Results in 387 patients over 15 years [abstract 1428]. *Am J Transplant* 2006;6:546.

- 2 O'Grady JG. Liver transplantation alcohol related liver disease: (deliberately) stirring a hornet's nest! *Gut* 2006;55:1529–31.
- 3 ICD Version 2006: World Health Organization 2006.
- 4 Yates WR, Booth BM, Reed DA, et al. Descriptive and predictive validity of a high-risk alcoholism relapse model. *J Stud Alcohol* 1993;54:645–51.

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 ICC 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

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

Genotype		Patients (n=256)	Controls (n=242)	
<i>RET</i> -5G/A	<i>PHOX2B</i>	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.

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 *PHOX2B* SNPs affect the risk of Hirschsprung's disease. However, the essential mechanisms behind our finding need to be investigated.

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References

- 1 Amiel J, Lyonnet S. Hirschsprung disease, associated syndromes, and genetics: a review. *J Med Genet* 2001;38:729–39.
- 2 Brooks AS, Oostra BA, Hofstra RM. Studying the genetics of Hirschsprung's disease: unraveling an oligogenic disorder. *Clin Genet* 2005;67:6–14.
- 3 Iwashita T, Kruger GM, Pardoll R, et al. Hirschsprung disease is linked to defects in neural crest stem cell function. *Science* 2003;301:972–6.
- 4 Angrist M, Kauffman E, Slaugenhaupt SA, et al. A gene for Hirschsprung disease (megacolon) in the pericentromeric region of human chromosome 10. *Nat Genet* 1993;4:351–6.
- 5 Garcia-Barceló MM, Sham MH, Lui VC, et al. Chinese patients with sporadic Hirschsprung's disease are predominantly represented by a single *RET* haplotype. *J Med Genet* 2003;40:e122.
- 6 Garcia-Barceló M, Ganster RW, Lui VC, et al. TTF-1 and *RET* promoter SNPs: regulation of *RET* transcription in Hirschsprung's disease. *Hum Mol Genet* 2005;14:191–204.
- 7 Pattyn A, Morin X, Cremer H, et al. The homeobox gene *Phox2b* is essential for the development of autonomic neural crest derivatives. *Nature* 1999;399:366–70.
- 8 Baccetti T, Borghini S, Ravazzolo R, et al. An in vitro approach to test the possible role of candidate factors in the transcriptional regulation of the *RET* proto-oncogene. *Gene Expr* 2005;12:137–49.
- 9 Garcia-Barceló M, Sham MH, Lui VC, et al. Association study of *PHOX2B* as a candidate gene for Hirschsprung's disease. *Gut* 2003;52:563–7.
- 10 Kleinbaum DG. Epidemiologic methods: the "art" in the state of the art. *J Clin Epidemiol* 2002;55:1196–200.

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