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# A DNA study in twin total colonic aganglionosis

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Hirschsprung's disease is a frequent congenital malformation of unknown origin resulting in intestinal obstruction in neonates. In the majority of the cases (80%), the aganglionic tract involves the rectum and the sigmoid colon, while in 7% of cases it extends toward the entire colon (total aganglionosis). Recently, study of the pathogenesis of Hirschsprung's disease has concentrated on the genetic aspects. In the most recent studies, some groups were able to identify mutations of the RET protooncogene, thus suggesting a possible cause of the disease.

We report here a pair of twins, one member of which was effected by total colonic aganglionosis while the other was entirely normal. The monozygoticity was supported by using common DNA polymorphism short tandem repeat loci. Their parents' DNA polymorphism patterns were also studied.

**Index** :Total colonic aganglionosis

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**บทคัดย่อ**

การศึกษาหา pathogenesis ของ Hirchsprung's disease ในระยะหลัง ๆ เน้นความสำคัญของปัจจัยทาง genetic มากขึ้น พบว่า mutation ของ RET protooncogenes มีความสัมพันธ์อย่างใกล้ชิดกับ การเกิดโรค Hirschsprung's Disease

ผู้เขียนได้รายงานการศึกษาทาง DNA ในผู้ป่วยคู่แฝดซึ่งแฝดผู้ที่ได้รับการวินิจฉัยว่าเป็น Total Hirschsprung's Disease พบว่าด้วยวิธีการใหม่ของการศึกษา monozygoticity ของคู่แฝดโดยใช้ common DNA polymorphism short tandem repeat loci ได้ผบว่าแฝดคู่นี้เป็น monozygotic twins ดังนั้น pathogenesis ของการเกิด Total Hirschsprung's Disease ในรายนี้น่าจะเป็น sporadic somatic mutation ของ RET protooncogenes การศึกษาเปรียบเทียบในคู่แฝด discordant twins เพิ่มเติมจะสามารถบ่งบอกถึงความสำคัญ RET protooncogenes กับการแสดงอาการของโรคได้โดยชัดเจนขึ้น

## CASE REPORT

Twin females were born at 37 weeks gestation. Caesarean section was performed due to the twin pregnancy. Their mother had attended a full course of antenatal care without any complications of the pregnancy. There was no history of congenital malformation in the family.

Twin one weighted 2200 gm. The Apgar scores were 9 at 1 minute and 10 at 5 minutes. She failed to pass meconium after 40 hours and she was noted to have a distended abdomen. A provisional diagnosis of intestinal atresia or meconium ileus was clinically made, However, the result of a B.A. study revealed dilatation of the proximal small bowel with microcolon. Midgut obstruction was suggested.

The child was then operated on. At laparotomy, there were distended loops of small bowel down to the ileum at 15 cm. from the I-C valve. Frozen section biopsies showed few ganglion cells up to 40 cm. from the I-C valve. A double-barrel colostomy was performed.

At the age of 6 months, the child underwent a definitive abdominoperineal pull-through procedure (Modified Martin - Duhamel). The operation was uneventful.

The patient is now at ten months old, she had episodic diarrhea which improved after hospitalization and conservative treatment. Otherwise, she is in a healthy condition.

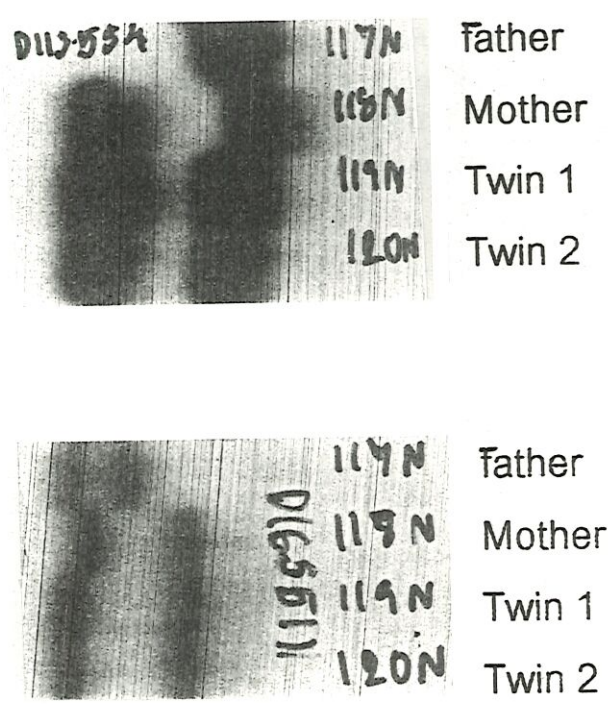


Figure 1. Examples of result from zygosity testing in 2 markers

## Determination of Zygosity

We determined the zygosity of twins by using short tandem repeat loci. DNA was extracted from blood samples collected from the twins and their parents.

A total of 12 markers were used for the zygosity testing. These included the STR markers at D2S102, GLUT2, TCRD, D11S534, GABRB3, D10S169, D13S119, D5S82, IL2RB, D8S88, D10S141, D12S82, D14S267, D18S35, D20S120, and D21S267 (table 1)

## Result

By molecular genetics personal identification by short tandem repeat loci, the child with Hirschsprung's disease and her twin sister had identical alleles of 12 markers, thus there was a 99.999% likelihood that they were monozygotic twins ( table 1 and figure 1 ).

## Discussion

Until recently, the determination of monozygoticity was done by observation of the sharing of a single and common placenta and determination of identical blood groups and HLA system. The DNA typing method was developed for more accuracy. The short tandem repeat loci is an easy and reliable method. The accuracy depends on the number of STR loci. The estimated probability of each informative locus is about 50-70%. When 12-13 markes are used, the probability increases up to 99.999%

The inheritance pattern has not yet been clearly defined. It was found that 4% of the patients with Hirschsprung's disease had a familial incidence. The extent of the aganglionosis seems to be related to the mode of inheritance. For the patients with

Table 1 STR Markers Studied in Twins and Autoradiographs Showing the Same Alleotypes

| STR Markers | Father | Mother | 1stTwin | 2ndTwin | Probability* |
|---|---|---|---|---|---|
| D10S141 | 1,2 | 3,4 | 1,3 | 1,3 | 1/4 |
| D12S82 | 1,1 | 1,2 | 1,1 | 1,1 | 1/2 |
| D14S267 | 2,3 | 1,3 | 1,3 | 1,3 | 1/4 |
| D11S354 | 1,2 | 2,2 | 2,2 | 2,2 | 1/2 |
| D13S119 | 1,2 | 1,1 | 1,2 | 1,2 | 1/2 |
| D5S82 | 1,2 | 1,3 | 1,1 | 1,1 | 1/4 |
| D18S35 | 1,1 | 2,2 | 1,2 | 1,2 | 1/2 |
| IL2RB | 1,1 | 2,3 | 1,2 | 1,2 | 1/2 |
| D8S88 | 2,3 | 1,4 | 1,3 | 1,3 | 1/4 |
| TCRD | 1,1 | 1,2 | 1,1 | 1,1 | 1/2 |
| D11S534 | 1,2 | 2,3 | 2,3 | 2,3 | 1/4 |
| GABRB3 | 1,3 | 1,2 | 1,1 | 1,1 | 1/4 |

* Probability for independent segregation.

aganglionosis beyond the sigmoid colon, this is compatible with a dominant gene with incomplete penetrance, whereas for patients in which the aganglinosis extending no further than the sigmoid colon, the inheritance pattern was likely to be multifactorial or a recessive gene with very low penetrance

In 1992, a deletion on the long arm of chromosone 10 was described in a newborn patient with total aganglionosis. Recent reports suggest that both Hirschsprung and MEN type 2 are associated with mutations within the same RET proto-oncogene.

Concordant and discordant twins with Hirschsprung's desease have been reported in previous studies.However, this is a very first set of discordant twins with total aganglionosis in which monozygoticity was proven by a DNA study. The mechanism of the disease may involve sporadic somatic mutation of RET protooncogene. More studies of mutations of the affected gene, including more cases of twins, may clarify the contribution gene mutations to the pathogenesis of Hirschsprung's disease..

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