calcium and subsequent precipitation, and this may be a mechanism shared between different types of stones.

We noticed in the study of Maurer and colleagues[2] that cholesterol gallstone formation was only detected after infection with the urease positive species *H hepaticus* and *H bilis* but not with the urease negative species *H rodentium* or *H cinaedi*.[2] This prompted us to investigate whether *Helicobacter* urease activity is involved in precipitation of calcium. For this purpose we developed a precipitation agar that allows for simultaneous growth of *Helicobacter* species and testing of their ability to precipitate calcium. We tested four different calcium concentrations (30, 10, 5, and 1 mM $CaCl_2$); best results were seen at 30 mM of calcium (fig 1) but calcium precipitation also occurred at more physiologically relevant calcium concentrations (10, 5, and 1 mM)[11] although the crystals were smaller. All four urease positive *Helicobacter* species tested (*H hepaticus*, *H bilis*, *H pylori*, and *H mustelae*) were capable of precipitating calcium in our assay (fig 1) whereas isogenic urease negative mutants of three species as well as urease negative *Helicobacter* species (*H pullorum* and *H cinaedi*) were unable to do so (fig 1). Purified urease enzyme alone was also capable of precipitating calcium.

This suggests that urease positive *Helicobacter* species that are able to survive in or colonise the bile ducts (which excludes *H pylori*[12]) may induce the formation of gallstones both directly via their urease activity and indirectly via the immune response. Our observations extend those previously reported,[2–4 12] and provide a possible mechanism to explain the association between hepatobiliary colonisation with urease positive *Helicobacter* species and gallstone formation.

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No association between the functional *CARD4* insertion/deletion polymorphism and inflammatory bowel diseases in the German population

Inflammatory bowel diseases (IBD, OMIM 601458) are represented by two main subtypes, Crohn's disease (CD, OMIM 266600) and ulcerative colitis (UC, OMIM 191390). The first and most widely replicated susceptibility gene for CD is *CARD15* (caspase recruitment domain family, member 15) that encodes NOD2 (nucleotide binding oligomerisation domain protein 2), a protein involved in the pathogen associated molecular pattern recognition system (for review see Schreiber and colleagues[1]). Identification of functional *CARD15* variants revealed the important role of impaired barrier integrity and host defence in the pathogenesis of CD and other inflammatory diseases.[1] Recently, an association between IBD and variants in the *CARD4* gene, which encodes NOD1, a structural homologue of NOD2, has been demonstrated in the British population.[2] In that study, McGovern *et al* identified the common (deletion) allele of the marker ND_1+32656 as a risk factor for IBD. Conversely, the minor ND_1+32656 allele, in haplotype combination with its adjacent marker rs2907748, exhibited a protective effect.[2] ND_1+32656 may alter the splicing of *CARD4* by affecting the binding of an unknown nuclear factor.[3] The more frequent deletion variant could result in a NOD1 protein with reduced numbers of the leucine rich repeats that are essential for pathogen recognition. The odds ratio (OR) associated with this allele was calculated to be 2.0.[2] Association of *CARD4* polymorphisms has also been observed with asthma and atopic eczema,[3 4] suggesting that *CARD4* may be a common barrier disease susceptibility gene.

In the present study, we evaluated whether the variants previously reported in the British population are also associated with IBD in Germany. In addition to ND_1+32656, we included the adjacent single nucleotide polymorphisms (SNPs) ND_1+27606 (rs2075822) and ND_1+45343 (rs2907748) in our analyses. ND_1+233 and ND_1+21984, which were only weakly associated with IBD in the study of McGovern *et al*, were not examined here. SNP genotyping was undertaken on a case control (1015 IBD patients (676 CD, 344 UC), 886 unrelated controls) and an independent family based panel (775 mother-father affected child trios for IBD (328 CD, 447 UC)) using TaqMan Assays-by-Design (Applied Biosystems, Foster City, California, USA). Given our sample sizes, our study provided >96% power to detect an OR of 2.0, assuming a risk allele frequency of 79% and 1% type I error. However, no significant association was detected with either the case control or independent family based CD/UC samples (tables 1, 2). McGovern *et al* also reported a strong association with specific disease subgroups.[2] We therefore stratified our sample for patients with early onset IBD (n = 449 <25 years) and patients with fistulae and stenoses (n = 491). Analyses of both subgroups showed no significant associations (data not shown).

In conclusion, there is no evidence for an association of the IBD phenotype with the previously reported *CARD4* susceptibility variants in the German population. The discrepant findings in the German and British samples may reflect true genetic heterogeneity, as has been demonstrated for *CARD15* associations.[5] This heterogeneity is partly evident in the different linkage disequilibrium (LD) patterns of the three

Table 1 Summary of single marker association statistics for *CARD4* (caspase recruitment domain family, member 4)

				CCA‡	CCG§	TDT¶
Phenotype	Variant*	$MAF_{controls}$†	MAF_{cases}†	p Value	p Value	p Value
CD	rs2075822	0.81	0.80	0.569	0.308	1.000
	ND_1+32656	0.79	0.79	0.847	0.977	0.091
	rs2907748	0.79	0.77	0.420	0.578	0.306
UC	rs2075822	0.81	0.78	0.152	0.357	0.480
	ND_1+32656	0.79	0.76	0.124	0.295	0.951
	rs2907748	0.79	0.75	0.073	0.195	0.956
IBD	rs2075822	0.81	0.79	0.263	0.327	0.580
	ND_1+32656	0.79	0.78	0.510	0.804	0.248
	rs2907748	0.79	0.77	0.132	0.279	0.547

*All three assays had a call rate >95% and showed no significant deviations from the Hardy-Weinberg equilibrium in the control sample.
†MAF, major allele frequency in cases and controls.
‡CCA, case control analysis for alleles.
§CCG, case control analysis for genotypes.
¶TDT, transmission disequilibrium test.

Table 2 Two marker haplotype frequencies, transmission, and association statistics for *CARD4* (caspase recruitment domain family, member 4) in inflammatory bowel diseases

Variation	Haplotype*	$f_{controls}$†	f_{cases}†	p Value‡	f_T§	f_{NT}§	p Value¶	D′**
rs2075822 +	1 – 1	0.771	0.762	0.846	0.455	0.486	0.094	0.91
ND_1+32656	1 – 2	0.042	0.040		0.140	0.101		
	2 – 1	0.013	0.015		0.026	0.057		
	2 – 2	0.174	0.184		0.380	0.357		
ND_1+32656+	1 – 1	0.784	0.775	0.727	0.473	0.519	0.719	0.98
rs2907748	1 – 2	0.002	0.002		0.011	0.011		
	2 – 1††	0.006	0.004		0.014	0.008		
	2 – 2	0.208	0.220		0.503	0.462		

*Allele 1 is defined as the major allele.
†Frequencies of haplotypes in cases and controls estimated by the expectation maximisation algorithm using COCAPHASE.[6]
‡Global significance value obtained after 10 000 permutations with COCAPHASE.
§Frequencies of transmitted (f_T) and non-transmitted (f_{NT}) haplotypes observed using TDTPHASE.[6]
¶Global significance value obtained after 10 000 permutations with TDTPHASE.
**D′ value as a measure of linkage disequilibrium in the control sample.
††Protective haplotype previously identified by McGovern and colleagues.[2]

polymorphisms analysed. While LD was incomplete in the British,[2] there was strong LD in the German patients (table 2). ND_1+32656 could therefore be a population specific marker for an as yet unidentified causative variant in the vicinity. Replication in other British and European samples will be necessary to further examine the role of *CARD4* variants in IBD susceptibility.

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Preliminary data on the use of intraductal optical coherence tomography during ERCP for investigating main pancreatic duct strictures

Optical coherence tomography (OCT) is an optical imaging technique that uses infrared light reflectance and produces high resolution microstructural cross sectional images of tissues in vivo.[1–3] The OCT probe can be inserted inside a standard transparent endoscopic retrograde cholangiopancreatography (ERCP) catheter. To date, only the epithelium of the main pancreatic duct (MPD) has been examined by OCT in humans in three studies: one post mortem[4] and two ex vivo.[5 6] The aim of the present prospective pilot study was to assess the feasibility of intraductal OCT in vivo during an ERCP procedure, its ability to identify changes in MPD wall structure in vivo, and its ability to differentiate non-neoplastic from neoplastic tissue in the presence of MPD strictures.

Fifteen consecutive patients with documented or suspected MPD strictures at a previous computed tomography scan or magnetic resonance cholangio-pancreatography (MRCP) were investigated by endoscopic ultrasonography (EUS) and ERCP; the two procedures were done at the same time under propofol sedation. Mean age of the patients was 61.9 years (range 38–78); there were 11 men and four women. Fine needle aspiration biopsy (FNAB) was planned during EUS when pancreatic neoplasia was suspected; intraductal OCT followed by brush cytology was scheduled during ERCP in all cases when the MPD stricture was confirmed. OCT findings were compared with EUS, cytology (FNAB and/or intraductal brushing), and histopathological findings in those undergoing surgical pancreatic resection. All patients gave informed consent to the endoscopic procedures and the institutional ethics committee approved OCT use in humans.

A near focus OCT probe (Lightlab Imaging, Westford, Massachusetts, USA) was used with a penetration depth of about 1 mm, resolution of approximately 10 μm, and outer diameter of 1.2 mm. An MPD stricture was confirmed by EUS and/or ERCP in 12 of 15 patients. The three patients in whom the stricture was not confirmed were excluded from the study. EUS findings suggested a neoplastic lesion in seven cases, chronic pancreatitis with segmental MPD stricture in three, neuroendocrine tumour compressing the MPD in one, and normal tissue in the remaining case. EUS guided FNAB was performed in eight patients with findings suggesting neoplasia or neuroendocrine tumour. Three patients with neoplastic findings were judged unfit for surgery at EUS. In 10 cases, MPD segments not affected by the stricture showed normal morphology at ERCP, with mild upstream dilatation (4–6 mm); in two cases with EUS findings suggesting chronic pancreatitis, minimal ductal changes were also documented at ERCP.

Both intraductal OCT and brush cytology were performed in 11 patients; in one patient with adenocarcinoma the stricture was too tight to pass the ERCP catheter upstream of the lesion. FNAB findings confirmed the diagnosis of adenocarcinoma and neuroendocrine tissue in all cases. Brush cytology was concordant with FNAB findings in 4/6 patients with pancreatic adenocarcinoma (66.7%) and negative for neoplastic cells in the five cases in which EUS findings did not suggest ductal adenocarcinoma. Five patients were operated on and pancreatic adenocarcinoma and neuroendocrine tumour were confirmed in the surgical specimens. OCT imaging showed a recognisable three layer structure in cases with a normal MPD and chronic pancreatitis whereas in all cases with ductal adenocarcinoma the layer structure was totally unrecognisable (fig 1).

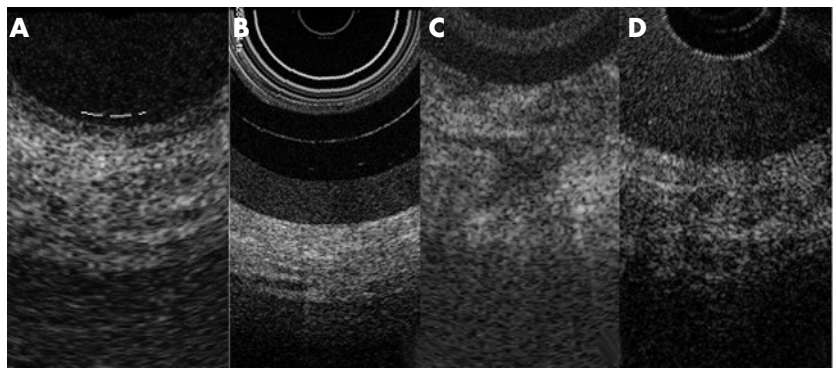

Figure 1 Magnification of an optical coherence tomography (OCT) image from a segmental stricture of the main pancreatic duct with non-neoplastic and neoplastic stricture, with the OCT probe outside (A, B) and inside (C, D) the endoscopic retrograde cholangiopancreatography catheter.