行政院國家科學委員會專題研究計畫　成果報告

台灣地區罹患低磷酸鹽血性佝僂症家族之 PHEX 基因突變研究

計畫類別： 個別型計畫
計畫編號： NSC93-2314-B-002-135-
執行期間： 93 年 08 月 01 日至 94 年 07 月 31 日
執行單位： 國立臺灣大學醫學院小兒科

計畫主持人： 蔡文友

計畫參與人員： 蘇怡寧

報告類型： 精簡報告

處理方式： 本計畫可公開查詢

中　華　民　國 94 年 10 月 28 日

中文摘要

低磷血性佝僂症是因為腎臟無機磷酸鹽傳送障礙導致無機磷酸鹽流失及低磷血症的疾患。本研究收集 18 名低磷血性佝僂症及其家人的血液進行 *PHEX* 基因的突變分析，結果於其中 13 名患童發現 *PHEX* 基因突變，這些突變中有 5 名為無意義突變，4 名為缺失，2 名為插入及 2 名為誤義突變。其中 83% *PHEX* 基因突變導致 PHEX 蛋白質產物的截短，而且 69%的突變為這些家庭內無中生有的新突變。本研究顯示 *PHEX* 基因突變所導致的性聯低磷血性佝僂症為台灣地區低磷血性佝僂症最常見的原因，而此種突變大多數為無中生有的新突變。

英文摘要

Hypophosphatemic rickets consists of a group of disorders characterized by a defect in renal phosphate transport resulting in phosphate wasting and hypophosphatemia. Eighteen unrelated Taiwanese patients with hypophosphatemic rickets and their families were enrolled for the mutational analysis of their *PHEX* gene in this study. Mutations of *PHEX* gene was detected in 13 out of 18 patients (72%) with hypophosphatemic rickets. These mutations include 5 nonsense mutations, 4 deletions, 2 insertions and 2 missense mutations. The majority (83%) of these mutations are predicted to result in truncation of the PHEX protein product and 69% of these mutations have arisen *de novo*. Our data revealed that X-linked hypophosphatemic rickets due to mutations of *PHEX* gene is the most common cause of hypophosphatemic rickets in Taiwan and most of such mutations of *PHEX* gene occurred *de novo*.

報告內容

Introduction

Hypophosphatemic rickets consists of a group of disorders characterized by a defect in renal phosphate transport resulting in phosphate wasting and hypophosphatemia. The most common mode of inheritance is transmitted as an X-linked dominant disorder (MIN 307800) with an incidence of 1 per 20,000 individuals in Caucasians [1-6].

The genetic defect responsible for X-linked hypophosphatemia (XLH) has been identified on Xp22.1 by linkage analysis and named as *PHEX* gene [7-11]. Recent studies have provided compelling evidence for the causative role of *PHEX* in XLH. Such mutations include nonsense mutations, missense mutations, splice site mutations, insertions and deletions [8-10, 12-17].

To elucidate the characteristics of *PHEX* gene mutations in Taiwanese patients with hypophosphatemic rickets, we have carried out an extensive mutational analysis in 18 unrelated families with familial or sporadic cases of hypophosphatemic rickets.

Subjects and Methods

A total of 18 unrelated Taiwanese patients with hypophosphatemic rickets and their families were assessed. Among them, seven individuals had a family history of hypophosphatemic rickets. There were 21 affected members (7 males and 14 females) and 18 unaffected members (14 males and 4 females) enrolled in the present study. On the other hand, eleven individuals were sporadic cases without any affected family members elicited. In addition to these eleven affected members (4 males and 7 females), there were 35 unaffected family members (17 males and 18 females) also enrolled in the present study.

After informed consents were obtained, venous blood samples were collected and DNA were extracted from these 32 affected individuals and 52 unaffected family members. The DNA samples were used with 22 pairs of primers for the PCR amplification of the 22 exons and their respective exon-intron boundaries of *PHEX* gene. The primer pairs were made based on the genomic sequence of human *PHEX* gene published by Francis et al [9]. Direct sequencing of PCR products was performed from both strands using an automated sequencer (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA).

Results

Thirteen patients were found to have mutations of *PHEX* gene in 18 patients with hypophosphatemic rickets. Among them, five (71%) of the *PHEX* mutations were detected in seven probands with familial XLH and eight (73%) of the *PHEX* mutations were detected in the 11 probands with sporadic XLH.

In this study, five nonsense mutations including two 58C→T, one 871C→T, one 1589C →T and one 2104C→T transversion were detected. A 1151-T insertion in codon 384 and a 1783TGAT insertion in codon 595 were also detected in two unrelated families. Deletional

mutations of *PHEX* gene including 133TT in codon 45, 547AG in codon 183, and 2155G in codon 719 were detected in three unrelated families. All these mutations lead to a frameshift and stop codon in the following codons of *PHEX* gene. Another girl had a large deletion of 23bp at 3'-terminal end of intron 21, which was -7 upstream to the splice acceptor consensus sequence of exon 22. Two missense mutations, conversion of 2237G to A in codon 746 and conversion of 2240G to A in codon 747, were found in two unrelated sporadic patients with XLH.

Discussion

Mutational analysis of the *PHEX* exons in 18 individuals with hypophosphatemic rickets revealed mutations in 13 patients (72%). These data are comparable to the mutations rate previously reported [8-10, 12, 14]. Twelve different mutations were found because one mutations (R20X) was detected in two unrealed families. Four of these 12 mutations (R20X, R291X, C1583 in TGAT, and R702X) have been previously described [9, 10, 12, 14, 18]. Otherwise , the rest eight mutations were novel mutations of *PHEX* gene. Three (25%) of the 12 mutations in this study were detected in exon 22. Apart from this clustering, mutations were scattered widely throughout the entire *PHEX* gene and there are no apparent hot spots detected.

Our findings showed that *de novo* mutation was detected in the mother of one familial XLH and eight patients with sporadic XLH. Our result indicated that 69% of *PHEX* mutations in Taiwanese patients with XHL may have arisen *de novo*, which is higher than those previously reported [12, 14]. These data suggest that *PHEX* gene appears to be particularly prone to have mutations for unknown reasons.

In this study, the mutations of *PHEX* gene consisted of 4 nonsense mutations (33%), 4 deletion (33%), 1 duplication (8%), 1 insertion (8%) and 2 missense mutations (17%). The majority (83%) of these 12 PHEX mutations are predicted to result in truncation of the PHEX protein products leading to a functional loss of the PHEX protein.

In conclusion, our data showed that XLH due to mutations of *PHEX* gene is the most common cause of hypophosphatemic rickets in Taiwan and most of such mutation of *PHEX* gene occurred *de novo*. Because about one quarter of patients with hypophosphatemic rickets have normal genetic study in *PHEX* gene, further studies are indicated to clarify the cause and pathogenesis of hypophosphatemic rickets in Taiwanese children.

References

1. Bianchine JW, Stambler AA, Harrison HE: Familial hypophosphatemic rickets showing autosomal dominant inheritance. Birth Defects 1971; 7:287-294.
2. Econs MJ, McEnery PT: Autosomal dominant hypophosphatemic rickets/ osteomalacia: Clinical characterisation of a novel renal phosphate wasting disorder. J Clin Endocrinol Metab 1997; 82:674-681.
3. Stamp TC, Baker LR: Recessive hypophosphatemic rickets, and possible aetiology of the 'vitamin D – resistant' syndrome. Arch Dis Child 1976; 51:360-365.
4. ADHR Consortium: Autosomal dominant hypophosphatemic rickets is associated with mutations in FGF23. The ADHR Consortium. Nat Genet 2000; 26:345-348.
5. Winters RW, Grahan JB, Williams TF et al.: A genetic study of familial hypophosphatemia and vitamin D resistant rickets with a review of the literature. Medicine 1958: 37:97-142.
6. Burnett CH, Dent CE, Harper C et al.: Vitamin-D resistant rickets. Am J Med 1964; 36:222-232.
7. HYP consortium: A gene (PEX) with homologies to endopeptidase is mutated in patients with X-linked hypophosphatemic rickets. The HYP Consortium. Nat Genet 1995; 11:130-136.
8. Rowe PS, Oudet CL, Francis F et al.: Distribution of mutations in the PEX gene in families with X-linked hypophosphatemic rickets (HYP). Hum Mol Genet 1997; 6:539-549.
9. Francis F, Strom TM, Henning S et al.: Genomic organization of the Human PEX gene mutated in X-linked dominant hypophosphatemic rickets. Genome Res 1997; 7:573-585.
10. Holm IA, Huang X, Kunkel LM: Mutational analysis of the Pex gene in patients with X-linked hypophosphatemic rickets. Am J Med Genet 1997; 60:790-797.
11. Turner AJ, Tanzawa K: Mammalian membrane metallopeptidase: NEP, ECE, KELL, and PEX. FASEB J 1997; 11:355-364.
12. Dixon PH, Christie PT, Wooding C et al.: Mutational analysis of PHEX gene in X-linked hypophosphatemia. J Clin Endocrinol Metab 1998; 83:3615-3623.
13. Econs MJ, Friedman NE, Rowe PSN et al.: A PHEX gene mutation is responsible for adult-onset vitamin D-resistant hypophosphatemic osteomalacia: evidence that the disorder is not distinct entity from X-linked hypophosphatemic rickets. J Clin Endocrinol Metab 1998; 83:3459-3462.
14. Holm IA, Nelson AE, Robinson BG et al.: Mutational analysis and genotype-phenotype correlation of the PHEX gene in X-linked hypophosphatemic rickets. J Clin Endocrinol Metab 2001; 86:3889-3899.
15. Miyamura T, Tanaka H, Inoue M et al.: The effects of bone marrow transplantation of X-linked hypophosphatemic mice. J Bone Miner Res 2000; 15:1451-1458.
16. Shih NR, Jo O, Yamagawa N: Effect of PHEX antisense in human osteoblast cells. J Am Soc Nephrol 2002; 13:394-399.
17. Sabbagh Y, Boileau G, Campos M et al.: Structure and function of disease-causing

missense mutations in the PHEX gene. J Clin Endocrinol Metab 2003; 88:2213-2222.
18. Sato K, Tajima T, Makae J et al.: Three novel PHEX gene mutations in Japanese patients with X-linked hypophosphatemic rickets. Pediatr Res 2000; 48:536-540.