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Apolipoprotein e4 allele and cognitive decline in elderly men

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Abstract

***Objectives*—To determine whether polymorphism of apolipoprotein E—notably, the e4 allele—predicts cognitive deterioration in the general population.**

***Design*—Population based cohort investigated in 1990 and in 1993.**

***Setting*—Zutphen, the Netherlands.**

***Subjects*—Representative cohort of 538 Dutch men aged 70-89 at baseline.**

***Main outcome measures*—Cognitive function assessed by mini mental state examination, change in cognitive function and incidence of impaired cognitive function at three years.**

***Results*—The baseline prevalence of impaired cognitive function (mini mental state examination score ≤25) was higher among carriers of the e4 allele compared wth men without the allele (41·0% (55) *v* 31·1% (122) P=0·03), and this result was still valid after adjustment for age, occupation, smoking, alcohol use, and cardiovascular diseases. The decline in cognitive function at three years was largest in men homozygous for e4 (−2·4 points), intermediate in those heterozygous for e4 (−0·7 points), and lowest in men without e4 (−0·1 points), and it was independent of other risk factors (P=0·02). The risk of developing impaired cognitive function during follow up was significantly increased in allele carriers compared with non-carriers (27·6% (16/58) *v* 15·5% (32/207)). The adjusted odds ratio was 2·87 (95% confidence interval 1·29 to 6·42). Twenty two per cent of the risk of developing impaired cognitive function in this population may be attributable to the e4 allele.**

***Conclusions*—The apolipoprotein e4 allele predisposes to cognitive decline in a general population of elderly men.**

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Introduction

Cognitive performance is an important indicator of health and functioning in elderly people and is, as such, of increasing importance in our aging society. Cognitive decline can be regarded as a marker of a dementing process, and current knowledge of the aetiology of dementia may point at potential risk factors for reduced cognitive function with aging.

The genetic heterogeneity of apolipoprotein E may have a functional role in the pathogenesis of both late and early onset familial Alzheimer's disease.[1,2] Apolipoprotein E is a plasma protein involved in the metabolism of cholesterol and triglycerides. It is present in particles such as chylomicrons, very low density lipoprotein and their remnants, and high density lipoprotein. It serves as ligand for receptor mediated uptake of these lipoproteins in the liver and other organs.[3] It is a polymorphic protein with e2, e3, and e4 as common isoforms. The gene is localised on the long arm of chromosome 19.[4,5] Several studies have shown that apolipoprotein E polymorphism affects plasma concentrations of lipid and lipoprotein,[6-8] and the e4 isoform was found to be associated with an increased risk of premature coronary heart disease.[6,9]

Apolipoprotein E is also involved in the regenertive response of injured nerve tissue, and it is present in amyloid plaques of various amyloid forming diseases.[1,10] Linkage between markers in the apolipoprotein E gene region and late onset familial Alzheimer's disease has been reported.[11] Recently, it has been shown that the prevalence of the e4 allele is increased in subjects with late onset familial and sporadic Alzheimer's disease,[1,12-15] as well as in early onset cases.[2,16] In addition, increased prevalence of the e4 allele was reported from patients with vascular dementia.[17]

It is not yet clear to what extent apolipoprotein E affects overall cognitive performance, and population based studies are currenty lacking. Because of its potential role in atherosclerosis and vascular dementia as well as in Alzheimer's disease we hypothesised that the e4 allele is a risk factor for cognitive dysfunction, and we investigated this issue in a three year longitudinal study of a population based cohort of elderly men in the Netherlands.

Subjects and methods

SUBJECTS

The Zutphen elderly study is a longitudinal investigation of risk factors for chronic diseases in elderly men carried out in Zutphen, a town in the eastern part of the Netherlands.[18] It represents a continuation of the Zutphen study, the Dutch contribution to the seven countries study.[19] In 1985, 555 men of the original cohort born between 1900 and 1920 were still alive and were invited for new examinations together with an additional random sample of 711 men of the same age group (65-84 years). In 1985, 939 men (response rate

74%) entered the study, and this group formed the cohort of the Zutphen elderly study. In 1990, 544 out of 718 (76%) surviving men underwent further investigation, including a mini mental state examination. Complete information on all risk factors was available for 538. The examination was repeated on 378 of the 553 survivors in the spring of 1993.

MINI MENTAL STATE EXAMINATION

During the survey in 1990 the mini mental state examination was administered by two trained nurses in a hospital setting. In 1993 the examination took place at the subject's home with academic and medical staff who had been trained intensively by the same instructors as in 1990. For five participants information on dementia was obtained from close relatives by using a structured interview,[20] and dementia was reported for two of them. Nurses and staff were randomly assigned to take the interviews. When we compared the results of the tests no evidence for interview bias was found. The Dutch translation of the examination developed by Folstein *et al* was used.[21] When individual (sub)items were missing they were rated as errors,[22] with the exception of those that could not be done because of physical disability, and in that case a weighted total score was given. The overall score (maximum of 30) is an indicator of cognitive function. A score of 25 or less is indicative of reduced cognitive function.[23] Men who had a score higher than 25 in 1990 and 25 or less in 1993 were considered as incident cases of impaired cognitive function. Change in cognitive function was defined as the difference in score between 1990 and 1993. For the two men with dementia a change of −10 was arbitrarily assigned.

APOLIPOPROTEIN E PHENOTYPE

Serum samples were obtained during the examination in 1990 and frozen at −20°C until determination of phenotype in 1993. For 19 subjects stored samples from 1985 were used. The apolipoprotein E phenotype was determined by isoelectric focusing of delipidated plasma samples followed by immunoblotting.[24] The validity of the use of stored serum for this purpose has been extensively described before.[24]

RISK FACTORS

During the examination in 1990 subjects had their weight and height measured while in their underwear. Systolic and diastolic blood pressure (V Korotkoff phase) were measured in duplicate in the right arm, with the men in supine position. Hypertension was defined as subjects having a systolic blood pressure of 160 mm Hg or more, having a diastolic blood pressure of 95 mm Hg or more, or using antihypertensive drugs.[25] Total cholesterol concentration was determined enzymatically[26] in non-fasting serum samples by a standardised laboratory lipid test. Information on amount and duration of smoking was assessed by using a standardised questionnaire and converted into pack years of cigarettes (packs of cigarettes a year × years of smoking). Habitual alcohol intake (g per day) was assessed from the cross check dietary history adapted for the Dutch.[27] Occupation was used as an indicator of education since education itself is not a good marker of intellectual capacity in a population with a relatively low level of education because of little free access to education of this age group (70-89 years). Lifelong occupation was obtained from a self administered questionnaire and coded from class I (professionals and managers, teachers) to class IV (manual workers).

Information on a history of myocardial infarction, angina pectoris, and intermittent claudication was obtained from a questionnaire developed at the London School of Hygiene and Tropical Medicine[28] administered by trained physicians at the surveys in 1985 and 1990. Standardised information on history of stroke, transient ischaemic attack, and diabetes mellitus was also obtained. Diagnosis of each disease was verified with hospital discharge data and with written information from the subjects' general practitioners. All information was uniformly coded by two trained medical staff members.

STATISTICAL METHODS

SAS statistical programs were used for the analyses.[29] The men were classified according to the e4 allele in two ways. Firstly, to analyse dose-response relations a trend was tested over three groups: men without the e4 allele, men heterozygous for e4, and men homozygous for e4. In addition, the heterozygous and homozygous men were combined into one group and compared with the men without the allele to show an overall effect of e4. Mean scores of the mini mental state examination were compared between the different phenotypes, and this was done by using a non-parametric test (Kruskal-Wallis). When the change in cognitive function was investigated as an outcome, analysis of variance was used. Analysis of covariance was used to adjust for potential confounding factors. The association between presence of e4 alleles and the prevalence and incidence of reduced cognitive function (examination score ≤ 25) was assessed by using multiple logistic regression analyses. All reported P values are based on two sided tests.

Results

The mean (SD) age of the participants at baseline in 1990 was 75·0 (4·6) years. Table I shows the distribution of the apoliprotein E phenotypes and the allele prevalences in the population. One man was found to have a rare phenotype, E3/E1. The distribution of the different phenotypes was in Hardy-Weinberg equilibrium ($\chi^2=7{\cdot}1$; $P<0{\cdot}05$ at $\chi^2>11{\cdot}0$, df=5). The allele prevalences were not different from those found in a random sample of 507 men aged 35 years and living in the same region ($\chi^2=4{\cdot}1$; $P<0{\cdot}05$ at $\chi^2>6{\cdot}0$, df=2) (table I). The distribution of phenotypes in the cohort members who took part in the three year follow up survey (n=378) was not significantly different from the distribution in those who died in the meantime or refused to participate in the second examination (P=0·04) (not shown).

A quarter of the subjects carried an e4 allele (n=134), and 3% (16) were homozygous. Total cholesterol concentrations and the prevalence of diabetes mellitus were different according to the e4 allele, but the presence of e4 was not associated with any other general characteristic (table II).

The average baseline score on the mini mental state examination was lower in the e4 allele carriers (P=0·10) (table III). The prevalence of reduced cognitive function (score ≤ 25) varied significantly

TABLE I—*Prevalences of phenotypes and alleles of apolipoprotein E in sample of elderly men and young men. Figures are numbers (percentages)*

Type	Zutphen elderly study* (n=538)	Dutch men aged 35 years† (n=507)
Phenotype:		
E2/E2	1 (0·2)	2 (0·4)
E3/E2	45 (8·3)	61 (12·0)
E3/E3	358 (66·4)	305 (60·2)
E4/E2	12 (2·2)	9 (1·8)
E4/E3	106 (19·7)	112 (22·1)
E4/E4	16 (3·0)	18 (3·6)
Allele prevalence:		
e2	0·055	0·073
e3	0·806	0·772
e4	0·139	0·155

*χ^2 Hardy-Weinberg distribution 7·07 (df=5), one man with rare phenotype (E3/E1) excluded.
†χ^2 Hardy-Weinberg distribution 4·51 (df=5).

TABLE II—*Selected characteristics of 538 men aged 70-89 years at baseline according to presence e4 allele*

Characteristic	No e4 allele (n=404)	Carriers of e4 allele: Heterozygous (n=118)	Carriers of e4 allele: Homozygous (n=16)	Carriers of e4 allele: All (n=134)
Mean (SD) age (years)	75·1 (4·6)	74·9 (4·6)	73·4 (3·7)	74·7 (4·6)
Mean (SD) total cholesterol concentration (mmol/l)	6·01 (1·11)	6·19 (1·11)	7·17 (1·32)*	6·31 (1·17)†
Mean (SD) cigarettes (pack-years)×1000	8·5 (8·8)	8·5 (9·7)	4·7 (6·3)	8·1 (9·3)
Mean (SD) alcohol intake (g/day)	10·8 (14·1)	9·4 (12·9)	15·0 (13·0)	10·0 (13·0)
No (%) highest occupation class	112 (27·8)	30 (25·0)	5 (28·6)	34 (25·4)
No (%) with hypertension	155 (38·4)	44 (37·3)	5 (31·3)	49 (36·6)
No (%) with stroke	18 (4·5)	5 (4·2)	1 (6·3)	6 (4·5)
No (%) with transient ischaemic attack	17 (4·2)	3 (2·5)	0	3 (2·2)
No (%) with myocardial infarction	62 (15·4)	10 (8·5)	1 (6·3)	11 (8·2)
No (%) with diabetes mellitus	29 (7·3)	10 (8·5)	4 (25·0)‡	14 (10·5)

*P<0·05 for trend over groups no e4 allele, heterozygous, and homozygous.
†P<0·01 for all e4 carriers *v* no e4 allele.
‡P<0·001 for trend over groups no e4 allele, heterozygous, and homozygous.

according to the e4 allele from 31·1% (126/404) in men without the allele to 50% (8/16) in the homozygous men. This result did not change when potential confounding factors such as age, occupation, cigarette smoking, or alcohol use were taken into account in logistic regression analysis (P for trend=0·03). Also total cholesterol concentrations and the presence of stroke, transient ischaemic attack, myocardial infarction, angina pectoris, diabetes mellitus, and hypertension could not explain this result (table III). Compared with men without the e4 allele the adjusted odds ratio for carriers of the e4 allele was 1·56 (95% confidence interval 1·01 to 2·39).

The change in three years in cognitive functioning was significantly different between the groups with the largest decline in men homozygous for e4 and an intermediate decline in those heterozygous for e4 (table III). These values remained essentially the same after adjustment for other risk factors including a history of stroke and other cardiovascular diseases (table III). Additional adjustment for baseline score on the mini mental state examination did not alter these results, with a reduction of 0·07 points in men without e4 and a reduction of 0·72 and 2·71 points in men heterozygous and homozygous for e4, respectively (P=0·001). Even exclusion of men with a history of stroke did not affect the findings (P=0·01). Also the incidence of impaired cognitive function among the men who had normal cognitive function at baseline (n=265) was associated with the presence of the e4 allele (P=0·03); the adjusted odds ratio was 2·87 (1·29 to 6·42). The attributable risk, calculated from this odds ratio and the prevalence of e4, was 0·22—that is, 22% of the risk of developing impaired cognitive function in this population may be attributed to the e4 allele.

Discussion

Several researchers have suggested that genetic heterogeneity of apolipoprotein E has a role in the development of atherosclerosis,[6-9] Alzheimer's disease,[1 2 12-16] and possibly other forms of dementia.[17] These studies all referred to groups of patients, and until now the impact of the e4 allele on cognitive decline has not been studied in a general population. We therefore investigated this issue in our population based cohort of elderly men. The prevalence of impaired cognitive function at baseline as well as the cognitive decline after three years and the risk of developing reduced cognitive function during follow up was significantly higher in carriers of the e4 allele. Cognitive decline was greatest in men homozygous for e4 and intermediate in those heterozygous, providing evidence for a gene-dose effect.

Our population based cohort consisted of elderly men with a mean age of 75 years. The prevalences of e2 and e4 alleles were somewhat lower compared with the prevalence in a population of 35 year old men from the same area.[7] The prevalences of stroke and other cardiovascular diseases were no higher in the e4 carriers, in contrast with what was expected.[6 9] The difference in prevalences of alleles in the younger men was, however, not significant, and the association between apolipoprotein E phenotypes and total cholesterol concentrations was similar to that in other reports.[6-8] This indicates that selective survival had only a limited impact on the distribution of the phenotypes. Also, within our cohort there was no evidence that selective drop out because of death or other reasons according to phenotype had occurred during the three years of follow up. This indicates that the association between apolipoprotein E phenotype and cognitive function is unlikely to be due to a selective survival phenomenon.

Because of associations with atherosclerosis and Alzheimer's disease some selective survival according to phenotype, however, was expected in advance. A recent French study showed that the e4 allele was two times less common in centenarians compared with younger controls, indicating a higher mortality in subjects with the e4 allele.[30] Several explanations for the apparent discrepancy with our findings on survival can be offered. The three year follow up of our study may be too short and the prevalence of severe atherosclerosis or Alzheimer's disease too low to show clear survival differences. The men in our cohort were also

TABLE III—*Baseline and longitudinal results of mini mental state examination and impaired cognitive function according to presence of e4 allele*

Detail	No e4 allele	Carriers of e4 allele: Heterozygous	Carriers of e4 allele: Homozygous	Carriers of e4 allele: All
Baseline study:				
No of subjects	404	118	16	134
Mini mental state examination score (95% confidence interval)	26·0 (25·7 to 26·3)	25·7 (25·1 to 26·2)	25·1 (23·5 to 26·6)	25·6 (25·1 to 26·1)
No (%) with impaired cognitive function	126 (31·1)	47 (39·8)	8 (50·0)	55 (41·0)*
Adjusted odds ratio‡ (95% confidence interval)	1·0	1·48 (0·95 to 2·32)	2·31† (0·75 to 7·07)	1·56 (1·01 to 2·39)
Longitudinal study (at three years):				
No of subjects	285	83	10	93
Change in mini mental state examination	−0·06 (−0·36 to 0·24)	−0·84 (−1·41 to 0·28)	−2·10† (−3·72 to 0·48)	−0·98§ (−1·51 to 0·44)
Adjusted change‡	−0·10	−0·68 (−1·26 to 0·11)	−2·38† (−4·16 to 0·59)	−0·84* (−1·39 to 0·29)
No (%) with impaired cognitive function‖	32 (15·5)	13 (25·5)	3 (42·9)†	16 (27·6)*
Adjusted odds ratio‡	1·0	2·38 (1·03 to 5·50)	12·53 (1·55 to 101·1)†	2·87 (1·29 to 6·42)

*P<0·05 for all carriers of e4 *v* subjects with no e4 allele.
†P<0·05 for trend over groups no e4 allele, heterozygous, and homozygous.
‡Adjusted for age, occupation, cigarette smoking, alcohol use, total cholesterol, stroke, transient ischaemic attack, myocardial infarction, diabetes mellitus, and hypertension.
§P<0·01 for all carriers of e4 *v* subjects with no e4 allele.
‖No of subjects=207, 51, 7, 58, respectively.

considerably younger than the centenarians in the French study. Finally, in this age group the overall mortality is fairly high, which limits the possibility for large survival differences and relative risks. An extended follow up of our cohort and additional survival studies in other populations are necessary to clarify this issue.

COGNITIVE FUNCTION

Cognitive function was assessed by using the mini mental state examination, which measures a selected set of cognitive functions. It was originally developed as a short screening instrument for clinical purposes.[21] Later studies showed that it adequately reflects the distribution of cognitive function in a population,[23 31 32] and the examination has been used extensively in epidemiological studies to screen for dementia and to characterise cognitive function.[33] The cross sectional association between apolipoprotein E phenotype and cognitive functioning was most pronounced for the lower end of the distribution of cognitive function. Low scores are indicative of dementia.[21 23] As regards the results of the prospective analysis, both the risk of developing impaired cognitive function as well as the overall cognitive decline was associated with the presence of the e4 allele. Change in cognitive function generally develops gradually until a clinical threshold is reached and dementia is diagnosed. Asymptomatic changes in cognitive performance may thus point at a dementing process, and our results indicate that this process develops faster in carriers of the e4 allele.

In addition, cognitive performance and decline is of major importance as indicators of general health status in elderly people.[31] Our finding that over a fifth of the risk of developing impaired cognitive function in our population can be attributed to presence of the e4 allele indicates that the public health impact of this association is large.

Several factors may affect the relation between apolipoprotein E polymorphism and cognitive decline. Age and occupation, as indicators of educational level, are strongly associated with cognitive function[34] but did not correlate with phenotype and thus played a minor part. Also cigarette smoking and alcohol use, behavioural factors that may affect cognitive function,[31] did not modify the observed associations. Other factors that were investigated can be considered as intermediate factors in the causal pathway, such as stroke and other cardiovascular diseases. When these factors were taken into account in the analyses the results remained essentially the same. This indicates that these factors are probably only partly responsible for the association between the e4 allele and cognitive decline. Especially regarding the role of stroke this would suggest that the observed associations are not due to the presence of vascular dementia. This was supported by an additional analysis that excluded patients with stroke, which resulted in identical findings for the association between the e4 allele and cognitive function. This result, however, remains to be confirmed in other studies since the diagnoses of cardiovascular diseases were based on clinical signs only and subclinical stage could not be taken into account.

Clinical implications

- Cognitive decline can be regarded as a marker of a dementing process and is an important indicator of health and functioning of elderly people
- Genetic heterogeneity of apolipoprotein E may have a functional role in the pathogenesis of both late and early onset familial Alzheimer's disease
- Elderly men with the apolipoprotein e4 allele are at a two fold increased risk of developing impaired cognitive function and are susceptible to cognitive decline
- A quarter of elderly men carry the e4 allele, known to confer susceptibility to hypercholesterolaemia, Alzheimer's disease, and also general cognitive impairment

CONCLUSION

Our study shows that apolipoprotein E phenotype is associated with cognitive decline in a general population. Thus, the susceptibility to dementia and dementia related disorders is partly determined by the genetic heterogeneity of apolipoprotein E. Elderly men with the e4 allele are at twice the risk of developing impaired cognitive function.

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Does sedation help in fibreoptic bronchoscopy?

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Although sedation is associated with major complications[1,2] sedative drugs are often given immediately before fibreoptic bronchoscopy in the belief that patients' comfort is improved. Uncontrolled studies have shown that fibreoptic bronchoscopy is well tolerated without sedation.[3,4] Most comparative studies of premedication have looked at different drug regimens; we are aware of only one study that included an unsedated control arm.[5]

Opiates and benzodiazepines are frequently used for sedation during fibreoptic bronchoscopy; we compared two such regimens with placebo.

Patients, methods, and results

Of 184 patients undergoing routine diagnostic fibreoptic bronchoscopy without transbronchial biopsy, 182 consented to enter a consecutive double blind comparison of (*a*) intravenous phenoperidine and droperidol with saline palcebo or (*b*) intravenous midazolam with saline placebo. Three doctors performed the bronchoscopy; all patients received supplemental oxygen, topical lignocaine, intravenous atropine 600 μg, and the trial drugs through individual syringes prepared by the pharmacy department. The dose given was varied to produce light sedation, with most patients receiving phenoperidine 1 mg and droperidol 5 μg or midazolam 70 μg/kg.

A visual analogue scale (100 mm) was used to score the answer to five questions about the ease and comfort of bronchoscopy, high scores indicating an unfavourable response. Results are expressed as medians with differences, 95% confidence intervals, and the significance of the differences (Mann-Whitney U test; Minitab). Immediately after bronchoscopy doctors stated whether they thought active drug or placebo had been used and rated the ease of performing the procedure. At the same time they and the attendant nurses rated how comfortable the patient had been during the procedure. Patients were assessed by a doctor and when fully recovered (usually around six hours) rated how comfortable they had been during the procedure and their willingness to undergo a repeat procedure if one was clinically indicated.

Fifty patients were randomly assigned phenoperidine and droperidol and 51 placebo. Midazolam and placebo were randomly assigned in the ratio of two to one to 51 and 30 patients respectively. For 180 patients the study bronchoscopy was their first such procedure, two patients having undergone one several years previously. Doctors correctly identified active treatment in 41 (82%) of those receiving phenoperidine and droperidol and in 27 (53%) of those given midazolam. The table shows median scores and 95% confidence intervals.

Age, sex ratio, and visual analogue scores of patients in placebo controlled comparison of sedation with phenoperidine and droperidol or with midazolam during fibreoptic bronchoscopy

	Drug	Placebo	Difference (95% confidence interval	P value*
	Phenoperidine and droperidol			
Mean age of patients (years)	64·1	64·1		
No of men:women	31:19	36:15		
Median visual analogue scores (mm) for:				
Ease of procedure (doctor)	11	22	11 (5 to 19)	0·001
Comfort of procedure:				
Doctor	11	20	7 (2 to 13)	0·01
Nurse	12	30	16 (5 to 19)	0·001
Patient	45	45	−1 (−13 to 11)	0·9
Willingness to repeat (patient)	46	24	−18 (−34 to −3)	0·008
	Midazolam			
Mean age of patients (years)	63·3	61·9		
No of men:women	37:14	19:11		
Median visual analogue scores (mm) for:				
Ease of procedure (doctor)	19	30	9 (2 to 16)	0·016
Comfort of procedure:				
Doctor	11	14	2 (−2 to 16)	0·285
Nurse	20	36	6 (−3 to 19)	0·192
Patient	30	36	7 (−3 to 19)	0·166
Willingness to repeat (patient)	19	8	0 (−9 to 5)	0·936

*Mann-Whitney U test.

Comment

One of the main objects of sedation is to make fibreoptic bronchoscopy less unpleasant for patients. Several different regimens have been described; phenoperidine and droperidol and, more recently, midazolam are frequently used.[2]

In our study phenoperidine and droperidol produced sedation, with 82% of the injections being correctly identified. Doctors and nurses found that bronchoscopy was more easily performed and patients more comfortable with this regimen than with placebo. Patients, however, found no difference in comfort between the active and placebo regimens, and they were less willing to have the test repeated when they had received these drugs. Although this combination has the theoretical advantages of amnesia, analgesia, euphoria, and cough suppression, the dysphoric effects probably taint patients' perception of the procedure.

The benzodiazepine midazolam would be expected to be helpful by producing amnesia and anxiolysis. In the doses we used, however, there was little obvious effect, doctors correctly identifying its use on only 52% of occasions. Furthermore, although doctors found the procedure easier with midazolam, the drug was not significantly better than placebo in making patients comfortable or willing to have the test repeated.

These sedative regimens fail in their primary