Research letters

Concentrations of dioxin 20 years after Seveso

M T Landi, L L Needham, G Lucier, P Mocarelli, P A Bertazzi, N Caporaso

In 1976 an industrial accident contaminated the area around Seveso, Italy, with the highest concentrations of the potent toxicant 2,3,7,8-tetrachlorodibenzo-(*p*)-dioxin (TCDD) ever recorded in man. TCDD is important because of its persistence in the environment, long half-life in man, and its toxic effects,[1] which may be teratogenic, carcinogenic, hormonal, immunological, and affect the sex ratio.[2] On the basis of soil TCDD measurements, the contaminated area was divided into three zones (A, B, R) with progressively lower levels of contamination. Mortality and cancer incidence has been studied in a cohort, including all persons ever resident in the area from the date of the accident onward (age range 20–75 years, about 700 individuals in zone A, 4800 in zone B, and 31 600 in zone R). About 180 000 individuals from the surrounding non-contaminated area (non-ABR) were followed up as a reference population.

Almost 20 years after the accident, 62 individuals (7 from zone A [2 women, 5 men], 55 from zone B [28 women, 27 men]) from the TCDD-contaminated zones (A and B) and 59 from non-ABR (32 women, 27 men) were randomly selected from the cohort and frequency matched by gender, age, and smoking history. Written informed consent was obtained and approval given by the local institutional review board. We gave the participants a questionnaire surveying demographic, lifestyle, and accident-related factors, and medical history. We assayed plasma TCDD concentrations by high-resolution gas chromatography/high resolution mass spectrometric analysis, with results reported as parts per trillion (ppt), lipid adjusted.[3]

Raised TCDD concentrations persist in individuals from the exposed areas (range 1·2–89·9 ppt; geometric mean (GM) concentration in zone A=53·2 ppt, in zone B=11·0 ppt, in non-ABR=4·9 ppt; p=0·0001) and correspond to zone categorisation based on soil TCDD measurements determined in 1976.[1] Women from zone B (small numbers in zone A precluded a sex comparison) had significantly higher TCDD concentrations than did men (table). Presence in the contaminated area at the time of the accident, number of years spent in the zone, occupation, and distance from the accident site within the zone (taking into account the course of the toxic cloud during the accident), did not explain the gender difference. Even in non-ABR, where typical population values were present, women had significantly higher TCDD concentrations than men. We observed a weak, non-significant increase in TCDD concentrations with age, possibly attributable to declining steroid hormones, or changes in body-fat distribution or amount. TCDD concentrations were unrelated to percentage body fat, body mass index (BMI), height, weight, or reported weight loss for each sex. Among the reproductive variables studies in women of zone B, only oral contraceptive use was significantly associated with lower TCDD concentrations (never users n=13, GM=23·4; ever users n=13, GM=13·2, p=0·05, *t*-test).

	Zone B		Non-ABR	
	Women	Men	Women	Men
Number	26	25	28	24
Geometric mean	17·6	6·7	6·1	3·7
GSD*	2·1	2·7	1·7	2·0
p-values	0·0003		0·007	

*Geometric standard deviation.

Current TCDD concentrations (ppt) by zone and gender

In a multivariate analysis of individuals in zone B, we also examined the effect of sex, age, smoking status, BMI, and food consumed during the accident period. Sex, distance from the accident site, and meat consumption were significantly associated with TCDD concentration (p=0·0002, p=0·02, and p=0·05, respectively).

As far as we know, a gender difference has not been previously reported in TCDD-exposed populations, perhaps because many groups have consisted mostly of men exposed as a result of their occupation or military service, but one study that included women has reported similar findings.[4] Increased TCDD concentrations in women may be due to variation in metabolism or elimination, or to differences in body fat or hormone-related factors. Gender differences in cancer occurrence have also been reported in Seveso.[1] The recorded TCDD concentrations raise concern about adverse reproductive, developmental, and cancer outcomes in women and their offspring.

This work was supported by Regione Lombardia and Fondazione Lombardia per l'Ambiente.

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Genetic Epidemiology Branch, NCI/NIH, Bethesda, MD 20892, USA (M T Landi)**; Centers for Disease Control, Atlanta, USA; NIEHS, Research Triangle Park, NC, USA; and Desio Hospital, University of Milan, Italy: and EPOCA, University of Milan, Milan**

Molecular diagnosis of Wilson's disease

Norikazu Shimizu, Hiroki Nakazono, Atsuko Watanabe, Yukitoshi Yamaguci, Hiromichi Hemmi, Tsugutoshi Aoki

The diagnosis of Wilson's disease is usually based on such biochemical findings as low serum caeruloplasmin, excess urinary copper excretion, and/or a high hepatic copper content. However, difficulties may arise in very young patients with this disease, since some of these biological features may not be present. We have diagnosed the disease in an 8-month-old baby on the basis of gene analysis.

An 8-month-old baby boy presented with hypocaeruloplasminaemia. His serum total caeruloplasmin was 9·5 mg/L and holocaeruloplasmin (active caeruloplasmin)

was 8·7 mg/L. Possible diagnoses included Wilson's disease, caeruloplasmin deficiency, and others. He had no clinical symptoms or signs. His liver function was normal and he was not anaemic. Urinary copper excretion was 17 µg per day, which is within normal limits. We screened for the Wilson's disease gene (*WD* gene or *ATP7B*). Genomic DNA was isolated from peripheral blood. 25 sets of oligonucleotide primers were prepared to amplify all exons except exon 1, as described.[1] PCR was carried out as described[2] except for a modification in the total volume (20 µL). In addition, DNA sequencing analysis was done essentially as described elsewhere.[2] A mutation was found in exon 8 of *ATP7B*. A one-base insertion of C was detected at nucleotide 2302, homozygously. This mutation (2302 ims C) is believed to alter the reading frame of the gene product and to result in truncation of the protein. As a result, the protein becomes non-functional. The same mutation has been reported in one Italian and one British patient.[1] We thus diagnosed this 8-month-old boy to have a presymptomatic type of Wilson's disease.

Usually, low urinary copper excretion in a patient with presymptomatic Wilson's disease is thought to indicate copper accumulation in the liver. However, little has been reported about the hepatic copper content at this age, and a liver biopsy is highly invasive. By contrast, only a small amount of peripheral blood is required to the isolate genomic DNA for genetic analysis. We are following up this baby carefully and will begin treatment before liver cirrhosis occurs.

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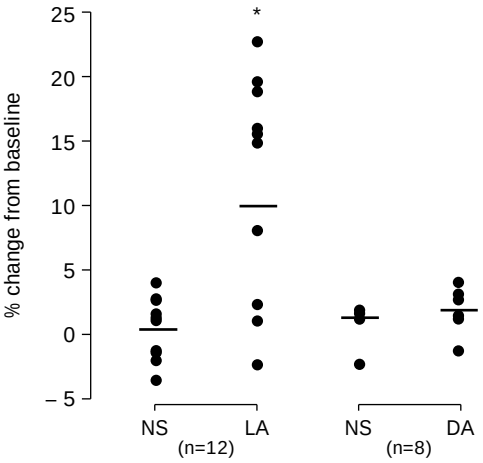
2nd Department of Paediatrics (N Shimizu) **and Department of Molecular Biology, Toho University School of Medicine, Ohashi Hospital, Tokyo 153, Japan**

Coronary stenosis dilatation induced by L-arginine

Dimitris Tousoulis, Graham Davies, Costas Tentolouris, Tom Crake, Pavlos Toutouzas

L-arginine, a substrate in the production of endothelium-derived nitric oxide (NO), may stimulate the release of NO. L-arginine improves the coronary blood flow response to acetylcholine in patients with normal coronary arteries and hypercholesterolaemia.[1] However, it is unknown whether local administration of L-arginine dilates coronary stenoses in patients with stable angina. This study assessed the effects of L-arginine and D-arginine at the stenotic site in patients with coronary artery disease and stable angina.

15 patients with chronic stable angina, coronary artery disease, and a positive treadmill exercise test (⩾0·1 mV ST segment depression) at between 5 and 7 METS with the modified Bruce protocol, were studied in the cardiac catheterisation laboratory of the Hippokration Hospital, Athens University. The protocol was approved by the research ethics committee of the Hippokration Hospital and each patient gave written informed consent. Antianginal medication was stopped 24 h before the study. The patients were allowed to use sublingual glyceryl trinitrate as necessary, but no study was done within 3 h of its administration. None of the study patients had taken glyceryl trinitrate on the day of the study. Following the diagnostic coronary angiogram, an optimum radiographic projection was selected and kept constant for subsequent angiograms. In ten patients (seven male, three female, mean age 58 [SD 8] years) intracoronary infusion of 0·9% saline (2 mL/min) for 2 min was followed by intracoronary infusion of 150 µmol/min of L-arginine for 8 min. In five patients (four male, one female, mean age 61 [10] years) the same protocol was used, substituting 150 µmol/min of D-arginine for L-arginine. The chosen dose and the infusion time of L-arginine administration was consistent with a study showing a response after L-arginine administration in the brachial artery.[2] The arterial segments in each frame were analysed at the Hammersmith Hospital in random order with quantitative computerised analysis, with an automated edge contour detection analysis system. The percentage change in luminal diameter from baseline was calculated at the end of the 8 min infusions and the values for L-arginine and D-arginine and normal saline were compared with Student's *t*-test.



Responses of coronary stenoses to normal saline (NS) and L-arginine (LA) in ten patients, and to NS and D-arginine (DA) in five patients
Horizontal bars=mean values, *p=0·004 *vs* DA, p=0·001 *vs* NS, n=number of stenoses.

The infusions of L-arginine and D-arginine did not affect systolic blood pressure (mean [SE]) (L-arginine: mean 152 [7·1] *vs* 150 [7·2] mm Hg, p=0·82 D-arginine: 154 [11·2] *vs* 157 [11·6] mm Hg, before and after infusions, respectively, p=0·27). L-arginine administration was associated with significant dilatation of stenoses (11·7 [2·3]%: p=0·004 *vs* D-arginine, p=0·001 *vs* normal saline) (figure), but there was no significant change with D-arginine (2·0 [0·04]%) (figure). No patients were receiving longacting nitrates, and the previous antianginal treatment did not affect the response to L-arginine (β-blockers: 11·1 [6·0]%; calcium antagonists 10·5 [4·7]%; β-blockers and calcium antagonists 12·5 [3·4]%).

Arginine, a semiessential aminoacid serves as the substrate for the enzyme NO synthase, which converts arginine to citrulline and NO, reducing vascular tone.[3] A recent study[4] in experimental models suggested possible beneficial effects of L-arginine administration. Long-term oral therapy with L-arginine reduced endothelial dysfunction and inhibited the development of atherosclerosis,[5] but the mechanisms by which it exerts its vasodilator effect are not completely understood. Possible explanations include an effect on endothelial synthesis and release of NO, and direct or indirect physiological actions on the vascular smooth muscle to augment its responsiveness to NO. In our study a significant dilation was found in coronary stenotic segments after L-arginine administration in patients with coronary artery disease and stable angina. These data suggest that the

endothelial intracellular availability and/or mobilisation of L-arginine is defective in atherosclerotic coronary arteries in man. Augmentation of the activity of the NO synthase pathway merits further evaluation as a novel therapeutic target in the treatment of atherosclerosis and ischaemic heart disease.

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Cardiology Unit, Hippokration Hospital, Athens University Medical School, Athens, Greece; and Cardiology Unit, Hammersmith Hospital, London W12 0NN, UK (G J Davies)

Borna disease virus in brains of North American and European people with schizophrenia and bipolar disorder

*Mirella Salvatore, Sergei Morzunov, Martin Schwemmle, W Ian Lipkin, and the Bornavirus Study Group**

The pathogenesis of schizophrenia is unknown. Although a genetic component is likely, discordance for disease in 50% of monozygotic twins and epidemiological studies suggest that there are environmental co-factors.[1] Borna disease virus (BDV) is an RNA virus characterised by low productivity, non-cytolytic replication, broad host range, neurotropism, and the potential to alter movement and behaviour through selective effects on brain monoamine circuits.[2] BDV RNA has been detected in peripheral blood mononuclear cells of patients with neuropsychiatric disorders in Germany and Japan with prevalence rates for schizophrenia ranging from 12% to 64%.[3]

We examined the prevalence of BDV P gene mRNA in postmortem brain samples from 75 North American and European individuals with various brain disorders and healthy controls by means of nested reverse transcription PCR experiments (table). On the basis of knowledge of BDV tropism in animals, samples were taken from limbic structures (prefrontal cortex, hippocampus, amygdala, basal ganglia, cingulate gyrus) to increase the probability of detecting BDV RNA. At the University of California Irvine (UCI), P gene transcripts were detected in ten people: eight with schizophrenia (two from Germany, two from Southern California, USA, and four from New England, USA; and two with bipolar disorder (from New England). None were detected in brains of people with multiple sclerosis, Alzheimer's Disease, Parkinson's Disease, or any of the controls.

Samples from 13 of the 75 brains examined were sent from New England to the University of Nevada Reno (UNR) for independent analysis. Amplification products were obtained from two of five brains positive at UCI. One sample from a patient with schizophrenia negative at UCI was positive at UNR. Detection was RT-dependent indicating that products did not represent amplification of DNA from plasmids or products of other RT-PCR reactions. All products were of the expected length and hybridised with internal P-gene probes. Considering assays at both UCI and UNR, BDV nucleic acids were present in brains of nine of 17 patients with schizophrenia (53%, 95% CI: 0·27–0·78) and two of five patients with bipolar disorder (40%, 95% CI: 0·05–0·86). The amplification products had sequence divergence in the range of one of four nucleotides with respect to previously reported human isolates[4] or animal isolate He/80-1.[5]

Diagnosis (DSM IV)	BDV+/total	m/f	Mean age at death in years (SD)
Schizophrenia (295·xx)	9/17	15/2	60·6 (18·0)
Bipolar disorder (296·xxx)	2/5	3/2	53·8 (22·9)
Major depressive disorder (296·30)	0/6	1/5	72·3 (16·7)
Psychotic disorder not otherwise specified (298·9)	0/2	2/0	67·0 (17·0)
Alzheimer's disease	0/19	8/11	81·8 (6·1)
Parkinson's disease	0/11	7/4	71·5 (6·9)
Multiple sclerosis	0/5	1/4	60·0 (11·7)
No neurological disease	0/10	6/4	67·1 (11·4)
Total	11/75	43/32	

Borna disease virus in human brains

The nested RT-PCR method we used is sensitive to 100 copies of RNA template yet functions near the threshold for detection of BDV nucleic acids. This limitation together with differences in viral nucleic-acid distribution in brain may account for discordance between laboratories with some samples. The observation that sequences of P gene messages from brain were highly conserved with respect to previously described isolates might argue that positive samples represent amplification of low-level contaminants. However, samples sent directly from brain banks to two independent laboratories yielded similar results. Furthermore, amplification was RT-dependent. The finding of sequence conservation is consistent with previous analyses of well-characterised isolates from different host species and geographic locations.[5]

In non-human species, BDV causes either an immune-mediated disease with profound disturbances in monoamine metabolism, movement, and behaviour, or persistent infection with disturbances in brain development and subtle abnormalities in learning and behaviour.[2] The capacity for BDV to alter neural circuits in non-human species that are also reported to be impaired in schizophrenia suggests the possibility of common pathways for brain dysfunction. However, BDV and BDV mRNA can be detected in brains of non-human species without recourse to cocultivation or amplification. Thus, if BDV can be implicated as a primary or co-factor in the pathogenesis of schizophrenia, the mechanisms in human beings are likely to be different.

We cannot exclude the possibility that BDV in the brains of people with schizophrenia reflects the influence of psychotropic drugs on BDV-gene expression, or increased probability of exposure to infectious agents including BDV, particularly in that subgroup of patients with lower socioeconomic status. These questions and others including identification of reservoirs for BDV and modes for natural transmission need further study.

This work was supported by awards from the National Institute of Health, National Multiple Sclerosis Society, Wayne and Gladys Valley Foundation, UCI-Markey Program in Human Neurobiology, and Deutsche Forschungsgemeinschaft. We thank P Staeheli, M Hornig, S Lee and T Kurosaki for comments and F Benes and S Vincent of the Harvard Brain Tissue Resource Center contributing necropsy brain tissues.

A detailed description of the study may be found at http://www.ucihs.uci.edu/neurovirol

*Bornavirus Study Group: Departments of Neurology, Anatomy and Neurobiology, Microbiology and Molecular Genetics, Psychiatry and Human Behaviour, University of California Irvine, Irvine, CA, USA

(William E Bunney, Carl W Cotman, Chen Even, Carolyn G Hatalski, W Ian Lipkin, Steven G Potkin, Michelle L Portlance, Mirella Salvatore, Martin Schwemmle); Special Pathogens Branch, Division of viral and Rickettsial Disease, Centers for Disease Control and Prevention, Atlanta, GA, USA (Stuart T Nichol); National Neurological Research Specimen Bank, West Los Angeles VA Medical Center, Los Angeles, 90073, USA (Wallace W Tourtelloutte); University of Nevada, Reno, NV, USA (Sergei Morzunov); Universität Würzburg, Würzberg, Germany, Department of Psychiatry; Institut für Virologie und Immunbiologie (Peter Riederer, Volker ter Meulen).

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University of California, Irvine, CA 92697-4290, USA (W I Lipkin)

Protein kinase C expression in salivary gland acinar epithelial cells in Sjögren's syndrome

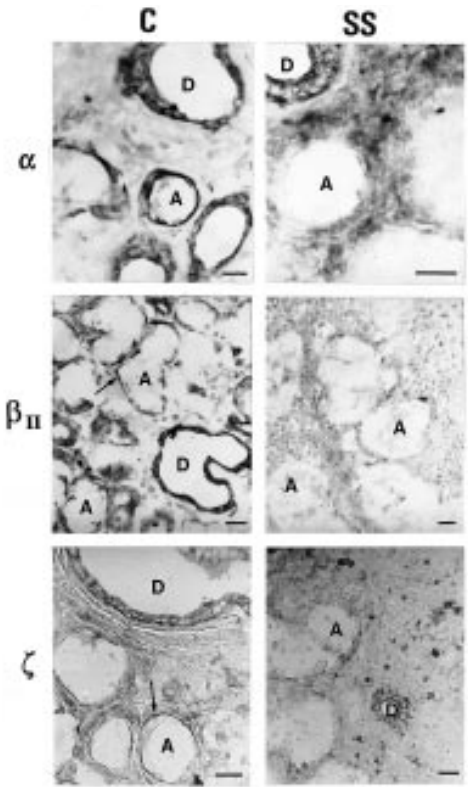
J Törnwall, Y T Konttinen, R K Tuominen, M Törnwall

Sjögren's syndrome (SS) is associated with autoimmune features, such as a focal adenitis and SS-A/Ro and SS-B/La autoantibodies,[1] but the mechanisms underlying the diminished function of exocrine glands is unknown. A characteristic feature of salivary-gland tissue in SS is focal adenitis, which is not found in all labial salivary glands or in all lobules of individual glands. This focal and local involvement contrasts with the functional impairment of the salivary gland. It has been shown that in an animal model for SS (NOD mice) sialopenia develops independently of lymphocyte infiltration,[2] suggesting that defective intracellular signalling, rather than lymphocyte-mediated destruction of the acinar epithelial cells, is responsible for the sialopenia.

We postulated that acinar epithelial cells in SS have a diminished capacity to respond to stimuli, such as activation of α_1-adrenergic, M1 and M3 type muscarinic, and NK-1-type substance P receptors. All these receptors converge on one common intracellular signalling pathway—protein-kinase C (PKC). Because formation of the saliva depends on the normal function of the acinar epithelial cells and is partly dependent on PKC,[3] we looked for the expression of PKC in patients with SS compared with controls.

Seven patients with primary SS and six controls gave informed consent to a labial salivary gland biopsy. The diagnosis of SS was made with EU criteria.[4] The specimens were labelled with both mouse monoclonal and rabbit polyclonal antibodies which recognise PKC α, β_I, β_{II}, γ, δ, ϵ, and ζ isoforms. Binding of the primary antibodies was visualised by avidin-biotin-peroxidase complex staining and western blotting.

In control sections, PKC α and β_{II} isoforms were found in acinar and ductal epithelial cells. PKC ζ and β_{II} isoforms were found in myoepithelial cells, which surround the acini but are located inside the basal lamina. PKC ζ isoform was also found in ductal epithelial cells. By contrast, in the SS sections, PKC β_{II} isoform showed no detectable staining and α isoform staining was weak in the acinar epithelial cells.



Protein kinase C isoforms in control (C) and SS labial salivary glands.
Notice the defect and/or weak expression of PKC β_{II} and α in acinar epithelial cells (a) and PKC β_{II} and ζ in myoepithelial cells (arrows) in patients compared with controls. PKC staining of the ductal epithelial cells (d) is similar in patients and controls. In addition to the structural salivary gland cells, some immigrant cells, such as periductal lymphocytes and mast cells, show positive staining. Bar=50 μm.

Furthermore, ζ and β_{II} isoforms did not show any staining in myoepithelial cells in patients (figure). However, α, β_{II} and ζ isoform staining of the ductal epithelial cells was similar in patients and in controls. Acinar and ductal epithelial cells of patients and controls did not contain any detectable PKC γ, β_I, δ, or ϵ isoform immunoreactivity. Western blotting confirmed the presence of PKC α, β_{II}, and ζ isoforms in salivary tissue.

Our findings show a defect in the signal-transduction system of the acinar epithelial cell due to PKC isoform deficiencies. PKC is a family of enzymes that modulate protein activity via serine/threonine phosphorylation.[5] Conventional PKC isoforms (α, β_I, β_{II}, and γ) depend on calcium diacylglycerol for full activation. Unusual isoforms (δ, μ, ϵ, η, and θ) do not need calcium for activation. A third class, so called atypical PKC isoforms (ζ, λ, and ι) require phosphatidylserine but not calcium or diacylglycerol.[5] In the present study two conventional (α, β_{II}) and one phosphatidylserine-dependent (ζ) PKC isoforms were found to be defectively expressed in the acinar epithelial and myoepithelial cells in patients with SS.

Activation of the PKC enzyme by stimuli from hormones, neurotransmitters, and growth factors is essential for cell proliferation, differentiation, membrane transport, and gene expression.[5] In salivary-gland acinar cells, activation of M1 and M3, α_1, or NK-1 receptors by acethylcholine, noradrenaline, or substance P, respectively, is linked to phospholipase C via GTP-dependent proteins. This leads to the hydrolysis of phosphatidylinositol 4,5-biphosphate to inositol 1,4,5-trisphosphate and *sn*-1,2-diacylglycerol, which raise intracellular free (Ca^{2+}) concentration and activate PKC, respectively.[5] The elevation of intracellular (Ca^{2+})

activates Ca^{2+}-sensitive ion channels leading to saliva formation. Myoepithelial cells are believed to play a central part in the reflex salivary flow by concentration, which may stimulate the release of acinar cell contents and exert some counterpressure during stimulated salivary flow.

These results show a new cellular defect of potential pathogenetic importance in an autoimmune disease.

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Departments of Anatomy (J Törnwall) **and Pharmacology and Toxicology, University of Helsinki, FIN-00014, Finland; and Department of Medicine, Helsinki University Central Hospital, Helsinki**

5-HT_{2A} receptor polymorphism and steady state receptor expression in schizophrenia

Alexander P Kouzmenko, Wendy L Hayes, Avril M Pereira, Brian Dean, Phillip W J Burnet, Paul J Harrison

The 5-HT_{2A} receptor has been implicated in the pathogenesis and pharmacotherapy of schizophrenia.[1] A genetic association has been found between a polymorphism at position 102 in the coding region of the gene and schizophrenia as well as with non-response to the atypical antipsychotic clozapine.[2] Thus the allelic variant is a conservative substitution (T to C) which does not alter the aminoacid sequence of the encoded receptor. The pathogenic effect of the polymorphism may occur as a result of its effect on receptor expression, possibly by changing the secondary structure of the transcript and thence affecting its stability or translational activity. We have examined whether central 5-HT_{2A} receptor densities in schizophrenia and controls are affected by T102C genotype.

Frontal cortex tissue was taken from 63 patients with DSM-III-R schizophrenia and 62 controls matched for age, sex, and post-mortem interval. None had been treated with clozapine or other atypical antipsychotics. Genotyping at position 102 of the 5-HT_{2A} receptor gene was by PCR amplification and *Msp*I digestion.[2] Quantitative autoradiography of 5-HT_{2A} receptor binding sites was carried out in dorsolateral prefrontal cortex (Brodmann area 9/46) with ^{3}H-ketanserin, as described previously.[3,4] 5-HT_{2A} receptor density was significantly lower in the schizophrenic individuals than in controls (*t*-test, $p<0{\cdot}00001$, table) in accordance with results reported from smaller samples.[3,4] However, stratification of the groups showed that there was no effect of genotype upon 5-HT_{2A} receptor densities in either controls or schizophrenics. The reduction of ^{3}H-ketanserin binding in schizophrenia was significant in each genotype group (all $p<0{\cdot}001$).

These data show that allelic variation at position 102 of the 5-HT_{2A} receptor gene does not affect the density of the receptor in the frontal cortex. It is known that the T102C variant is tightly linked to a polymorphism in the gene promoter, and it may be the latter that causes the genetic

5-HT_{2a} receptor gene at position 102	Controls	(n)	Schizophrenics	(n)
T/T, T/C, or C/C	60·4 [13·3]	62	40·3 [13·0]	63
T/T	62·7 [14·4]	10	36·4 [12·3]	8
T/C	58·8 [13·6]	35	40·7 [13·7]	38
C/C	62·3 [12·2]	17	41·1 [11·8]	17

Values are mean [SD] ^{3}H-ketanserin densities (fmol/mg tissue).

5-HT_{2A} receptor densities and T102C polymorphism

associations reported with the T102C variant (M J Owen, personal communication). Promoter polymorphisms must, however, like conservative substitutions in a coding region, exert their functional effect through gene expression rather than upon the receptor itself. Thus, our conclusion that the 5-HT_{2A} receptor gene allelic variants do not act by producing different steady-state concentrations of central 5-HT_{2A} receptors, remains valid. As such, there is no apparent biological mechanism to explain the effect of the polymorphisms. But the promoter region of the gene is complex,[5] and it is possible that the polymorphisms might alter expression of the receptor in discrete cell types, or in response to specific regulatory signals.

We thank Maria Arranz for genotyping some cases.

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Rebecca L Cooper Research Laboratories, Mental Health Research Institute of Victoria, Victoria, Australia; University Department of Clinical Neurology, Radcliffe Infirmary, Oxford, and University Department of Psychiatry, Warneford Hospital, Oxford OX3 7JX, UK (P J Harrison)

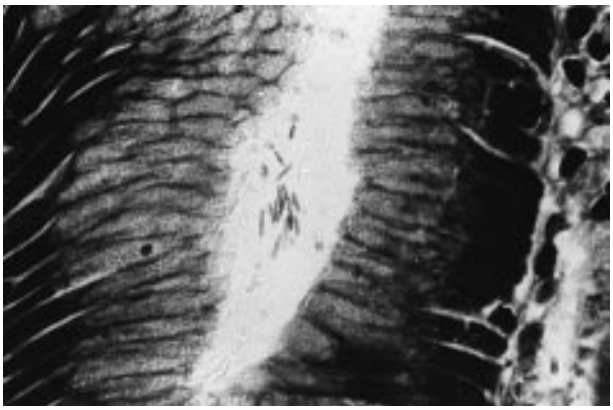
Healing of duodenal ulcer after eradication of *Helicobacter heilmannii*

Andrew F Goddard, Robert P H Logan, John C Atherton, David Jenkins, Robin C Spiller

Helicobacter pylori is the main cause of duodenal ulcer, and eradication of this organism leads to ulcer healing and prevents relapse. We describe successful healing of duodenal ulcer after eradication of another *Helicobacter* spp, *H heilmannii*.

A 47-year-old man was referred for gastroscopy with a 6-month history of epigastric pain. He had been previously well, smoked less than five cigarettes daily, and did not use non-steroidal anti-inflammatory drugs (NSAIDs). On endoscopy, we observed florid haemorrhagic erosive duodenitis with four small duodenal ulcers (3–5 mm across with obvious depth). Biopsy specimens were taken from the antrum and corpus for histological assessment (haematoxylin and eosin and toludine blue staining), culture (chocolate agar under microaerophilic conditions for 10 days), and rapid urease testing (CLO test, Delta West, Perth, Australia).

Despite a positive urease test, *H pylori* was not isolated by culture or identified by histology. The biopsy samples showed atrophic gastritis, intestinal metaplasia, and large mucosal-associated spiral bacteria morphologically identical



Gastric antral mucosa from case showing large, tightly spiralled bacteria (*H heilmannii*) within gastric gland
Stained with toludine blue ×635.

to *H heilmannii* (figure). He was then given twice daily omeprazole 20 mg, clarithromycin 250 mg, and metronidazole 400 mg for 1 week, but this had no effect on his symptoms. Repeat endoscopy showed persistent ulceration, CLO tests remained positive, the histological features did not change, and *H pylori* cultures were negative. Duodenal histology showed no evidence of Crohn's disease, and gastrinoma and occult NSAID use were excluded (fasting serum concentration of gastrin 83 mU/L and serum concentrations of thromboxane 34·8 ng/100 μ/L were within the normal range). He was then treated with omeprazole 40 mg twice daily, De-Nol 120 mg four times daily, tetracycline 500 mg four times daily, and metronidazole 400 mg three times daily for 2 weeks. This treatment resulted in good relief of symptoms. 10 weeks later, endoscopy was normal, CLO tests from the antrum and corpus were both negative, and histology showed no evidence of *H heilmannii*. 6 months later he remains well.

H heilmannii (formerly known as *Gastrospirillum hominis*, the new nomenclature has yet to be formally validated) is found in up to 0·2% of patients undergoing gastroscopy.[1–3] In such patients, *H heilmannii* is usually associated with chronic gastritis, and has been found in the absence of *H pylori* or NSAID use in three patients with duodenal ulcer.[2,3] Bismuth monotherapy can eradicate *H heilmannii* and lead to the resolution of gastritis,[1,4] but this is the first report of duodenal ulcer healing after eradication of *H heilmannii*. The organism expresses variable low concentrations of urease activity, which gives a misleading positive result on urease-based *H pylori* diagnostic tests. Culture of *H heilmannii* with traditional *H pylori* culture techniques is difficult, although one research group has been successful.[5] However, *H heilmannii* and *H pylori* differ morphologically and on microscopy, and can readily be distinguished in histological sections. These two organisms also shown substantial phenotypic differences, especially with regard to adherence and cytotoxin production. These differences illustrate the diversity of mechanisms involved in the microbiological pathogenesis of gastritis and duodenal ulcer.

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Department of Medicine, Division of Gastroenterology, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH, UK
(A F Goddard)

Vero-cytotoxin-producing *Escherichia coli* O157 in a farmer handling horses

R M Chalmers, R L Salmon, G A Willshaw, T Cheasty, N Looker, I Davies, C Wray

Transmission of vero-cytotoxin-producing *Escherichia coli* O157 (O157 VTEC) is usually food-borne, particularly from undercooked beef or raw milk, or person-to-person.[1] Direct or indirect contact with various farmed or companion animals has also resulted in human infection.[2–4]

We report sporadic infection with O157 VTEC in an 84-year-old farmer with bloody diarrhoea, who had no contact with the cattle on his farm. However, his symptoms appeared a few days after he had cleaned up faeces from two horses with diarrhoea from which *Salmonella typhimurium*, definitive type 104, was isolated. *Salmonella* spp were not detected in the farmer's faeces. Faecal samples taken 2 weeks later from these two horses, three other healthy horses, and 22 of the 70 beef cattle owned by the farmer resulted in isolation of O157 VTEC from one of the horses who had had diarrhoea. All the other samples were negative for O157 VTEC by immunomagnetic separation and culture on sorbitol MacConkey agar.[1]

The O157 VTEC isolates from the horse and the farmer belonged to phage type 2 and were sensitive to all the commonly used antimicrobial agents. They produced vero cytotoxin (VT) 2, and possessed only the VT2 sequence subtype. They were indistinguishable by pulsed field gel electrophoresis and restriction fragment length polymorphism analysis of DNA hybridising with a VT-encoding bacteriophage probe.[1] Thus either there was a common source of infection for the farmer and the horse, or the horse was the source of infection for the farmer. There is only one other report of *E coli* O157 from horses in the UK, in which a case of bloody diarrhoea due to O157 VTEC in a child was linked to contact with a horse and a dog both carrying a strain indistinguishable from that of the patient.[5] However, horses are not usually investigated for carriage of O157 VTEC. Given the high degree of handling and the large numbers of people, particularly children, who have contact with them there is a need for awareness that horses can be a vehicle of O157 VTEC infection in human beings.

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PHLS Communicable Disease Surveillance Centre (Welsh Unit), Cardiff CF4 3QX, UK (R Chalmers)**; Laboratory of Enteric Pathogens, Central Public Health Laboratory; Rhyl PHL, Rhyl; Veterinary Laboratories Agency, Veterinary Investigation Centre, Shrewsbury; Veterinary Laboratories Agency, Weybridge, Surrey**