

**LETTERS**

**Effect of deprivation on time to hospital in acute stroke**

Acute stroke is the leading neurological cause of death and disability. After years of therapeutic nihilism there is now some optimism that effective treatments might become more widely available. It appears likely that very early thrombolysis is of benefit,<sup>1</sup> and there is good evidence for the use of aspirin.<sup>2</sup> As well as gains from the early initiation of these "disease modifying" treatments, it is likely that early admission to a safe clinical environment, with attention to simple physiological variables such as temperature and blood glucose, will improve outcome.<sup>3</sup> For these reasons, it seems probable that the time taken for patients to reach hospital following stroke onset will have a significant effect on outcome.

We have shown higher rates of intensive care admission for epilepsy, stroke, or head injury among individuals from affluent areas.<sup>4</sup> The impact of social deprivation on disease management might result from biases within health care systems, or from differences in how individuals perceive disease and access health care systems, and in particular how quickly they access care.

As acute stroke services are developed to meet the challenges ahead, any impact of deprivation on the delay to treatment should be identified. We have examined time to hospital admission by deprivation category for patients enrolled in the Lothian stroke registry (LSR).

**Participants, methods, and results**

The LSR prospectively identified stroke patients admitted to the medical unit of our hospital (excluding those with subarachnoid

haemorrhage), and all patients were assessed by a stroke physician. We extracted time of symptom onset and of hospital admission; home postcode; computed tomography (CT) findings; and whether symptoms were first apparent on waking from sleep. Carstairs DepCat scores<sup>5</sup> were determined from the home postcode and, to maximise statistical power, patients were grouped into approximate quartiles of deprivation (DepCats 1 and 2; 3; 4; and 5-7). Prespecified analyses were the proportion of patients reaching hospital within three hours of symptom onset by deprivation quartile, CT findings, and whether symptoms were present on waking. During the period of this study there were considerable developments in the provision of in-hospital stroke services, and for this reason analysis of the proportion of patients from each deprivation quartile admitted to a stroke unit were though likely to be unreliable.

Data were obtained for Lothian for mean general practitioner list size (1999; Lothian Health Board, personal communication), for mean ambulance response times (2000; Scottish Ambulance Service, personal communication), and for mean distance to hospital (derived from the distance "as the crow flies" from the centre of each postcode sector to the centre of the postcode sector in which the hospital lies, using online mapping tools (<http://www.streetmap.co.uk>)). The Information and Statistics Department of the Scottish Health Service provided data for hospital admissions and for death by deprivation category for patients with a main diagnosis of stroke (ICD 9 codes (to March 1996) 430-438; ICD 10 codes (from April 1996) I60-I69, G450, G451, G458, G459) for the years 1995 to 1999, and from these, age and sex adjusted rates of admission and death were calculated. This analysis was restricted to persons under 80 years of age because of concerns regarding the accuracy of death certification data among the very elderly.

Of 1927 patients, data on time to hospital and home postcode were available for 1416; patients with incomplete data were evenly distributed between deprivation quartiles (table 1). The number of patients reaching hospital within three hours fell from 105/407 (25.8%) in the most affluent quartile to 57/317 (17.9%) in the most deprived quartile ( $p = 0.037$ ,  $\chi^2$  on ranks, table 1), most of the observed difference lying between the most affluent quartile and the rest. Among 1316 patients with CT data available, 164 (12.5%) had haemorrhagic strokes, but there was no difference in the proportion reaching hospital within three hours. Of 1374 patients with data available, 428 (31.1%) had their symptoms present on waking, and fewer of these reached hospital within three hours (16.4% v 23.7%,  $p = 0.002$ ,  $\chi^2$ ); 10.5% of such patients from the most deprived quartile arrived at hospital within three hours, compared with 25.4% of those from the most affluent quartile ( $p = 0.002$ ,  $\chi^2$  on ranks).

There were no significant differences between deprivation quartiles for GP list size, distance to hospital, or ambulance response times (table 1). There were higher rates of hospital admission in patients from more deprived areas, and this reflected higher rates of death from stroke in persons resident in such areas (table 1).

**Comment**

Patients from affluent areas get to hospital sooner following stroke. This does not reflect differences in the distance to hospital, ambulance response times, or GP list size. Public recognition of stroke symptoms and understanding of the importance of early hospital admission may be greater in those living in affluent areas. Efforts to reduce time to hospital following stroke should include specific attention to public education targeted at those living in deprived areas.

**Table 1** Patients reaching hospital within three hours of symptom onset

	Quartiles of deprivation										Total	
	DepCats 1 and 2		DepCat 3		DepCat 4		DepCats 5-7		DepCat unknown			
	n	%	n	%	n	%	n	%	n	%	n	%
Number reaching hospital within 3 hours	105/407	25.8	58/310	18.7	87/380	22.9	57/319	17.9	7/42		307/1416	21.7
By CT findings												
Normal or infarct	78/313	24.9	47/255	18.4	71/309	23.0	48/275	17.5	6/36		244/1152	21.3
Haemorrhage	15/55	27.3	6/35	17.1	10/47	21.3	5/27	18.5	1/4		36/164	22.0
Not done/not known	12/39		5/20		6/24		4/17		0/1			
By symptom onset												
Present on waking	29/114	25.4	14/91	15.4	15/109	13.8	12/114	10.5	5/26		70/428	16.4
While awake	70/277	25.3	41/210	19.5	69/262	26.3	44/197	22.3	1/14		224/946	23.7
Not known	6/16		3/9		3/9		1/8		1/2			
Missing time to hospital data	127/534	23.8	96/406	23.6	124/504	24.6	109/428	25.5	13/55	23.6	469/1927	24.3
Other factors	Mean		Mean		Mean		Mean				Mean	
GP list size	1722		1618		1550		1595				1603	
Distance to hospital (km)	9.61		7.57		10.51		11.56				9.95	
Ambulance response times (min)	9.28		8.56		8.79		8.87				8.77	
Stroke admissions/1000 population	13.57		15.41		19.31		22.17				17.74	
Stroke deaths/1000 population	2.91		3.05		3.93		3.95				3.49	

## Acknowledgements

We are grateful to all of those who have contributed to the Lothian Stroke Register. MRM acknowledges the support of the Brain and Spine Foundation.

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## References

- 1 **Stroke Study Group.** The National Institute of Neurological Disorders and Stroke rt-PA Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med* 1995;**333**:1581-7.
- 2 **Chen ZM, Sandercock P, Pan HC, et al.** Indications for early aspirin use in acute ischemic stroke: a combined analysis of 40 000 randomized patients from the Chinese Acute Stroke Trial and the International Stroke Trial. *Stroke* 2000;**31**:1240-9.
- 3 **Bhalla A, Wolfe CD, Rudd AG.** Management of acute physiological parameters after stroke. *QJM* 2001;**94**:167-72.
- 4 **Macleod MR, Andrews PJD.** Effect of deprivation and gender on the incidence and management of acute brain disorders. *Intensive Care Med* 2002;**28**:1729-34.
- 5 **Carstairs V, Morris R.** Deprivation and mortality: an alternative to social class? *Community Med* 1989;**11**:210-19.

## Paraoxonase 1 promoter and coding region polymorphisms in Parkinson's disease

Parkinson's disease (PD) is thought to be caused by a combination of genetic and environmental factors. Epidemiological studies have found associations of PD with pesticide exposure, or suspected pathways of pesticide exposure, such as rural residence and well water consumption. Many organophosphorous insecticides (for example, chlorpyrifos and diazinon) are bioactivated to potent cholinesterase inhibitors by the cytochromes P450, and the resulting toxic oxon forms are hydrolysed by paraoxonase (PON1). Genetic variants of detoxifying enzymes or pesticide metabolising enzymes, such as paraoxonase, may confer a predisposition to PD and thus are considered candidate genes for association studies.

Multiple polymorphisms have been identified in the *PON1* gene. The coding region contains two common polymorphisms, at amino acid codons 55 and 192. The glutamine (Q) to arginine (R) substitution at codon 192 causes

substrate dependent differences in the kinetics of hydrolysis: compared with the *PON1* 192Q isoform, *PON1* 192R has higher activity towards paraoxon and chlorpyrifos oxon, but lower activity towards diazoxon, soman, and sarin. The leucine (L) to methionine (M) substitution at codon 55 does not affect the catalytic efficiency of substrate hydrolysis by the enzyme, but the *PON1* 55M allele is correlated with decreased mRNA and protein levels, because of linkage disequilibrium with a single nucleotide polymorphism (SNP) at position -108 of the promoter region of the gene. Five SNPs have been identified in the promoter region, at positions -108, -126, -162, -832, and -909. The -108 SNP has been shown to have the greatest effect on arylesterase activity, accounting for about 22% of the total variance, followed by the polymorphisms at positions 192, 55, and -162, which account for about 5.7%, 4.1%, and 1.1% of the total variance in arylesterase activity, respectively. Cell culture studies indicate an approximately twofold change in *PON1* gene transcription attributable to the -108C/T and -162G/A SNPs, with the -108C and -162A providing more efficient transcription.

A significant association of the 192R allele with PD was found in a population of patients of Japanese ethnicity with comparatively low mean age of onset.<sup>1</sup> In contrast, no difference in genotypes distributions was found between PD cases and controls for the *PON1* Q192R polymorphism in an Australian study<sup>2</sup> nor in another study on subjects of Russian ethnicity.<sup>3</sup> In a more recent study by Akhmedova *et al*<sup>4</sup> on the same Russian population, the M55 allele was found to be associated with PD. No associations for the amino acid codon 55 and 192 polymorphisms were found in a study from China.<sup>5</sup>

In this study, we examined associations of two promoter (G-162A and C-108T) and two coding region (M55L and Q192R) polymorphisms in *PON1* with PD. Newly diagnosed idiopathic PD patients (n=150; 91 men and 59 women), aged 37 to 88 years, were identified by neurology and general medical practice clinics of the group health cooperative (GHC) from the Puget Sound area in western Washington State. Inclusion criteria for the cases were the presence of at least two of the four cardinal signs of PD: bradykinesia, resting tremor, cogwheel rigidity, and postural reflex impairment. Exclusion criteria included the use of certain drugs during the 12 months preceding symptom onset, history of multiple cerebrovascular events, or another explanation for parkinsonism symptoms.

Controls (n=244; 158 men and 86 women), aged 44 to 84 years, were identified from GHC enrollees without past histories of PD or other neurodegenerative disorders. Controls were matched to cases by birth decade, sex, and year of enrolment in GHC. All subjects were of non-Hispanic white ethnicity. Study subjects were volunteers who were informed of the purpose of the study. Study forms and procedures were approved by the Institutional Review Board committees on Human Subjects Research at the University of Washington and GHC Center for Health Studies.

A PCR/dye terminator cycle sequencing based assay was used to detect the -162 G/A and -108 C/T genetic variants within the paraoxonase 1 (*PON1*) gene. TaqMan Detection System based assays were developed to identify the *PON1* 55 T/A and *PON1* 192 A/G variants. Odds ratios and  $\chi^2$  tests were calculated using of SPSS software for Windows;  $\alpha=0.05$  was taken as the level of significance. Logistic regression models were used to calculate adjusted odds ratios and to test for statistical significance of interactions. Haplotypes were inferred using EH software.

Among controls, we observed the following allelic frequencies: -162 A = 0.23, G = 0.77; -108 T = 0.46, C = 0.54; 55M = 0.35, L = 0.65; 192R = 0.30, Q = 0.70. As shown in table 1, there were no significant differences in the genotype distributions of cases and controls for any of the four *PON1* polymorphisms. The distribution of three marker haplotypes was not significantly different between cases and controls ( $\chi^2_{(7)}=4.78$ , p=0.69). There also was no difference in the distribution of four marker (including -162) haplotypes between cases and controls ( $\chi^2_{(15)}=9.98$ , p=0.82).

We also tested for interactions between *PON1* genotypes and age (<60, =60), sex, and smoking. No conclusive evidence of interaction between any *PON1* genotype and either smoking or sex was found. However, we did detect an interaction between *PON1* 192 genotype and age. Interestingly, among cases, *PON1* 192 QQ genotype frequency increased with age ( $\chi^2_{(4)}$  for genotype distribution =  $3.9 \times 10^3$ ), whereas among controls, 192 QQ genotype frequency decreased with age ( $\chi^2_{(4)}$  for genotype distribution = 0.09). Mean age at diagnosis, however, did not differ appreciably between *PON1* 192 genotypes (mean (SD) age for Q/Q cases = 68.9 (9.1), Q/R cases = 65.7 (10.4), R/R cases = 66.5 (8.3)).

In contrast with the reports of Kondo and Yamamoto<sup>3</sup> and Akhmedova *et al*,<sup>4</sup> our results do not indicate that PD is associated with specific *PON1* genotypes. In addition to the

**Table 1** *PON1* -162, -108, 55, and 192 genotype frequencies in cases and controls

SNP	Genotype			$\chi^2$ Genotype distribution	OR* (95% CI)	OR* (95% CI)	
-162	GG	GA	AA	2.68 p=0.26	GA v GG 1.28 (0.82 to 1.98)	AA v GG 1.72 (0.80 to 3.68)	
	Cases (%)	79 (52.7)	56 (37.3)				15 (10)
	Controls (%)	145 (60.2)	80 (33.2)				16 (6.6)
-108	CC	CT	TT	1.34 p=0.51	CT v CC 1.09 (0.67 to 1.78)	TT v CC 0.75 (0.41 to 1.38)	
	Cases (%)	43 (31.6)	67 (49.3)				26 (19.1)
	Controls (%)	71 (31.1)	102 (44.7)				55 (24.1)
55	LL	LM	MM	0.57 p=0.75	LM v LL 1.19 (0.77 to 1.86)	MM v LL 0.99 (0.52 to 1.89)	
	Cases (%)	60 (40.0)	70 (46.7)				20 (13.3)
	Controls (%)	105 (43.2)	104 (42.8)				34 (14)
192	QQ	QR	RR	0.76 p=0.68	QR v QQ 0.89 (0.58 to 1.37)	RR v QQ 0.71 (0.33 to 1.51)	
	Cases (%)	81 (54.0)	57 (38.0)				12 (8.0)
	Controls (%)	121 (50.0)	97 (40.1)				24 (9.9)

\*OR, odds ratio, adjusted for age (<60, ≥60) and sex; CI, confidence intervals.

coding region polymorphisms investigated previously, we assessed the role of two promoter mutations but found no evidence of association. These findings suggest that *PON1* genotypes may not be predictive of PD, although there remains the possibility of interactions with pesticide exposures. Considerably larger studies will be required to investigate such interactions.

### Acknowledgements

The authors would like to thank the PD patients and control subjects that participated in this study, Ms Janet Petersen for administering the questionnaire to study subjects, and the neurologists of GHC and UW for referral of PD cases, in particular Drs Robert Gotshall, Ann Hunt, Erik Kraus, Richard Mesher, Steve Pugh, Bruce R Ransom, Ali Samii, Timothy Searc, Kurt Seiffert, Ken Uchino, Thurman Wheeler.

Funding: this research was supported by National Institute for Environmental Health Sciences Grants ES-04696, 10750, 07033, 09601, 07032 and Environmental Protection Agency Grant 826886-02.

Competing interests: none declared.

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### References

- 1 **Kondo I**, Yamamoto M. Genetic polymorphism of paraoxonase 1 (PON1) and susceptibility to Parkinson's disease. *Brain Res* 1998;**806**:271-3.
- 2 **Taylor MC**, Le Couteur DG, Mellick GD, *et al*. Paraoxonase polymorphisms, pesticide exposure and Parkinson's disease in a Caucasian population. *J Neural Transm* 2000;**107**:979-83.
- 3 **Akhmedova S**, Anisimov S, Yakimovsky A, *et al*. Gln → Arg 191 polymorphism of paraoxonase and Parkinson's disease. *Hum Hered* 1999;**49**:178-80.
- 4 **Akhmedova SN**, Yakimovsky AK, Schwartz El. Paraoxonase 1 Met-Leu 54 polymorphism is associated with Parkinson's disease. *J Neural Sci* 2001;**184**:179-82.
- 5 **Wang J**, Liu Z. No association between paraoxonase 1 (PON1) gene polymorphisms and susceptibility to Parkinson's disease in a Chinese population. *Mov Disord* 2000;**15**:1265-7.

### Multifocal myoclonus secondary to tranexamic acid

Myoclonus is characterised by sudden and brief involuntary movements. We describe a

patient who developed myoclonus and altered mental status following tranexamic acid overdose. The patient was a 61 year old man on chronic ambulatory peritoneal dialysis for adult polycystic kidney disease and had been prescribed lisinopril and metoprolol for long standing hypertension. He presented to hospital because of bloody effluent from his peritoneal catheter. Examination did not reveal any neurological abnormalities. Haemoglobin level was 6.2 g/dl, calcium 2.28 mmol/l, urea 20.3 mmol/l, and creatinine 1109 µmol/l, which was similar to the values taken one month before in the outpatient clinic (urea 20.2 mmol/l and creatinine 1112 µmol/l). Blood and peritoneal fluid were taken for microbiological analyses and were unrevealing.

He was transfused two units of packed cells and started on oral tranexamic acid 500 mg four times daily in order to reduce the bleeding. Six days later he became dull and developed spontaneous, arrhythmic, and multifocal myoclonus. On repeat renal function testing, the urea and creatinine levels were 20.6 mmol/l and 1190 µmol/l, respectively. An ECG and urgent brain computed tomography were unremarkable but an electroencephalogram (EEG) showed intermittent spike waves over both parasagittal regions. No other cause of epilepsy was found. Anticonvulsants were not started as it was felt that the involuntary movements were not disabling and likely to be a transient adverse drug effect. Four days after tranexamic acid had been stopped, the patient had regained his premonitory mental state and the myoclonus had ceased. Normal posterior dominant alpha activity was recorded on a repeat EEG, without further epileptiform discharges. Six months after this event the patient remains free from seizures.

### Comment

Drug induced myoclonus usually occurs with encephalopathy and is often a diagnosis of exclusion. In addition, other neurological signs such as ataxia, coma, generalised seizures, and headache may be present with certain agents. Implicated drugs include antibiotics, anaesthetics, calcium channel blocking drugs, antidepressants, and anti-epileptic drugs. Tranexamic acid (TAMCA; 4-(aminomethyl)cyclohexanecarboxylic acid) is commonly used in the treatment of disorders that predispose to bleeding. It is a synthetic lysine analogue that has strong antifibrinolytic activity. Plasminogen binds to fibrin to form plasmin, which in turn degrades fibrin into fibrin degradation products. TAMCA blocks the lysine binding site on plasminogen and prevents interaction with fibrin. Clinical trials have shown that TAMCA reduces blood loss in patients with primary menorrhagia, and in those undergoing cardiopulmonary bypass, prostatectomy, hip replacement, and liver transplantation.

TAMCA is generally well tolerated. Side effects are mainly gastrointestinal, such as nausea, vomiting, diarrhoea, and abdominal pain. There are, however, experimental studies of neurotoxicity.<sup>1-4</sup> Direct application of TAMCA to the cortex of cats produces spike-wave bursts on EEG similar in appearance to that found in feline generalised epilepsy.<sup>4</sup> The ability to elicit epileptic activity depended on the concentration of the drug and the area of cortex exposed. Intravenous injection causes intracranial and systemic hypertension as well as epileptiform discharges on the EEG.<sup>3</sup> Drugs may induce seizures by modulating neurotransmission, as application of excitatory transmitters such as

kainic acid and inhibitory receptor blockers such as penicillin have been shown to evoke seizures. By applying TAMCA to the spinal cord of rats, Furtmüller was able to demonstrate a dose dependent hyperexcitability.<sup>3</sup> This is blocked by the addition of muscimol, a γ-aminobutyric acid (GABA) receptor agonist, which indicates that tranexamic acid induces convulsions by blocking inhibitory GABAergic neurotransmission.

Clinically, an increase in cerebral ischaemia has resulted when TAMCA has been given for the treatment of subarachnoid haemorrhage.<sup>3</sup> TAMCA induced seizures have been reported in a man who was inadvertently given a 50 mg intrathecal dose during spinal anaesthesia and developed status epilepticus.<sup>6</sup> The patient required thiopentone infusion as the seizures were not controlled with phenytoin and midazolam; the clinical course was complicated by multiorgan dysfunction and critical illness polyneuropathy. Our patient with end stage renal failure was given an accidental overdose of oral TAMCA which resulted in myoclonus that resolved after the drug was stopped. As excretion of this drug depends on renal function, dosage adjustment is required in patients with renal failure; the efficacy of haemodialysis in removing the drug has not been studied. This case suggests that TAMCA overdose should be considered as a cause of drug induced myoclonus.

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### References

- 1 **Schlag MG**, Hopf R, Zifko U, *et al*. Epileptic seizures following cortical application of fibrin sealants containing tranexamic acid in rats. *Acta Neurochir* 2002;**144**:63-9.
- 2 **Fodstad H**. Convulsive seizures following subdural application of fibrin sealant containing tranexamic acid in a rat model. *Neurosurgery* 2001;**49**:479-80.
- 3 **Yamaura A**, Nakamura T, Makino H, *et al*. Cerebral complication of antifibrinolytic therapy in the treatment of ruptured intracranial aneurysm. Animal experiment and a review of literature. *Eur Neurol* 1980;**19**:77-84.
- 4 **Pellegrini A**, Giaretta D, Chemello R, *et al*. Feline generalized epilepsy induced by tranexamic acid (AMCA). *Epilepsia* 1982;**23**:35-45.
- 5 **Furtmüller R**, Schlag MG, Berger M, *et al*. Tranexamic acid, a widely used antifibrinolytic agent, causes convulsions by a gamma-aminobutyric acid(A) receptor antagonistic effect. *J Pharmacol Exp Ther* 2002;**301**:168-73.
- 6 **De Leede-van der Maal MGJ**, Hilken P, Bosch F. The epileptogenic effect of tranexamic acid. *J Neurol* 1999;**246**:843.

### Immunohistochemical study of caveolin-3 in idiopathic hyperCKaemia

With the increasing concern about malignant hyperthermia and with the inclusion of creatine kinase determination in the automated blood chemistry profile, performed as part of health screening, the number of subjects with raised serum creatine kinase (hyperCKaemia) without clinical signs of neuromuscular disease is continuously increasing. In 1980 Rowland *et al* coined the term "idiopathic hyperCKaemia" to describe patients with



consistently increased serum creatine kinase who may complain of myalgia or tiredness but do not have weakness or other abnormalities on neurological examination, electromyography, and muscle biopsy.<sup>1</sup> Extensive ancillary investigations may lead to the diagnosis of a subclinical neuromuscular disorder in a variable percentage of subjects but in others hyperCKaemia remains unexplained even after complete studies.

Merlini *et al*<sup>2</sup> reported on an 18 year old man and his 49 year old mother with persistent hyperCKaemia, no muscle symptoms and signs, muscle caveolin-3 deficiency, and a novel mutation (P28L) in the caveolin-3 (CAV3) gene. Recently two unrelated asymptomatic children (4 and 6 years old) with hyperCKaemia, reduced expression of caveolin-3 in muscle fibres and the same de novo CAV3 mutation (R26Q) have been also described.<sup>3</sup> Although the absolute number of reported subjects was small caveolin-3 deficiency has been indicated as a cause of sporadic and familial "isolated" hyperCKaemia without any signs or symptoms of myopathy.<sup>2,3</sup>

To investigate the relative frequency of caveolin-3 deficiency in hyperCKaemia we reviewed the clinical records and muscle biopsy specimens of 56 consecutive subjects with persistent hyperCKaemia (more than twice the normal value) and normal neurological examination referred to our centre for neuromuscular diseases. Extensive investigations showed that subclinical neuromuscular disorders (dystrophinopathy and carrier state of dystrophinopathy, adult maltase acid deficiency, inflammatory myopathy, mitochondrial myopathy, motor neurone disease, hypothyroidism, hypoparathyroidism) accounted for 20 cases, not included in the study. In the remainder 36 subjects (34 male), with CK increased from 2 to 18 times, muscle biopsy was completely normal (15 cases) or showed only minimal and non-specific alterations such as mild increase variability of fibre

size or increased percentage of internal nuclei. Mean age was 27 years (range 2-62). Three patients were younger than 15 years. Nine subjects belonged to nine different families with autosomal dominant hyperCKaemia without clinical signs reported in at least two subjects of two generations. Ten subjects complained of mild myalgias or feeling fatigued. EMG was normal in 35 of 35 tested subjects. Histochemistry for myofosforilase and myoadenilate deaminase, immunohistochemistry for dystrophin, sarcoglycans, laminins, desmin, and biochemical investigations for glycogen and lipid metabolism enzymes were all normal. In these 36 subjects, we labelled as idiopathic hyperCKaemia, we also performed immunofluorescence on frozen muscle sections using monoclonal antibody against caveolin-3 (BD Transduction laboratories). Biopsy specimens from normal subjects and patients with known diseases were used as controls. All 36 subjects showed a uniform sarcolemmal staining for caveolin-3, not different from controls. No staining was found inside muscle fibres in both groups.

CAV3 gene mutations have been associated with an autosomal dominant form of limb-girdle muscular dystrophy (LGMD-1C),<sup>4</sup> rippling muscle disease (RMD),<sup>5</sup> distal myopathy,<sup>6</sup> and isolated hyperCKaemia.<sup>2,3</sup> Different phenotypes may share the same mutation. For example, the P104L mutation may lead to LGMD-1C or RMD phenotype and the R26Q mutation, found in the two children with isolated hyperCKaemia,<sup>3</sup> also causes RMD.<sup>5</sup> This implies that other genes or regulating factors may be involved in determining the clinical phenotype and that, especially in paediatric cases, isolated hyperCKaemia may be a presymptomatic stage of other caveolinopathies.

All the muscle caveolinopathies described up to now showed a reduced intensity of sarcolemmal staining, with sometimes abnormal punctuated cytosolic staining.<sup>2,6</sup> Therefore immunohistochemistry, by itself, should be a

reliable enough technique to exclude in our series caveolin-3 deficiency.

In conclusion, our study on a large consecutive series of patients with idiopathic hyperCKaemia suggests that caveolin-3 deficiency is not a common cause of sporadic and familial hyperCKaemia without muscular signs. The other possible causes of this intriguing phenomenon remain to be unveiled.

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## References

- 1 Rowland LP, Willner J, Cerri C, *et al*. Approaches to the membrane theory of Duchenne muscular dystrophy. In: Angelini C, Danielli GA, Fontanari D, eds. *Muscular dystrophy research: advances and new trends*. Amsterdam: Excerpta Medica, 1980:3-13.
- 2 Merlini L, Carbone I, Capanni C, *et al*. Familial isolated hyperCKaemia associated with a new mutation in the caveolin-3 (CAV-3) gene. *J Neurol Neurosurg Psychiatry* 2002;**73**:65-7.
- 3 Carbone I, Bruno C, Sotgia F, *et al*. Mutation in the CAV3 gene causes partial caveolin-3 deficiency and hyperCKemia. *Neurology* 2000;**54**:1373-6.
- 4 Minetti C, Sotgia F, Bruno C, *et al*. Mutations in the caveolin-3 gene cause autosomal dominant limb-girdle muscular dystrophy. *Nat Genet* 1998;**18**:365-8.
- 5 Vorgerd M, Ricker K, Ziemssen F, *et al*. A sporadic case of rippling muscle disease caused by a de novo caveolin-3 mutation. *Neurology* 2001;**57**:2273-7.
- 6 Tateyama M, Aoki M, Nishino I, *et al*. Mutation in the caveolin-3 gene causes a peculiar form of distal myopathy. *Neurology* 2002;**58**:323-5.