Journal of Pediatric Surgery (2010) **45**, 709–713

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Journal of Pediatric Surgery

www.elsevier.com/locate/jpedsurg

Lack of association between *nNOS* –84G>A polymorphism and risk of infantile hypertrophic pyloric stenosis in a Chinese population

Xiaoping Miao[a,b,1], Maria-Mercè Garcia-Barceló[b,c,1], Man-ting So[b], Wai-kiu Tang[b], Xiao Dong[a], Bin Wang[a], Jianxiong Mao[a], Elly Sau-wai Ngan[b], Yan Chen[b], Vincent Chi-hang Lui[b], Kenneth Kak-yuen Wong[b], Lei Liu[a,*], Paul Kwong-hang Tam[b,c,*]

[a]*Department of Surgery, Shenzhen Children's Hospital, Shenzhen, China*
[b]*Division of Paediatric Surgery, Department of Surgery, Queen Mary Hospital, University of Hong Kong Medical Center, Hong Kong SAR, China*
[c]*Genome Research Centre, The University of Hong Kong, Hong Kong SAR, China*

Received 18 June 2009; revised 31 July 2009; accepted 31 July 2009

Key words:
Infantile hypertrophic pyloric stenosis;
nNOS;
Polymorphism;
Susceptibility

Abstract

Background: Infantile hypertrophic pyloric stenosis (IHPS) is one of the most common gastrointestinal obstructions in the infancy requiring surgery. Reduced expression of neuronal nitric oxide synthase (nNOS), which plays an important role in the regulation of the human pyloric muscle, is thought to underlie IHPS. The role of *nNOS* in IHPS has been supported by the genetic association of a functional regulatory *nNOS* polymorphism (*−84G>A*) with IHPS in whites. We reasoned that the corroboration of this association in a population of different ethnic origin would prompt follow-up studies and further investigation of the IHPS pathology at molecular level. Thus, we attempted to reproduce the original findings in a Chinese population of comparable size in what would be the first genetic study on IHPS conducted in Chinese.

Methods: *nNOS −84G>A* genotypes were analyzed in 56 patients and 86 controls by polymerase chain reaction and DNA sequencing. Logistic regression was used to compute odds ratios.

Results: Our study could not corroborate the association previously reported. Although the frequency of the IHPS-associated allele (*−84A*) in controls (0.205) was similar to that reported for white controls, there was a dramatic difference in *−84A* frequencies between white and Chinese patients (0.198). Similarly, there was no difference in the *nNOS −84G>A* genotype distribution between patients and controls, even when the *GA* and *AA* genotypes were combined to compare *GG* genotype (odds ratio, 1.01; 95% confidence interval, 0.47-2.19).

* Corresponding authors. Paul Kwong-hang Tam is to be contacted at Division of Paediatric Surgery, Department of Surgery, Queen Mary Hospital, University of Hong Kong Medical Center, Hong Kong SAR, PR China. Tel.: +852 28554850; fax: +852 28173155. Lei Liu, Department of Surgery, Shenzhen Children's Hospital, Shenzhen 518026, China. Fax: +86 755 83508700.

E-mail addresses: liulei3322@yahoo.com.cn (L. Liu), paultam@hkucc.hku.hk (P.K. Tam).

[1] Mercè Garcia-Barceló and Xiaoping Miao contributed equally to this work.

doi:10.1016/j.jpedsurg.2009.07.067

Conclusions: Failure to replicate the initial finding does not detract from its validity, because genetic effects may differ across populations. Differences across populations in linkage disequilibrium and/or allele frequencies may contribute to this lack of replication. The role *nNOS* in IHPS awaits further investigation.

Infantile hypertrophic pyloric stenosis (IHPS) (OMIM179010) is one of the most common forms of neonatal gastrointestinal obstruction requiring surgery. Infantile hypertrophic pyloric stenosis is characterized by the hypertrophy and hyperplasia of the circular muscle layer of the pylorus, leading to projectile and persistent vomiting, weight loss, and dehydration [1,2]. Both open and laparoscopic pyloromyotomy are proved to be effective in relieving gastric outlet blockage [3,4]. The estimated population incidence is 1 to 5 per 1000 live births, although this is a representative value, with a marked preponderance of males to females in a 4:1 ratio [5]. Although the etiology of IHPS is still unclear, genetic predisposition to IHPS is well established. Infantile hypertrophic pyloric stenosis has been reported to be associated with a variety of chromosomal abnormalities, including translocation of chromosome 8 and 17 and a partial trisomy of chromosome 9, and a number of inherited syndromes, such as Smith-Lemli-Opitz and Cornelia de Lange syndromes [6-8]. Approximately 10% of IHPS cases are familial. Twin studies showed a concordance rate of 25% to 40% in monozygotic twins. The recurrence risks to sibs are around 10% and 2% in males and females, respectively, and the risk of IHPS is nearly 17-fold higher in first-degree relatives than the general population [9].

Infantile hypertrophic pyloric stenosis is a well-recognized multifactorial trait that results from the interaction between genetic and environmental factors [10]. Two approaches, linkage and association studies, have been widely used to uncover genetic susceptibility to IHPS. Linkage is based on the analysis of the genotypes of members of families that segregates the disease, whereas association compares the genotypes of affected individuals with those of the general population. To date, 5 IHPS susceptibility loci have been identified (Table 1). Capon et al [11] analyzed a single large multiplex pedigree, which included 10 affected individuals, and identified an underlying IHPS disease locus in chromosome 16p12-13. Everett et al [12], using an SNP-based high-density linkage analysis in a cohort of 81 IHPS pedigrees, uncovered 2 IHPS disease loci that mapped to chromosome 11q14-q22 and Xq23. In an extended IHPS family including 8 affected individuals, chromosome 16q24 was found to be linked with the disease [13]. The chromosome 12q24.2-q24.31 region, where the gene encoding the neuronal nitric oxide synthase (*nNOS*) maps, was found to be implicated in IHPS [14] through a linkage analysis conducted in 1996. However, it was not until 2004 that an association study taking *nNOS* as a candidate gene was carried out.

nNOS is a key enzyme in the synthesis of the nitric oxide (NO), which plays an important role in the relaxation of the pyloric smooth muscle [15]. There is a growing body of evidence indicating that reduced expression of nNOS is a common hallmark of IHPS [16-18]. It has been shown that the administration of exogenous NO donor to sphincter tissues with low NOS expression can restore the capacity of relaxation [19], indicating that the reduced expression of nNOS may act as a mechanism for the defective relaxation of pyloric muscle and formation of pyloric mass, thought to be responsible for the obstruction of upper alimentary tract. Moreover, animal studies in mice have shown that absence of *nNOS* gene causes a phenotype closely resembling that of human IHPS [20,21]. The *nNOS* gene, which can generate 9 distinct first-exon transcripts (exon 1a-1i) in human gut by alternative promoter use, is one of the most structurally diverse human genes identified [22-24] in terms of first-exon use and complexity of expression patterns, which suggests the existence of a temporospatial regulation of its expression under different physiologic or pathological conditions.

Recently, Saur et al [25] found significantly decreased expression of total *nNOS* messenger RNA in pyloric tissues of patients with IHPS when compared with that of normal

Table 1 Genetic studies on IHPS

References	Type	Ethnicity	Sample size	IHPS susceptibility loci
Chung et al [14]	Linkage analysis	White	27 families (229 individuals, 87 with IHPS)	*NOS1* (12q12.2-q24.31)
Capon et al [11]	Linkage analysis	White	3-generation family (10 affected)	1 16p12-p13
Everett et al [12]	Linkage analysis	White	81 families (302 individuals, 206 with IHPS)	11q14-q22 and Xq23i
Everett et al [13]	Linkage analysis	White	1 family (8 with IHPS)	16q24
Saur et al [25]	Association study	White	Case control, 16 IHPS and 81 controls	*−84(G>A)* of *nNOS* has been identified to be associated with IHPS