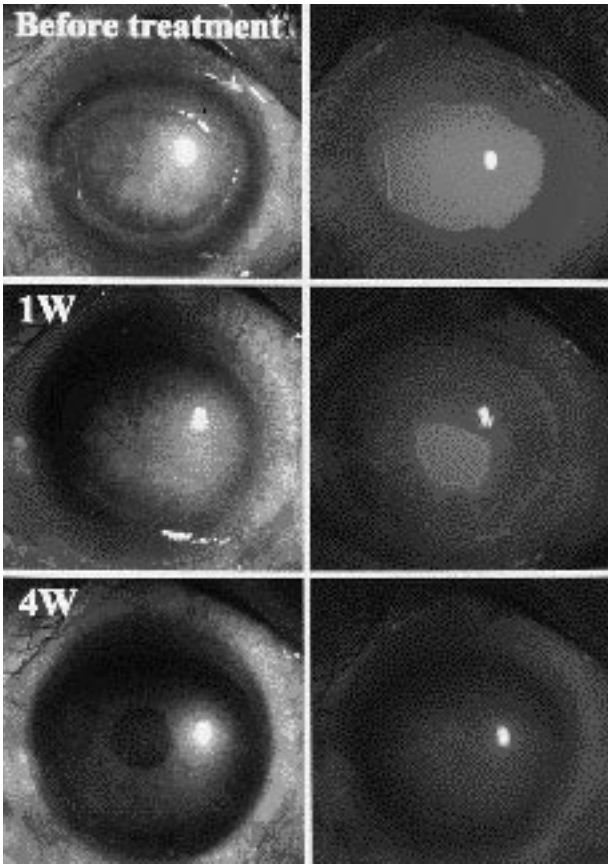
Research letters

Treatment of neurotrophic keratopathy with substance-P-derived peptide (FGLM) and insulin-like growth factor I

Tai-ichiro Chikama, Ken Fukuda, Naoyuki Morishige, Teruo Nishida

A 55-year-old woman was referred to us in October 1997. In January, 1997, she had experienced sudden onset of visual disturbance, pain, and hyperaemia in her left eye. She visited an ophthalmologist who diagnosed corneal epithelial erosion due to the hypolacrimation, and treated her with eye drops containing ofloxacin and hyaluronic acid. The epithelial erosion did not heal. She visited another ophthalmologist who added a therapeutic soft contact lens with no improvement.

On her visit to us, her visual acuity was 2/100. Large corneal epithelial defects (8×5·5 mm) and severe stromal oedema were evident in her left eye. There was complete loss of corneal sensation (<5 mm by Cochet-Bonnet aesthesiometer) and hypolacrimation. This suggested unilateral trigeminal nerve palsy. Her left eyebrow was lowered, but blinking was not impaired, which suggested incomplete facial nerve palsy. No sign of lagophthalmus or secondary corneal exposure was noted. Neurosurgeons at our institution discovered no abnormal findings with magnetic-resonance imaging. No signs, symptoms, or laboratory findings of collagen disease were found.



Clinical course after start of treatment with FGLM and IGF-I
Fluorescein staining (right panel) shows the epithelial defect.

We diagnosed of neurotrophic keratopathy due to trigeminal nerve palsy and started treatment with eye drops, combining a substance-P-derived peptide (phenylalanine-glycine-leucine-methionine amide [FGLM], 1 mg/mL) with insulin-like growth factor-I (IGF-I, 1 μg/mL), four times a day. 2 days after the start of treatment, clearly visible epithelial resurfacing had begun. After 1 week, the epithelial defects were reduced to less than 10% of their original size. Complete epithelial resurfacing was achieved within 2 weeks. Thereafter, the stromal oedema started to subside; corneal transparency was obtained after 4 weeks, and treatment was stopped. The patient's visual acuity returned to 20/50. Since then, her corneal surface epithelium has remained stable. During treatment her corneal sensation returned (50 mm). However, as soon as the treatment was stopped, corneal sensation was lost again (less than 5 mm). Despite the loss of corneal sensation, no epithelial defects have recurred in more than 5 months of follow-up.

Corneal epithelial wound healing is regulated by various factors: extracellular matrix proteins, cytokines, and growth factors.[1] Tears bathing the cornea supply cytokines and growth factors. The present case suggests that a sensory neurotransmitter substance P, is another regulatory factor in epithelial wound healing process. The cornea is the most heavily innervated tissue in the body. Trigeminal innervation is recognised as being important to the maintenance of a healthy corneal epithelium. Loss of corneal sensation often leads to neurotrophic keratopathy with persistent epithelial defects that do not respond to any currently available treatment, such as lubrication, ointment, or eye patches. Persistent epithelial defects result in damage to the underlying stroma and eventually to blindness.

We reported that substance P (a neurotransmitter of the trigeminal nerve) and IGF-I synergistically facilitate corneal epithelial migration in vitro[2] and in vivo,[3] although neither substance P nor IGF-I alone stimulates epithelial migration. These synergistic effects are mediated through NK-1 receptors for substance P.[4] The combination of substance P and IGF-I stimulates the expression of integrins α5 and β1 (fibronectin receptors) and the phosphorylation of focal adhesion kinase and paxillin, events that are essential to the attachment of epithelial cells to the extracellular matrix proteins.[5] Substance P is a member of tachykinin family and consists of 11 amino acids. We determined that the minimum essential-amino-acid sequence of substance P needed for the synergistic effect on corneal epithelial migration was the four amino-acid sequence, FGLM, at the C-terminus. Treatment with combined FGLM and IGF-I was as effective as treatment with substance P and IGF-I in rabbits in vivo (unpublished observation).

This case also raised an interesting question about the role of the sensory nervous system in the maintenance of epithelium. For example, we cannot explain why our

patient's healed epithelium remained stable even after the end of treatment with the substance-P-derived peptide. Perhaps presence of the sensory neural signal is required only to start the healing process.

The clinical application of the eye drops containing of FGLM and IGF-I was approved by the Internal Review Board of Yamaguchi University Hospital. Written informed consent was obtained from the patient.

1 Nishida T. Extracellular matrix and growth factors in corneal wound healing. *Curr Opin Ophthalmol* 1993; **4:** 4–13.

2 Nishida T, Nakamura M, Ofuji K, Reid TW, Mannis MJ, Murphy CJ. Synergestic effects of substance P with insulin-like growth factor-1 on epithelial migration of the cornea. *J Cell Physiol* 1996; **169:** 159–66.

3 Nakamura M, Ofuji K, Chikama T, Nishida T. Combined effects of substance P and insulin-like growth factor-1 on corneal epithelial wound closure of rabbit in vivo. *Curr Eye Res* 1997; **16:** 275–78.

4 Nakamura M, Ofuji K, Chikama T, Nishida T. The NK-1 receptor and its participation in the synergistic enhancement of corneal epithelial migration by substance P and insulin-like growth factor-1, *Br J Pharmacol* 1997; **120:** 547–52.

5 Nakamura M, Nagano T, Chikama T, Nishida T. Up-regulation of phosphorylation of focal adhesion kinase and paxillin by combination of substance P and IGF-1 in SV-40 transformed human corneal epithelial cells. *Biochem Biophys Res Comm* 1998; **242:** 16–20.

Department of Ophthalmology, Yamaguchi University Hospital and Yamaguchi University School of Medicine, Ube-City, Yamaguchi 755-8505, Japan (T Nishida)

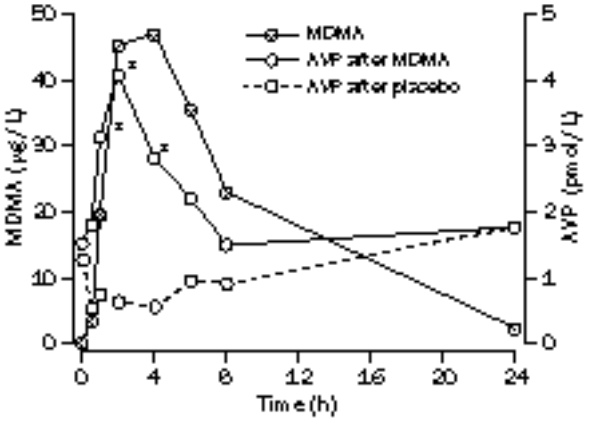
Low-dose MDMA ("ecstasy") induces vasopressin secretion

John A Henry, John K Fallon, Andrew T Kicman, Andrew J Hutt, David A Cowan, Mary Forsling

Abuse of 3,4-Methylenedioxymethamphetamine (MDMA, commonly called ecstasy) has been associated with acute symptomatic hyponatraemia with the syndrome of inappropriate antidiuretic-hormone secretion (SIADH).[1] In one case, raised antidiuretic-hormone (arginine vasopressin; AVP) concentration was found.[2] We investigated whether the hyponatraemic effect of MDMA is a direct effect of AVP secretion.

Approval was obtained from the UK Home Office for the purchase and administration of MDMA, and from King's College London Ethics Committee. Eight normally hydrated healthy male volunteers aged 22–32 years, were each given 47·5 mg MDMA hydrochloride (Sigma; equivalent to 40 mg MDMA base) in capsule form with 200 mL water at 1000 h. Three acted as untreated controls at least 2 weeks later. Blood was taken before ingestion and at 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, and 24 h after. Samples were analysed for MDMA by GC-MS and for AVP by RIA.[3] Cortisol and sodium concentrations were also measured. Statistical analysis between before and after data was by repeated-measures ANOVA or by paired *t* test.

Plasma AVP concentrations reached a maximum between 1 h and 2 h after drug administration. Baseline AVP concentrations (range 1·14–1·88 pmol/L) increased significantly at 2 h (range 2·46–9·16 pmol/L; paired *t* test p=0·006, one tail), mean AVP concentrations (figure) contrasting with the small decrease observed on control occasions. Despite increases in AVP and MDMA, a correlation was not shown (Spearman *r*=0·2, p=0·1), probably because of the rapid clearance of AVP, the initial elimination half-life of which is about 6 min,[4] compared with that of MDMA, which is measured in hours. Sodium concentrations changed significantly, ranges at baseline and 2 h after drug administration were 139–145 mmol/L and 140–142 mmol/L respectively; seven of eight volunteers showed a decrease of 1–3 mmol/L, whereas the remaining volunteer showed an increase of 1 mmol/L (paired *t* test p=0·025, one tail). Mean initial cortisol concentration was 331·4 nmol/L (range 208·8–603·4 nmol/L), which increased, although not significantly (p>0·05), to 377·2 nmol/L (range 268·4–583·3 nmol/L) at 2 h.



Plasma concentrations of AVP after MDMA (n=8) or placebo (n=3) in healthy volunteers. Concentrations of MDMA are also shown (n=8)

Results are exressed as geometric means; repeated measures ANOVA*, p<0·05 for AVP values for each subject compared with basal concentrations.

Street "ecstasy" frequently contains more than 100 mg of MDMA. In this study, a single relatively small dose caused an acute rise in AVP concentration at a time of day when it would not be expected to change. The rise in AVP was accompanied by a small fall in plasma sodium concentrations. The hyponatraemic illness experienced by some users is thus likely to be linked to the drug's ability to stimulate secretion of AVP. Hence, if fluid intake is excessive, even a relatively small dose of MDMA could lead to symptoms of hyponatraemia. The rise in AVP does not seem to be part of a generalised stress response because there was no significant change in plasma cortisol concentration. It therefore seems that MDMA-induced hyponatraemia is unlikely to be due to a rare and idiosyncratic reaction, but results from a pharmacological effect compounded by excessive fluid ingestion. Animal studies show that MDMA stimulates the output of serotonin by serotonergic neurones, and AVP secretion is regulated by serotonergic pathways.[5]

The message is that those who take "ecstasy" or similar drugs may be at risk of hyponatraemia and should, therefore, avoid drinking fluid in excess of the body's requirements. This may be difficult for users to estimate because MDMA reduces perception of thirst and impairs judgment.

We thank Peter Milligan for statistical advice.

1 Maxwell DL, Polkey MI, Henry JA. Hyponatraemia and catatonic stupor after taking "ecstasy". *BMJ* 1993; **307:** 1399.

2 Holden R, Jackson MA. Near-fatal hyponatraemic coma due to vasopressin over-secretion after "ecstasy" (3,4-MDMA). *Lancet* 1996; **347:** 1052.

3 Forsling ML. Measurement of vasopressin in body fluids. In: Baylis PH, Padfield PL, eds. The posterior pituitary: hormone secretion in health and disease. New York: Marcel Dekker, 1985: 161–92.

4 Fabian M, Forsling ML, Jones JJ, Pryor JS. The clearance and antidiuretic potency of neurohypophysial hormones in man and their plasma binding and stability. *J Physiol* 1969; **204:** 653–68.

5 Iovino M, Steardo L. Effects of substances influencing brain serotonergic transmission on plasma vasopressin levels in the rat. *Eur J Pharmacol* 1985; **113:** 99–103.

Academic Department of Accident and Emergency Medicine, Imperial College School of Medicine, St Mary's Hospital, London W2 1NY, UK (J A Henry)**; Drug Control Centre and Department of Pharmacy, King's College London, London; and Department of Physiology, United Medical and Dental Schools, St Thomas's Hospital Campus, London**

5-HT$_{2A}$ promoter polymorphism in anorexia nervosa

Sandro Sorbi, Benedetta Nacmias, Andrea Tedde, Valdo Ricca, Barbara Mezzani, Carlo Maria Rotella

A study[1] has shown an association between a polymorphism (–1438G/A) in the promoter region of the 5-HT$_{2A}$ gene and anorexia nervosa. Two independent groups failed to replicate these data.[2,3] We studied this polymorphism in two clinical subtypes of anorexia nervosa and in controls. We analysed the segregation of this polymorphism in 77 female patients with anorexia nervosa, according to DSM-IV criteria (43 restricting-type, body-mass index [BMI] 14·9 [SD 2·6] kg/m^2, and 34 purging type, BMI 16·0 [1·9] kg/m^2, 15–34 years (mean age 24·58 [5·75] years). All patients or their parents gave their informed written consent. We also studied 107 normal female age-matched controls (age 15–36 years, mean age 25·33 [5·57] years), obtained from the DNA bank of the CNR (National Research Council, Florence, Italy). All controls were carefully assessed to exclude any neurological or psychiatric disorders. DNA from affected and non-affected individuals was extracted from transformed lymphoblasts or peripheral blood samples with the phenol-chloroform procedure. Amplification from 200 ng of genomic DNA was done with a Thermal Cycler 9600 (Perkin Elmer). The −1438G/A polymorphism was analysed as previously described.[1] The frequencies of 1438G/A alleles were estimated by gene counting. Statistical analysis was done with the χ^2 test.

The table shows the distribution of −1438G/A alleles and genotypes in the different groups. The distribution of −1438G/A genotypes in all groups followed Hardy-Weinberg equilibrium. A significant difference in the distribution of −1438A/A genotype was observed in anorexia patients compared with the control group ($p<0·0001$). The restricting type showed a high frequency of the −1438A/A genotype (41·86% in anorexic patients *vs* 9·34% in controls; $p<0·0001$). Patients with the purging type had a genotype distribution similar to the control group (−1438A/A 14·7% *vs* 9·34%; $p>0·1$).

Our data independently confirm and extend previous results,[1] suggesting a role of the 5-HT$_{2A}$ gene in anorexia. Two other groups have reported negative results. However, one study[2] had no controls and did not report the −1438A/A genotype in anorexia nervosa. Indeed, the −1438A/A genotype is similar in all series of anorexia patients (0·3,[1] 0·25,[3] and 0·29), but Campbell's study showed a very high frequency in the control group (0·2) compared with the other series (0·12[1] and 0·9 in our study). This difference in controls may explain the difference in results. Our results also suggest that restricting and purging types of anorexia nervosa have a different involvement of the 5-HT$_{2A}$ gene promoter polymorphism. This finding may lead to a better understanding of the two types of this severe psychiatric disease and suggests a different implication of the serotonergic system in the pathogenesis and possibly in treatment of anorexia nervosa.

Samples	Genotype			Allele frequencies	
	−1438A/A	−1438G/A	−1438G/G	−1438A	−1438G
Anorexia nervosa (n=77)	23 (29·87%)*	41 (53·24%)	13 (16·88%)	0·565*	0·435
Anorexia restricting type (n=43)	18 (41·86%)*	21 (48·83%)	4 (9·30%)	0·662*	0·337
Anorexia purging type (n=34)	5 (14·70%)	20 (58·82%)	9 (26·47%)	0·441	0·558
Controls (n=107)	10 (9·34%)	56 (52·33%)	41 (38·31%)	0·355	0·644

*p<0·0001.

Distribution of −1438G/A genotypes and allele frequencies

1 Collier DA, Arranz MJ, Li T, Mupita D, Brown N, Treasure J. Association between the 5-HT$_{2A}$ gene promoter polymorphism and anorexia nervosa. *Lancet* 1997; **350:** 412.

2 Hinney A, Ziegler A, Nothen MA, Remschmidt H, Hebebrand J. 5-HT$_{2A}$ receptor gene polymorphism, anorexia nervosa, and obesity. *Lancet* 1997; **350:** 1324–25.

3 Campbell DA, Sundaramurthy D, Markham AF, Pieri LF. Lack of association between 5-HT$_{2A}$ gene promoter polymorphism and susceptibility to anorexia nervosa. *Lancet* 1998; **351:** 499.

Department of Neurological and Psychiatric Sciences, University of Florence, 50134 Florence, Italy (S Sorbi)**; Casa di Cura Villa dei Pini, Florence, Italy; and Department of Pathophysiology, University of Florence**

5-HT$_{2A}$ promoter polymorphism –1438G/A, anorexia nervosa, and obsessive-compulsive disorder

Mary-Anne Enoch, Walter H Kaye, Alessandro Rotondo, Benjamin D Greenberg, Dennis L Murphy, David Goldman

Hypothalamic serotonin (5-HT) dysfunction has been implicated in eating disorders and obsessive-compulsive disorder (OCD). Anorexia nervosa and OCD are heritable and often comorbid disorders[1,2] that share several personality traits, including harm avoidance, perfectionism, and obsessionality.[3] In this study we replicate an association of a 5-HT$_{2A}$-receptor promoter polymorphism, –1438G/A, with anorexia nervosa.[4] In addition, we show that the association extends to OCD but not to bulimia nervosa, a disorder in which the obsessive and perfection-seeking personality features seen in anorexia nervosa are less manifest.

We genotyped three independent sets of unrelated white people for the –1438G/A 5-HT$_{2A}$ polymorphism. One set was from the USA, with 68 anorexics, 22 bulimics, and 69 normal volunteers free of psychopathology; one set was from Italy, with 20 anorexics and 37 bulimics; and another set was from the USA, with 62 patients with OCD and 144 normal controls free of OCD but not excluded for other psychopathology. All participants gave informed consent under human-research protocols approved by review boards at the relevant institutions. All were diagnosed with the structured clinical interview for DSM-III-R criteria. We followed previously published methods[4] for genotyping.

As shown in the table, the –1438A 5-HT$_{2A}$ allele was more common in US anorexics (0·51) than in controls (0·36, $\chi^2=12·35$, $p<0·005$). The genotype frequencies were also different ($\chi^2=7·42$, $p<0·025$). However, in US bulimia patients the 5-HT$_{2A}$ –1438A allele frequency did not differ from controls and was lower (0·34) than in patients with anorexia (0·51, $\chi^2=16·71$, $p<0·005$). This finding was replicated in an independent dataset of Italian eating-disorder patients. Here the –1438 allele frequency in bulimics was 0·38 compared with 0·65 in anorexics ($\chi^2=12·53$, $p<0·005$) and

	n	Genotype frequency			Allele frequency	
		A/A	A/G	G/G	A	G
USA						
Anorexia	68	17 (0·25)	35 (0·61)	16 (0·24)	69 (0·51)	67 (0·49)
Bulimia	22	1 (0·05)	13 (0·59)	8 (0·36)	15 (0·34)	29 (0·66)
Controls	69	6 (0·09)	38 (0·55)	25 (0·36)	50 (0·36)	88 (0·64)
Italy						
Anorexia	20	9 (0·45)	8 (0·40)	3 (0·15)	26 (0·65)	14 (0·35)
Bulimia	37	6 (0·16)	16 (0·43)	15 (0·41)	28 (0·38)	46 (0·62)
USA						
OCD	62	17 (0·27)	28 (0·45)	17 (0·27)	62 (0·50)	62 (0·50)
Controls	144	25 (0·17)	69 (0·48)	50 (0·35)	119 (0·41)	169 (0·59)

All genotype frequencies were in Hardy-Weinberg equilibrium.

Genotype and allele frequencies of –1438G/A 5-HT$_{2A}$ promoter polymorphism in OCD and two populations of eating-disorder patients

genotype frequencies also differed (χ^2=6·784, p<0·05). Finally, there was an increase in the frequency of the –438A allele in patients with OCD (0·50) compared with controls (0·41, χ^2=3·88, p<0·05), although genotype frequencies did not differ.

These results suggest that the 5-HT_{2A} –1438G/A promoter polymorphism, or a variant with which it is closely linked, may contribute to a behavioural trait, for example perfectionism or obsessionality, common to both anorexia nervosa and OCD but uncommon in bulimia. The exact function of the 5-HT_{2A} receptor is unknown but it is thought to contribute to eating behaviours and anxiety. These traits are associated with anorexia nervosa and OCD.[3] 5-HT_{2A} is a G-protein-coupled receptor and controls signal transduction by activating phospholipase C. It is not known whether the –1438G/A alleles are functionally different. However, a functional promoter variant might differentially alter transcription, thereby affecting receptor number. Our results suggest that differences in 5-HT_{2A} function should correlate with –1438G/A genotype and that these differences may be detectable at either the level of 5-HT_{2A} binding or the downstream effects of 5-HT_{2A} activation.

1 Kaye WH, Weltzin TE, Hsu LKG, Bulik C, McConaha C, Sobkiewicz T. Patients with anorexia nervosa have evaluated scores on the Yale-Brown Obsessive-Compulsive Scale. *Int J Eat Disord* 1992; **12:** 57–62.

2 Pigott TA, Alternus M, Rubenstein CS, et al. Symptoms of eating disorders in patients with obsessive-compulsive disorder. *Am J Psychiatry* 1991; **148:** 1552–57.

3 Jarry JL, Vaccarino FJ. Eating disorder and obsessive-compulsive disorder: neurochemical and phenomenological commonalities. *J Psychiatry Neurosci* 1996; **21**(1): 36–47.

4 Collier DA, Arranz MJ, Li T, Mupita D, Brown N. Association between 5-HT_{2A} gene promoter polymorphism and anorexia nervosa. *Lancet* 1997; **350:** 412.

Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892-8110, USA (M-A Enoch)**; Eating Disorders Unit, Western Psychiatric Institute and Clinic, University of Pittsburgh Medical Center, Pittsburgh, PA, USA; Department of Psychiatry, University of Pisa, Pisa, Italy; and Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Health, Bethesda**

Follow-up of ventricular pre-excitation in Japanese schoolchildren

Souichi Sano, Sadayoshi Komori, Takazoh Amano, Kohji Tamura

We investigated changes in ventricular pre-excitation during growth with routine school medical examinations. All schoolchildren in Yamanashi prefecture (population 880 000) must undergo medical examination, including an electrocardiogram (ECG) when they enter elementary school (aged 6–7 years), junior high school (12–13 years), or high school (15–16 years). Ventricular pre-excitation was diagnosed if PR interval was less than 0·12 s, 0° less than p axis 90° or less, and there was a delta wave. We kept the records of all schoolchildren with ventricular pre-excitation since 1994. We have already reported that ventricular pre-excitation ECG in younger schoolchildren is less frequent than other reports,[1–3] and that the prevalence of ventricular pre-excitation and left-sided accessory pathway increase with age.[4]

In a longitudinal study, we compared the prevalence of ventricular pre-excitation in the same students in the first year of junior high school in 1994, and first year of high school in 1997. There were five students with pre-excitation in 1994; only two students had ventricular pre-excitation in 1994 and in 1997. One of those was diagnosed as having intermittent pre-excitation by ambulatory ECG. Two students with accessory pathway had a normal ECG in 1997. We do not know whether pre-excitation disappeared or became intermittent. One student diagnosed with pre-excitation in 1994 moved to another school district.

Eight students were newly diagnosed with pre-excitation in 1997; two of the eight students had a normal ECG in 1994. Six students were newly recognised with accessory pathways in 1997. There was no significant difference in the prevalence of pre-excitation in high-school students in 1997 and junior-high-school students in 1994. There was intermittent disappearance of accessory pathways in nine (75%) of 12 students.

In all, 13 students had pre-excited ECGs at one of two school medical examinations, and only five students were detected at first school examination; sensitivity of first school medical examination for diagnosis of pre-excitation was 38·5%. A single ECG at younger ages may not predict Wolff-Parkinson-White syndrome in later life. Based on these observations, we presume that more accessory pathways become manifest in the ECG as children grow because atrioventricular conduction through the AV node slows with age.

1 Klein GJ, Yee R, Sharma AD. Longitudinal electophysiologic assessment of asymptomatic patients with Wolff-Parkinson-White electrocardiographic pattern. *N Engl J Med* 1989; **320:** 1229–33.

2 Krahn AD, Manfreda J, Robert B, Tate RB, Mathewson FAL, Cuddy TE. The natural history of electrocardiographic preexcitation in men: The Manitoba follow-up study. *Ann Intern Med* 1992; **116:** 456–60.

3 Munger TM, Packer DL, Hammill SC, et al. A population study of the natural history of Wolff-Parkinson-White syndrome in Olmsted County, Minnesota, 1953–1989. *Circulation* 1993; **87:** 866–73.

4 Sano S, Komori S, Amano T, et al. The prevalence of ventricular pre-excitation in Japanese schoolchildren. *Heart* 1998; **79:** 374–78.

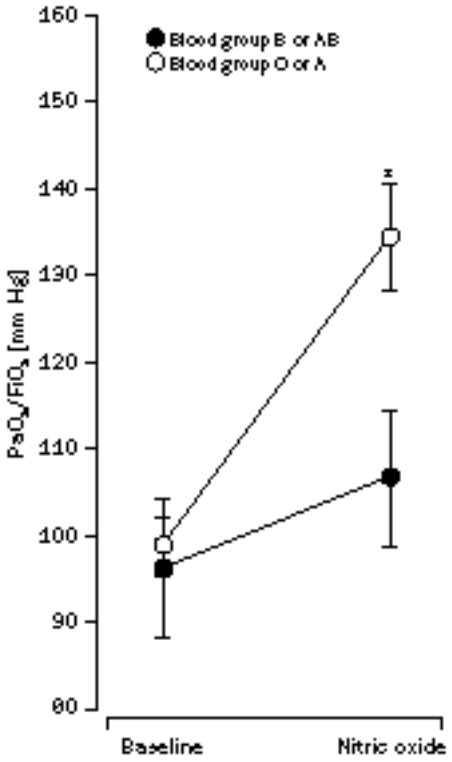
The Second Department of Internal Medicine, Yamanashi Medical University, Tamaho, Nakakoma, Yamanashi 409-38, Japan (S Sano) **and The Heart examination judgment committee of the medical association in Yamanashi prefecture**

ABO blood group and inhaled nitric oxide in acute respiratory distress syndrome

Jörg Weimann, Harald Bauer, Luca Bigatello, Kenneth D Bloch, Eike Martin, Warren M Zapol

Inhalation of low concentrations of nitric oxide augments arterial oxygenation in patients with the acute respiratory distress syndrome (ARDS).[1] However, 30–40% of patients with ARDS do not have an increase in arterial partial pressure of oxygen (PaO_2).[1–3] We investigated whether genetic factors contribute to the ability of inhaled nitric oxide to increase oxygenation in patients with ARDS.

We reviewed the medical records of 138 patients (95 at Massachusetts General Hospital and 43 at University Hospital Heidelberg) with ARDS (aged 20–85 years) who received inhaled nitric oxide in research protocols between 1991 and 1997. The aetiology of ARDS in these patients included infectious pneumonia (24), primary sepsis (19), lung resection (18), trauma and burn injury (15), aspiration (13), acute pancreatitis (four), and miscellaneous disorders (45). The blood-group distribution was similar to that in the normal population. Mean (SE) baseline values, including the ratio of PaO_2 to the fraction of inspired oxygen (PaO_2/FiO_2; 13·1 [0·4] kPa) as an index of the efficiency of arterial oxygenation, the mean pulmonary artery pressure (36 [1] mm Hg), the Murray lung injury score (3·0 [0·1], n=51),[4] and the APACHE II score (24 [1], n=103) did not differ among patients of either sex or ABO and Rhesus-factor blood groups. Patients inhaled between five parts per million and 40 parts per million nitric oxide. The oxygenation response to inhaled



PaO_2/FiO_2 ratio during nitric-oxide inhalation
Data are mean (SE). *p=0·03.

nitric oxide was measured as the percentage increase in PaO_2/FiO_2 ($\Delta PaO_2/FiO_2$). If more than one nitric-oxide dose was inhaled, the largest $\Delta PaO_2/FiO_2$ was recorded. Patients with a $\Delta PaO_2/FiO_2$ of less than 20% were classified as non-responders.[2,3]

The oxygenation response to inhaled nitric oxide was significantly decreased in patients with genotype B who express blood group B or AB ($\Delta PaO_2/FiO_2$ 13 [4]%, n=27) compared with patients without genotype B who express blood group O or A ($\Delta PaO_2/FiO_2$ 36 [4]%, n=111, p=0·03, ANOVA and post-hoc Scheffé's test; figure). 67% of patients with blood group B or AB were non-responders to inhaled nitric oxide compared with 27% of patients with blood group O or A (p<0·001, two-tailed Fisher's exact test). The oxygenation response to inhaled nitric oxide did not differ between men (32 [5]%, n=91) and women (32 [6]%, n=47), or between rhesus-positive (35 [4]%, n=109) and rhesus-negative patients (21 [5]%, n=29).

These results suggest a relation between the oxygenation response to inhaled nitric oxide and the ABO blood-group system. Possible explanations include a genetic linkage between the ABO gene locus (9q34) and another, as yet unknown, gene locus that is involved in the pulmonary vascular response to inhaled nitric oxide. Alternatively, ABO blood group may modify the severity or course of ARDS and alter responsiveness to nitric oxide. For example, patients with ARDS associated with sepsis more frequently fail to respond to inhaled nitric oxide than patients with ARDS associated with other processes.[2] Antibodies to blood group B are reported to possess antibacterial properties.[5] Since these antibodies are naturally present in individuals with blood groups O or A, who responded better to inhaled nitric oxide, it is possible that ABO blood group may modify the underlying process contributing to ARDS and, thereby, modulate responsiveness to inhaled nitric oxide.

This study was supported by USPHS grants HL 42397 (W M Z and K D B) and HL 55377 (K D B), and by the Deutsche Forschungsgemeinschaft WE 2114/1-1 (J W). K D B is an established investigator of the American Heart Association. We thank Yu Chiao Chang for statistical advice.

1 Rossaint R, Falke KJ, Lopez F, Slama K, Pison U, Zapol WM. Inhaled nitric oxide for the adult respiratory distress syndrome. *N Engl J Med* 1993; **328:** 399–405.
2 Manktelow C, Bigatello LM, Hess D, Hurford WE. Physiologic determinants of the response to inhaled nitric oxide in patients with acute respiratory distress syndrome. *Anesthesiology* 1997; **87:** 297–307.
3 Dellinger RP, Zimmerman JL, Taylor RW, et al. Effects of inhaled nitric oxide in patients with acute respiratory distress syndrome: results of a randomized phase II trial. Inhaled Nitric Oxide in ARDS Study Group. *Crit Care Med* 1998; **26:** 15–23.
4 Murray JF, Matthay MA, Luce JM, Flick MR. An expanded definition of the adult respiratory distress syndrome. *Am Rev Respir Dis* 1988; **138:** 720–23.
5 Springer GF. Importance of blood-group substances in interactions between man and microbes. *Ann N Y Acad Sci* 1970; **169:** 134–52.

Department of Anesthesia and Critical Care (J Weimann) **and the Cardiovascular Research Center and Cardiology Division of the Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA; and Klinik für Anaesthesiologie, Ruprecht-Karls Universität Heidelberg, Heidelberg, Germany**

Association at *LRP* gene locus with sporadic late-onset Alzheimer's disease

Jean-Charles Lambert, Fabienne Wavrant-De Vrièze, Philippe Amouyel, Marie-Christine Chartier-Harlin

The ε4 allele of the apolipoprotein E gene (*APOE*) is the only recognised genetic susceptibility factor for late-onset Alzheimer's disease (AD). However, the mechanism by which the *APOE* alleles affect disease development is not fully understood. The LDL receptor-related protein (LRP), located on chromosome 12, is the main apoE receptor in the brain. LRP mediates neurite outgrowth in an apoE-isoform-dependent manner. LRP is also responsible for the endocystosis of secreted APP, another molecule central to the pathogenesis of AD. These observations suggest a potential for this apoE receptor to have a role in the pathogenesis of AD. Four studies have investigated the association of a TTTC repeat polymorphism in the 5' end of the *LRP* gene with AD, two of them with negative results.[1-3] These discrepancies may be due to sample limitations (n<180 AD cases for all studies), different genetic background (not all whites, a mixture of familial or sporadic AD cases in some studies), or linkage disequilibrium with another gene. A report describing a silent CT polymorphism in exon 3 favours the hypothesis that variability in the *LRP* locus is associated with familial late-onset AD.[4] We tested this polymorphism in a large white sample composed of 558 sporadic probable AD—ie, cases without any formal pattern of family inheritance—(mean age 71·8 [SD 8·1] years; mean age at onset 68·6 [8·2] years; 38·2% men) and 596 controls (mean age 72·7 [8·3] years; 37·2% men). Patients characterised according to NINCDS-ADRDA criteria, were recruited in hospital. Controls were defined as those without DSM-III-R dementia criteria and with intact cognitive functions. Informed consent was obtained. Statistical analyses were done with SAS software (SAS institute, Cary, NC). Categorical data were tested with the Pearson χ^2 test. Multivariate logistic regression analyses were used to test for interactions between *LRP* genotypes and

	n	Genotype distribution*			Allele distribution†	
		CC	CT	TT	C	T
Control	596	407 (0·68)	168 (0·28)	21 (0·04)	982 (0·82)	210 (0·18)
AD	558	428 (0·77)	119 (0·21)	11 (0·02)	975 (0·87)	141 (0·13)

*p=0·004, †p<0·001.

Genotype and allele distributions of the LRP exon 3 CT polymorphism

age of onset or *APOE* status, and to estimate odds ratios adjusted for age and sex.

The genotype and allele distributions were similar to that described by Kang and colleagues[4] (table). The *LRP* CC genotype was over-represented in AD (odds ratio 1·5, [95% CI 1·2–2·0], p=0·002) compared with controls. Although no statistical interaction could be detected between *LRP* polymorphism and age at onset, the association of the *LRP* CC genotype was more pronounced in late-onset AD cases (1·7 [1·2–2·3], p=0·001, age at onset >65 years) than in cases with earlier ages at onset (1·2 [0·7–1·9], p=0·520, age at onset ≤65 years). Similarly, the level of association between the *LRP* CC genotype and AD tended to be stronger in those carrying at least one ε4 allele (2·2 [1·3–3·7], p=0·006), than in people without an ε4 allele (1·5 [1·0–2·3], p-0·080), despite the lack of significance of the statistical interaction.

Kang and colleagues[4] reported that the *LRP* CC genotype was a risk factor for familial late-onset AD and described a similar trend in sporadic late-onset AD cases, lack of significance in this latter group probably being due to the small sample size. Our results extend the possible effect of the *LRP* CC genotype to sporadic late-onset AD cases. However, we cannot reject the hypothesis that linkage disequlibrium exists between this *LRP* polymorphism and another polymorphism within *LRP* or neighbouring genes. In the light of linkage data implicating a new locus on chromosome 12 for late-onset AD,[5] the genetic association of *LRP* variability with both familial and sporadic late-onset AD may confirm involvement of the *LRP* locus in the disease.

We thank Jordi Pérez-Tur for his helpful discussion. J-CL is a recipient of the Ministère de l'Enseignement Supérieur et de la Recherche. This work was supported by the Institut National pour la Santé et la Recherche Médicale, the Institut Pasteur de Lille, the Conseil Régional du Nord-Pas de Calais 'axe régional de recherche sur les maladies neurodégénératives et le vieillissement cérébral' and the Fondation pour la Recherche Médicale.

1 Lendon CL, Talbot CJ, Craddock NJ, et al. Genetic association studies between dementia of Alzheimer's type and the three receptors for apolipoprotein E in Caucasian population. *Neurosci Lett* 1997; **222:** 187–90.

2 Wavrant-De Vrièze F, Pérez-Tur J, Lambert J-C, et al. Association between the low-density lipoprotein receptor-related protein (LRP) gene and Alzheimer's disease. *Neurosci Lett* 1997; **227:** 68–70.

3 Clatworthy AE, Gomez-Isla T, Rebeck GW, Wallace RB, Hyman BT. Lack of association of a polymorphism in the low-density lipoprotein receptor-related protein gene with Alzheimer disease. *Arch Neurol* 1997; **54:** 1289–92.

4 Kang DE, Saitoh T, Chen X, et al. Genetic association of the low-density lipoprotein receptor-related protein gene (LRP), an apolipoprotein E receptor with late-onset Alzheimer's disease. *Neurology* 1997; **49:** 56–61.

5 Pericak-Vance MA, Bass MP, Yamaoka LH, et al. Complete genomic screen in late-onset familial Alzheimer disease. *JAMA* 1997; **278:** 1237–41.

INSERM CJF 95-05, Institut Pasteur de Lille, BP 245, 59019 Lille, France
(M-C Chartier-Harlin)

Could lightning injury be magnetically induced?

Michael Cherington, Howard Wachtel, Philip R Yarnell

Lightning injuries are usually thought to be caused by direct strike, side flash, or ground current;[1,2] none of which explain lightning-related deaths where current does not apparently enter or leave the patient's body.

In June, 1996, a 32-year-old man and three other golfers stood under a tree during a lightning storm. When lightning struck the tree, the patient suffered a cardiac arrest. Cardiopulmonary resuscitation was given by a physician who was nearby. The patient was taken to hospital but remained comatose and died on the 18th day. Three other golfers under the same tree survived. One had surface burns on his head, neck, and abdomen at places where he wore metal objects: necklace, belt buckle, and metal button on a cap. The other two golfers had only brief loss of consciousness. This case and at least four other cases in the medical literature[3] are unusual in that patients succumbed to lightning but there was no evidence of external damage from lightning currents. Lightning-related hypoxic encephalopathy secondary to cardiac arrest is often fatal.[3] Most lightning casualties have external signs of damage from contact with electrical current, such as skin burns and Lichtenberg figures or "ferning".[4,5] However, some patients have a cardiac arrest as the result of a lightning strike without external signs of electrical burns.

Lightning "bolts" have very high peak currents (≥100 000 amps) which rise in μs and decay more slowly. They may produce intense nearby magnetic fields (several millitesla at a distance of about 1 m), which may induce large but short-lived (<1 ms) currents in a human body. The induced current wave-form is proportional to the change per time of the magnetic field. The lightning may induce a loop current within the human torso without evidence of current entering the body. If these currents occur during a vulnerable part of the cardiac cycle, they could cause asystole or ventricular fibrillation.*

This hypothesis may be tested on mice or rats exposed to rapidly rising magnetic field pulses that approximate the dynamics of lightning currents within cylindrical chambers enclosed by solenoid windings. This method would necessitate the use of fields on the order or ten times as great as the natural lightning fields. Our proposed mechanism may explain some unwitnessed and unexplained "heart attacks" among hikers found dead in the mountains.

Presented in part at the American Association of Physics Teachers Meeting in Denver, Colorado, August 14, 1997.

We thank Vincent W Vanek.

*Lightning stroke with peak current 100 000 amp gives peak magnetic field (B_p):

$$B_p=\frac{\mu_o I_p}{2\pi R}$$

Where μ_o=magnetic permeability of air $4\pi(10^{-7})$ Webers/A•m I_p=peak current (amps). R=distance from the lightning stoke (m).
For a distance of 1·0 m, $B_p=2(10^{-2})$ Tesla. Lightning current rises to its peak in about 10^{-6} s, so the rate of rise of dB/dt is $2(10^4)$ Tesla/s. Electrical field induced in a cylindrical object (human torso)

$$E=\left(\frac{a}{2}\right)\frac{dB}{dt}$$

Where E=electrical field (v/m) a=radius of the induced current path (m). Highest fields would be at the perimeter of the torso and the lowest ones produced in the centre. With an estimate of a=0·1 m yields E of about 1000 V/m for dB/dt=$2(10^4)$ Tesla, and current density of about 1000 A/m^2 through the ventricles (tissue resistivity 1 ohm/m). Long current pulses (of ms) or for 60 Hz, such a current density would lead to arrhythmias. For current pulses lasting a few μs, electrical impedance of cardiac-cell membranes is due almost entirely to the membrane capacitance (Cm) which is on the order of 0·01 F/m^2, the change in membrane potential (ΔVm) is

$$\Delta Vm=\frac{J(\Delta t)}{Cm}$$

For J=1000 A/m^2, and the duration of the current pulse, $\Delta t=10^{-6}$ s, predicts a ΔVm of 100 mV. If the cardiac cell is in a refractory state this current is not likely to re-trigger a discharge in the active phase corresponding to the ECG T wave. Ventricular fibrillation or asystole may result.

1 Kleinschmidt-DeMasters BK. Neuropathology of lightning-strike injuries. *Semin Neurol* 1995; **15:** 323–28.

2 Cooper MA. Emergent care of lightning and electrical injuries. *Semin Neurol* 1995; **15:** 268–78.

3 Wetli CV. Keraunopathology: an analysis of 45 fatalities. *Am J Forens Med Path* 1996; **17:** 89–98.

4 Cherington M, Yarnell PR, London SF. Neurological complications of lightning injuries. *West J Med* 1995; **162:** 413–17.

5 ten Dius HJ, Klasen HJ, Nijsten MWN, Pietronero L. Superficial lightning injuries: their "fractal" shape and origin. *Burns* 1987; **13:** 141–46.

Lightning Data Center, St Anthony Hospital, Denver, CO 80204, USA
(M Cherington)**; Department of Neurology, University of Colorado School of Medicine, Denver; and Electrical and Computer Engineering Department, Boulder, CO**