baseline was evaluated by a physician using information on self-reported medical diagnosis, medication records and dieting behavior. In ambiguous cases, the diagnosis was confirmed by personal communication with the participant and/or treating physician. Dietary habits including alcohol consumption during the preceding year were assessed by a validated self-administered food frequency questionnaire.

### 2.4. Blood collection and laboratory analysis

A total of 30 ml of venous blood was collected at baseline from participants at the Potsdam center; fractioned into serum, plasma, buffy coat, and erythrocytes; and was aliquoted into straws and stored until the time of analysis. All biochemical analyses were performed in one run at the Department of Internal Medicine, University of Tübingen in 2007. Plasma levels of GGT, ALT, HDL-cholesterol, total cholesterol, and high-sensitive CRP (hs-CRP) were measured with the automatic ADVIA 1650 analyzer (Siemens Medical Solutions, Erlangen, Germany).

### 2.5. Statistical analysis

Statistical analysis was performed using SAS software package, release 9.1 (SAS Institute, Cary, NC). All tests performed were two-sided with $p < 0.05$ considered as statistically significant. With respect to gender differences in liver enzyme activities sex-specific