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

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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