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Effect of *RET* c.2307T>G Polymorphism on the Outcomes of Posterior Sagittal Neurectomy for Hirschsprung Disease Procedure in Indonesian Population

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We investigated the effect of *RET* c.2307T>G polymorphism on the outcomes of posterior sagittal neurectomy for Hirschsprung disease (PSNHD) procedure in Indonesia. Hirschsprung disease (HSCR) is a neurocristopathy characterized by absence of enteric ganglia along variable lengths of the intestine in neonates. The *RET* c.2307T>G polymorphism has been shown to be associated with HSCR. Many surgical techniques with some advantage and disadvantage were established for HSCR. We have conducted PSNHD in short-segment HSCR patients.Thirty-one nonsyndromic HSCR patients underwent PSNHD. The polymorphism was determined using PCR-RFLP in genomic DNA. The rate of enterocolitis and constipation outcomes following PSNHD were 6 (19%) and 4 (13%) patients, respectively. The *RET* c.2307T>G polymorphism did not influence either enterocolitis or constipation outcome following PSNHD at *P* value of 0.07 (OR = 0.28; 95% CI = 0.08–1.05) and 0.67 (OR = 0.58; 95% CI = 0.12–2.76), respectively. Our study suggested that *RET* c.2307T>G polymorphism may not affect outcomes of PSNHD procedure in Indonesia. Furthermore, a multicenter study with a larger sample size is necessary to clarify this result.

Key words: Hirschsprung – Polymorphism – Posterior sagittal neurectomy – Constipation – Enterocolitis – Indonesian cases

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Hirschsprung's disease (HSCR, MIM #142623), characterized by the absence of ganglion cells (Meissner and Auerbach) along variable lengths of the distal gastrointestinal tract, is a common cause of neonatal intestinal obstruction that is of great interest to pediatric surgeons throughout the world.[1,2] Such aganglionosis is attributed to a failure of neural crest cells to migrate, proliferate, and/or differentiate during enteric nervous system (ENS) development in the embryonic stage.[2] It varies in length and is classified into 3 clinical groups of short-segment (S–HSCR: aganglionosis up to the upper sigmoid colon), long-segment (L–HSCR: aganglionosis up to the splenic flexure and beyond) and total colonic aganglionosis (TCA).[1] This birth defect shows population incidences of 15, 28, and 21 cases per 100,000 live births among Europeans, Asians, and Africans, respectively, with a marked sex-difference (3.9 male: female).[1]

The current treatment for HSCR is surgical resection of the aganglionic segment of the bowel. Many operative methods have been developed for HSCR such as Swenson, Soave, Duhamel, transanal endorectal pull-through, laparoscopic, transanal Swenson-like and posterior sagittal neurectomy for HSCR (PSNHD) procedures.[4–8] There is currently some debate over which technique offers the best outcome.[9] The absence of enterocolitis and constipation remains the most important marker of a good outcome.

Despite the central role played by *RET* in HSCR and the extensive mutation screenings performed by many groups in the last 10 years, the mutation rate remains quite low, and only about 50% of familial and 7–35% of sporadic cases present with coding sequence of *RET* mutations.[10,11] A common polymorphism of *RET* in exon 13, c.2307T>G, has been considered as a risk factor for HSCR.[12–16] We have already reported the frequency of *RET* c.2307T>G common variant in Indonesian HSCR patients.[17] In addition, our previous study showed that a common polymorphism within a gut enhancer of *RET* in intron 1, rs2435357, is present in 82% of all Indonesian HSCR patients.[18] In the present study, we investigated the effect of *RET* c.2307T>G polymorphism on the outcomes, enterocolitis and constipation, following PSNHD procedure in Indonesian HSCR patients.

Materials and Methods

The diagnosis of HSCR in our hospital was made by rectal biopsy, H&E staining, and AChE staining.

This study was reviewed and approved by the Institutional Review Board (IRB) of Faculty of Medicine, Universitas Gadjah Mada (KE/FK/24/EC). Written informed consent was obtained from all parents for this study.

Surgical procedure

All HSCR patients in this study underwent PSNHD by one experienced pediatric surgeon in a reasonably standard fashion. The patient was placed in a prone position. An incision of the intergluteal area was made. The incision was continued until the rectum was visible. The adventitia rectalis[19] was incised longitudinally at 3 sites. The mosquito clamp was used for disrupt the hypertrophic nerve fibers. This neurectomy maneuver was performed until the rectal submucosa bulged into the cleft. Following the completion of neurectomy, the bougienage was performed to compare the anorectal caliber before and after the PSNHD procedure.[8]

Outcomes assessment

After underwent the PSNHD procedure, the patients were followed up by telephone, outpatient clinic interviews, home visit, and sending the validated questionnaire to them regarding the outcomes of surgical treatment. Follow-up was scheduled for 1 month after the date of operation and then at regular 3- to 6-month intervals.

DNA isolation and genotyping

Genomic DNA was extracted from colonic tissue of the 31 HSCR probands, using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). The *RET* c.2307T>G was chosen based on previous studies.[12–15] Genotyping of *RET* c.2307T>G variant was performed using PCR–RFLP method.[13] Forward and reverse primers used for *RET* c.2307T>G genotyping were 5′-CTCTCTGTCTGAACTTG GGC –3′ and 5′-TCACCCTGCAGCTGGCCTTA –3′, respectively. The amplified fragment of 198 bp was digested by 10 U/sample of *TaqI* enzyme at 37°C for 18 h. The 2307T allele generated a restriction site in the amplified fragment and was digested into two fragments of 139 and 99 bp, respectively. Mutant-type allele (2307G) lacked the restriction site and therefore remained undigested (238 bp).[13]

Table 1 Patient characteristics, operative data and complications

Gender (male)	55% (17/31)
Segment length	
Short	100% (31/31)
Long/TCA	0
Delayed meconium passage (>24 hours)	90% (28/31)
Preoperative enterocolitis	58% (18/31)
Age at operation (month)	26.0 ± 5.8 (1–115)
Operative time (min)	30.3 ± 1.3 (20–50)
Postoperative complications	
Enterocolitis	19% (6/31)
Constipation	13% (4/31)
Hospital stay (day)	10.7 ± 0.6 (5–17)
Length of follow-up (month)	3–60

Statistical genetic analysis

Distribution of genotypes and alleles between groups were compared using Fisher's exact test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated and $P < 0.05$ was considered significant.

Results

In this study, we have analyzed the effect of *RET* c.2307T>G polymorphism on the outcomes of PSNHD procedure in Indonesian population. From January 2005 to December 2010, we ascertained 31 HSCR patients of whom 17 and 14 were males and females, respectively. All patients were sporadic nonsyndromic HSCR with degree of aganglionosis of short-segment. Twenty-eight (90%) patients demonstrated delayed meconium passage, while 18 (58%) patients showed preoperative enterocolitis. The mean age at operation and operative time was 26.0 ± 5.8 months (range, 1–115 months) and 30.3 ± 1.3 min (range, 20–50 min), respectively. The duration of follow-up ranged from 3 to 60 months. Postoperative hospitalization averaged 10.7 ± 0.6 days (range, 5–17 days) (Table 1).

The rate of enterocolitis and constipation outcomes following PSNHD were 6 (19%) and 4 (13%) patients, respectively. The frequencies of risk allele (G) in HSCR patients with and without enterocolitis were 50% (6/12) and 78% (39/50), respectively. The risk allele (G) frequency was not significantly difference between 2 groups ($P = 0.07$), with the OR of 0.28 (95% CI = 0.08–1.05; Table 2).

The frequencies of risk allele (G) in HSCR patients with and without constipation were 62% (5/8) and 74% (40/54), respectively. The risk allele (G) frequency was also not significantly difference between these groups ($P = 0.67$), with the OR of 0.58 (95% CI = 0.12–2.76; Table 3).

Discussion

To the best of our knowledge, this is the first study to show the effect of polymorphism on the outcomes after surgical treatment of Hirschsprung's disease. Previous studies showed that the polymorphisms might have an effect on the outcome of the surgery.[20,21] Therefore, we hypothesized that the *RET* c.2307T>G polymorphism might affect the outcomes of posterior sagittal neurectomy for Hirschsprung's disease procedure.

We show evidence that the *RET* c.2307T>G polymorphism did not influence the outcomes of PSNHD in the Indonesian population. Our previous study demonstrated no association between *RET* c.2307T>G polymorphism and HSCR in Indonesia.[17] The study revealed different results from previous studies.[12–15] These differences might relate to ethnicity since the *RET* c.2307T>G shows high variation throughout the world, with a range of 10%–51%.[16,22] Other reasons might cause these differences such as the heterogeneity of experimen-

Table 2 RET c.2307T>G genotype and allele frequencies in Hirschsprung patients with and without enterocolitis following PSNHD

	Enterocolitis		OR (95% CI); *P* value	
Genotype	(+)	(−)	Dominant (GG+TG versus TT)	Recessive (GG versus TG+TT)
TT	1 (17%)	2 (8%)	0.67 (0.05–8.52); 1.00	0.18 (0.03–1.09); 0.09
TG	4 (66%)	7 (28%)		
GG	1 (17%)	16 (64%)		
Allele				
T	6 (50%)	11 (22%)	0.28 (0.08–1.05); 0.07	
G	6 (50%)	39 (78%)		

The gene and variants genotyped, risk and non-risk alleles, allele frequency of the risk allele in Hirschsprung patients with and without enterocolitis after PSNHD, the odds ratio and its 95% confidence intervals (CI) with statistical significance (*P*) are shown. c: codon; T: thymine; G: guanine.

Table 3 RET c.2307T>G genotype and allele frequencies in Hirschsprung patients with and without constipation following PSNHD

	Constipation		OR (95% CI); *P* value	
Genotype	(+)	(−)	Dominant (GG+TG versus TT)	Recessive (GG versus TG+TT)
TT	0	3 (11%)	1.22 (0.09–33.6); 1.00	0.33 (0.05–2.18); 0.37
TG	3 (75%)	8 (30%)		
GG	1 (25%)	16 (59%)		
Allele				
T	3 (38%)	14 (26%)	0.58 (0.12–2.76); 0.67	
G	5 (62%)	40 (74%)		

The gene and variants genotyped, risk and nonrisk alleles, allele frequency of the risk allele in Hirschsprung patients with and without constipation after PSNHD, the odds ratio and its 95% confidence intervals (CI) with statistical significance (*P*) are shown. c: codon; T: thymine; G: guanine.

tal methods, the source of cases and controls in different studies, and the clinical classification of HSCR.[15] However, it is important to emphasize that a small number of respondents included in our study is not sufficient for generalizing the data on the entire population, due to the small sample size and limited power of the study.

The purpose of surgical treatment for HSCR is to achieve a good functional outcome of bowel motions. Constipation might become so severe that causes chronic laxative use, redo-pull through procedures and also predispose to a high incidence of enterocolitis. Our new technique, PSNHD, showed relatively low constipation rate (13%) compared with other techniques such as Soave (34%) and TEPT (25%) procedures.[9] The constipation rate ranges from 6% to 34%.[23]

Enterocolitis is one of the most severe complications following surgical treatment for HSCR that causes high morbidity and mortality when inappropriately treated. The enterocolitis rate of our PSNHD was 19%, which is similar with previous reports following other procedures (14%–20%).[7]

In conclusion, our study suggested that *RET* c.2307T>G polymorphism may not affect the outcome of PSNHD in Indonesia. Furthermore, a multicenter study with larger sample size is necessary to clarify this result.

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