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Role of *RET* and *PHOX2B* gene polymorphisms in risk of Hirschsprung's disease in Chinese population

Hirschsprung's disease (OMIM 142623) is a complex congenital disorder characterised by the absence of ganglion cells of the plexus myentericus and plexus submucosus in the variable lengths of the digestive tract.[1 2] Aganglionosis is attributed to a defect of the enteric nervous system, in which ganglion cells fail to innervate the lower gastrointestinal tract during embryonic development, resulting in failure to pass meconium, chronic severe constipation and colonic distention in the neonatal period.[3] The receptor tyrosine kinase gene *RET*, which is expressed in neural crest cells during enteric neurogenesis and is required for normal development of the enteric nervous system, is the major susceptibility gene for Hirschsprung's disease.[4] There is growing evidence indicating that functional single nucleotide polymorphisms (SNPs) of *RET* could act as low susceptibility factors for Hirschsprung's disease.[5 6] In addition, *PHOX2B* encodes a transcription factor that is involved in the development of the noradrenergic nervous system and that plays an important role in the regulation of *RET* transcription.[7 8] Our previous data showed that genotypes comprising allele A of the IVS2+100 A>G SNP of *PHOX2B* were associated with an increased risk of Hirschsprung's disease.[9] In view of the role of *RET* and *PHOX2B* in the development of the enteric nervous system, we hypothesised that *RET* and *PHOX2B* polymorphisms are likely to interact and have a joint effect in conferring susceptibility to Hirschsprung's disease.

This case–control study consisted of 256 ethnic Chinese patients histologically diagnosed with sporadic Hirschsprung's disease, including 13 patients with total colonic aganglionosis, 28 with long-segment aganglionosis and 215 with short-segment aganglionosis. Controls were 242 unselected, unrelated, ethnic Chinese subjects. At recruitment, informed consent was obtained from each subject. This study was approved by the institutional review board of the University of Hong Kong. Genotypes for *RET* promoter polymorphisms (5G>A and 1A>C) and *PHOX2B* were analysed using polymerase chain reaction and direct sequencing, as described previously.[6 9]

We observed an increased risk of Hirschsprung's disease in homozygous genotypes of the disease-associated RET alleles 5AA or 1CC when compared with 5GA and 5GG (OR = 7.78, 95% CI 5.21 to 11.70), or 1AC and 1AA (OR = 6.08, 95% CI 4.01 to 9.12) genotypes. The same risk was seen with the PHOX2B IVS2+100 AA genotype compared with the GG or GA genotypes (OR = 1.75, 95% CI 1.17 to 2.58). We then investigated the potential interaction between the *RET* and *PHOX2B* genotypes in the risk of Hirschsprung's disease using the additive interaction models (table 1).[10] Since the 5 and 1 *RET* SNPs were in almost complete linkage disequilibrium in our population ($D' = 0.986$, $p<0.001$), only the former was selected for further analysis. Among subjects carrying at least one *RET* 5G allele or at least one *PHOX2B* G allele, the OR for Hirschsprung's disease was 1.56 (95% CI 0.82 to 2.75) or 7.98 (95% CI 3.64 to 17.60). The OR increased, however, to 11.72 (95% CI 6.35 to 21.55) among subjects carrying both the RET 5AA and PHOX2B AA genotypes ($p<0.001$, test for homogeneity). The OR value of 11.72 is larger than the values of the RET 5AA and the PHOX2B AA genotypes independently (7.78+1.75−1 = 8.53), which indicates a more than additive interaction between the *RET* and the *PHOX2B* SNPs on risk of developing Hirschsprung's disease according to the statistical model.[10]

To our knowledge, this is the first study to demonstrate that the interaction between *RET* and *PHOX2B* polymorphisms has a substantial impact on risk of Hirschsprung's disease. This recognised multifactorial genetic disorder requires the interaction of several unlinked genes to produce the phenotype. Both RET and PHOX2B have important roles in the development of the enteric nervous system and PHOX2B is involved in the transcriptional regulation of RET in cell lines originated from neural crest. It is therefore biologically plausible that a joint effect of RET and PHOX2 SNPs affecct the risk of Hirschsprung's disease. However, the essential mechanisms behind our finding need to be investigated.

Table 1 Risk of Hirschsprung's disease associated with *RET* genotypes combined with *PHOX2B* genotypes

Genotype		Patients ($n=256$)	Controls ($n=242$)	
RET –5G/A	*PHOX2B*	*n* (%)	*n* (%)	OR* (95% CI)
GG + GA	*GG + GA*	19 (7.4)	65 (26.9)	1.00
GG + GA	*AA*	57 (22.2)	122 (50.4)	1.56 (0.82–2.75)
AA	*GG + GA*	36 (14.1)	15 (6.2)	7.98 (3.64–17.60)
AA	*AA*	144 (56.3)	40 (16.5)	11.72 (6.35–21.55)

* ORs and 95% CIs were calculated by unconditional logistic regression adjusted for gender.

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The incidence and risks of liver biopsy in non-cirrhotic patients: An evaluation of 3806 biopsies

Liver biopsy plays a crucial role in the diagnosis and management of liver diseases. For the past decade, this invasive procedure has become a safe one with the prevailing application of an ultrasound-guided method, the use of thinner gauge needles and improved operational techniques. Over the past years, the debatable issue of liver biopsy has mainly focused on the safety and suitability of a shorter observation time with respect to cost savings.[1–3] Referring to this issue, Beddy et al even shortened their observation time to 1 hour and indicated that only one haemorrhagic complication occurred within one hour amongst 500 liver biopsy occasions. There were no recorded delayed complications or deaths at follow up.[4]

Generally, we agree with the conclusion that outpatient liver biopsy is safe when done in a

Table 1 The characteristics of 12 patients with major complications after liver biopsy

Patient no.	Sex/age	Complications	Time of occurrence (hrs)	In-hospital days
1	M/35	Haemoperitoneum	6	3
2	M/53	Haemoperitoneum	6	3
3	F/55	Haemothorax	12	1
4	M/52	Haemothorax with empyema	6	7
5	M/53	Massive subcapsular haematoma with shock	12	1
6	M/35	Haemoperitoneum	12	1
7	F/60	Haemoperitoneum with shock	6	7
8	F/53	Haemoperitoneum	5	5
9	F/55	Haemoperitoneum	5	5
10	F/42	Haemoperitoneum, haemobilia, shock, and transient obstructive jaundice	24	7
11	M/65	Haemoperitoneum with shock	6	5
12	F/53	Haemoperitoneum	4	3

setting that provides close observation for 1 hour after a biopsy. However, we keep a conservative and different view on the sufficiency and safety of 1 hour observation time after a liver biopsy. We have performed a total of 3806 liver biopsies using 18-gauge needle on 2980 patients (males 1817, females 1163, mean age was 41.3 years, range 17–72 years), performed in a medical centre and in a core regional hospital in southern Taiwan from Jan 1996 through Sep 2006. Our outpatient biopsy was assigned to a 6-hour observation time. The reasons for the liver biopsy studies on these patients, were either a result of chronic viral hepatitis or a surveillance of abnormal liver function tests. There was no clinical evidence of cirrhosis diagnosed by high-resolution ultrasound before a biopsy. Patients who have thrombocytopenia or the presence of oesophageal varices, ascites, splenomegaly, signs of portal hypertension, or encephalopathy by other imaging studies as well as bleeding tendencies, were excluded. These procedures have been done by six well-trained, board certified hepatologists. Apart from minor complications such as pain, nausea, and vomiting, a total of 12 patients (0.32%) suffered from haemorrhagic complications 4 to 12 hours after the biopsy (table 1). Our results were comparable with those of Beddy et al in terms of major complications and mortality. Although there was no death at follow up of one week, four patients did experienced hypovolemic shock. In one patient, haemobilia, presenting with insidious onset of tarry stool, obstructive jaundice, and hypovolemic shock, developed 24 hours after the biopsy. The total in-hospital days therefore were extended and ranged from 1 to 7 days. In rare occasions haemorrhagic complications such as haemoperitoneum, haemothorax, and haemobilia did occur with potential risk of morbidity and mortality after the procedure. Noteworthy is that those with poor performance such as liver cirrhosis and bleeding tendency were excluded from our study. Major complications occurred in four patients after the regular observation time. This does, indicate a possible need of a longer time of observation. Therefore, our study with a 6-hour observation time basis concluded that although ultrasound-guided liver biopsy is generally a safe procedure, major haemorrhage complications may occur more than one hour after liver biopsy. A longer time of observation may be more suitable with this potential risky procedure.

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The role of gender on clearance of hepatitis C virus: a different story in an area endemic for hepatitis B and C

We read with interest the article by Bakr *et al* (*GUT* 2006;55:1183-7). The authors recruited 4720 residents aged 18–65 years from a rural community in Egypt, a country hyperendemic for hepatitis C virus (HCV) infection, which might be attributed to mass campaigns for intravenous antischistosomal treatment.[1] They found that the HCV antibody (anti-HCV) was positive in 910 individuals (19.3%), and 38.5% of the anti-HCV-positive individuals were negative for serum HCV RNA. Interestingly, the authors concluded that women had a significantly higher HCV clearance rate (44.6% vs 33.7%, respectively; $p = 0.001$, adjusted OR 1.77) than men, which was similar to reports by Inoue *et al*[2] and Yamakawa *et al*[3] from Japan.

We conducted a large-scale community-based study in southern Taiwan, a country hyperendemic for hepatitis B virus (HBV) infection (prevalence of hepatitis B surface antigen (HBsAg) 10–20%), and several villages were reported to be hyperendemic for anti-HCV (prevalence of anti-HCV 17–50%).[4–6] Among the general population of the Kaohsiung area aged 40–65 years, 11 239 subjects were enrolled. The prevalence of HBsAg and anti-HCV (detected using a third-generation, commercially available ELISA kit (Abbott Laboratories, Chicago, Illinois, USA)) as 13.7% and 6.3%, respectively, and 84 (0.7%) participants were positive for both HBsAg and anti-HCV. In all, 642 anti-HCV-positive participants were tested for HCV RNA using a polymerase chain reaction assay (Cobas Amplicor Hepatitis C Virus Test, V.2.0; Roche Diagnostics, Branchburg, New Jersey, USA; detection limit: 50 IU/ml), and 478 (74.5%) of them were positive for HCV RNA. In addition to the manufacturer's instructions, which suggest rechecking for anti-HCV if the data are <20% (0.8–0.99 signal to cut-off (SCO)) of

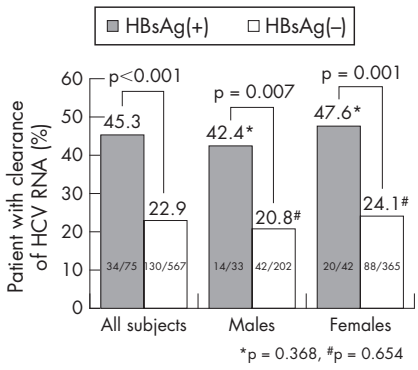

Figure 1 Proportions of clearance hepatits C virus (HCV) RNA in 642 anti-HCV-positive participants grouped by positive (+) and negative (−) hepatitis B surface antigen (HBsAg) are shown. Individuals with positive HBsAg had a significantly higher proportion of HCV RNA clearance than these negative for HBsAg, men and women. There was no significant difference in the proportions of clearance of HCV RNA between male and female patients in HBsAg-positive and HBsAg-negative groups.