Abstract PWE-164 Table 1

CLO results	Totals	%
Negative	167	35.3
Positive	27	5.7
Not done	279	59

Competing interests None declared.

PWE-165 CYP2C19*17 GAIN OF FUNCTION MUTATION IS ASSOCIATED WITH PEPTIC ULCER DISEASE

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Introduction Studies show that single nucleotide polymorphisms (SNPs) in non-steroidal antiinflammatory drug (NSAID)-metabolising enzymes (mainly CYP2C9 and CYP2C8) may predispose NSAID-users to peptic ulcer disease (PUD) or upper gastrointestinal bleeding (UGIB), but results have been inconclusive.

Methods We hypothesised that the eight closely-linked clinically important SNPs in the CYP2C family of genes, namely CYP2C8*3 (rs11572080 and rs10509681), CYP2C8*4, CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2C19*3, and CYP2C19*17 predispose to PUD via impaired NSAID metabolism as well as other potentially important mechanisms (eg, metabolism of arachidonic acid (AA) and proton-pump inhibitors-PPIs). Subjects diagnosed with PUD/UGIB at 13 hospitals in the UK between 2005 and 2011 were recruited and interviewed using a structured questionnaire, and categorised as either NSAID-users or non-users. UGIB was defined as haematemesis, melaena or anaemia, and endoscopic stigmata of recent bleeding. *H pylori* status was ascertained in most subjects. Following extraction of genomic DNA, genotyping was performed by KBioscience Ltd (UK). Logistic regression analysis was used to test for association between each SNP and risk of PUD/UGIB. Interaction terms were introduced to determine whether any observed genetic effects were influenced by factors including type of NSAID, PPI use and gender.

Results 1246 white patients were recruited and categorised as follows: 485 (39%) PUD+/NSAID+; 357 (29%) PUD+/NSAID-; 125 (10%) PUD−/NSAID+; 280 (22%) PUD−/NSAID−. Seven SNPs were included in the final analysis (CYP2C19*3 was monomorphic and excluded). All SNPs were in Hardy-Weinberg equilibrium. Logistic regression analysis of cases (PUD+; n=842) and controls (PUD−; n=405), assuming an additive mode of inheritance at each SNP, showed that only CYP2C19*17 was significantly associated with PUD (p=0.006), with suggestion of an allele-dose effect, even on classifying cases as those using only CYP2C9/CYP2C8-substrate NSAIDs (p=0.005). Post-hoc analysis showed no interaction between CYP2C19*17 and NSAID type, PPI use or gender. Subgroup analysis per ulcer type showed CYP2C19*17 was significantly associated with gastric ulcers (p=0.02), while only rs11572080 was associated with duodenal ulcers (p=0.04). No SNPs were associated with UGIB.

Conclusion Possession of CYP2C19*17 allele is associated with PUD, especially gastric ulcers, regardless of aetiology. We postulate that this could be through its effect on the metabolism of AA or other endogenous substances, leading to impairment of gastrointestinal mucosal defences. Further studies are needed to correlate the functional consequences of CYP2C19*17 in the pathogenesis of PUD.

Competing interests None declared.

PWE-166 INCREASING VACA TOXIN ACTIVITY ALTERS *HELICOBACTER PYLORI* COLONISATION DENSITY AND THE NATURE OF THE ACQUIRED IMMUNE RESPONSE IN A MOUSE MODEL OF INFECTION

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Introduction *Helicobacter pylori* (*Hp*) infection causes chronic asymptomatic gastritis, and may lead to peptic ulceration and gastric cancer. *Hp* strains expressing more active forms of the vacuolating cytotoxin VacA are more strongly associated with disease than strains producing less toxigenic VacA. *Hp* infection stimulates a strong immunosuppressive interleukin-10 (IL-10) response,[1] and VacA inhibits T cell activation and interleukin-2 (IL-2) production[2] in vitro, but exactly how VacA contributes to disease development remains unclear.

Methods The *vacA* allelic type of the mouse-colonising *Hp* strain SS1 was found to be the non-toxigenic s2/i2/m2 form by PCR-typing. To assess whether vacuolating activity affects colonisation, a variant of strain SS1 expressing the more active s1/i1/m2 form of VacA (SS1/s1i1) was constructed. Vacuolating activities were compared in vitro by incubating RK13 epithelial cells with water extracts from each strain for 4 h in the presence of 10 mM ammonium chloride, and counting the number of extensively vacuolated cells in random fields by light microscopy. C57BL/6 mice were infected with either SS1, SS1/s1i1 or given diluent as a placebo. After 3 weeks, gastric colonisation densities were assessed from weighed stomach tissue samples by quantitative culture on selective blood agar plates containing vancomycin, polymyxin B, bacitracin, nalidixic acid and amphotericin B. Immune responses were investigated by culturing spleen cells with and without *Hp* antigens in vitro and quantifying IL-2 and IL-10 concentrations in culture supernatants by ELISA.

Results As expected, the SS1 wild type strain induced virtually no vacuolation of RK13 cells (2% of cells vacuolated). In contrast, its isogenic variant, SS1/s1i1, expressing the more active form of the toxin, induced vacuolation in 37% of cells ($p<0.01$). Mutagenesis of SS1 *vacA* to a more active form had no effect on strain growth and viability in liquid culture media. Mice infected with SS1/s1i1 had approximately 100-fold lower colonisation densities compared to those administered the wild-type strain ($p<0.05$). Splenocytes from mice infected with SS1/s1i1 secreted 2.8-fold lower IL-2 concentrations and 1.5-fold more IL-10 than cells from wild-type SS1-infected animals in response to stimulation with *Hp* antigens.

Conclusion Our data show that *Hp* strains expressing active VacA colonise less densely than less toxigenic strains, and that VacA modulates the immune response in vivo. We speculate that further characterisation of these effects will uncover why strains with active VacA are common in patients developing gastric cancer.

Competing interests None declared.

REFERENCES

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