论文题目：两个遗传性白内障致病基因的定位与克隆

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摘　要

白内障是一个常见的人类眼科疾病，其临床表现为晶状体混浊。先天性白内障是常见的儿童致盲疾病。先天性白内障病因比较复杂，主要是由于遗传因素或环境因素造成的。大约 26%－51%的先天性白内障是因为遗传因素导致的。染色体异常和基因突变都可以造成个体的白内障。遗传性单纯性白内障具有很强地遗传异质性，有三种不同的遗传方式：常染色体显性遗传、常染色体隐性遗传和性染色体连锁遗传。在三种遗传型中，以常染色体显性遗传最多见，常染色体隐性遗传次之，性染色体连锁遗传型最少见。迄今为止，至少有 23 个遗传性单纯性白内障基因被定位或被克隆。但是由于白内障疾病的复杂性，目前的研究还远远不够，如在同一家系中相同基因突变而不同个体的白内障表型却可完全不同，遗传性白内障与老年性白内障之间的关系等许多问题还不是十分清楚，距离真正了解这一疾病的发病机制和根治该疾病还有很大距离，存在着许多未知，所以需要通过对遗传性单纯性白内障研究来深入了解可导致和影响晶状体病变的所有基因。

研究遗传性白内障致病基因，实验动物模型是一个非常好的方式。实验动物模型中小鼠模型有着实验动物易于饲养，实验样本易于得到，实验可广泛展开和小鼠与人类同源性较高的优点。我们建立了一个白内障隐性遗传小鼠模型，纯系白内障小鼠 rncat 10 天到 20 天，双眼晶状体内出现白色混浊；35 天后，小鼠晶状体核区完全白色混浊所覆盖，为核性白内障。运用荧光 AFLP 方法作为该隐性白内障基因定位的主要手段，对小鼠全基因组进行扫描。隐性遗传的白内障小鼠通过与近交系小鼠 B10/Sn 品系杂交得到的 F1 代，并与白内障小鼠回交后的 N2 代小鼠为遗传分析样本。N2 代小鼠基因组 DNA 经 EcoRI/MseI, EcoRI/TaqI 内切酶组合分别消化，连上各自的接头并进行两次 PCR 扩增。结果发现在一对 EcoRI/TaqI 引物组合所经 PCR 扩增获得的条带中，有一个多态性标记与隐性白内障基因相连锁。通过 Radiation Hybrid Panel 方法将此多态性标记定位于小鼠第 16 号染色体上，利用微卫星标记进一步将此小鼠品系的隐性白内障基因定位在小鼠基因组的 16 号染色体上 23.8 cM 的区域内。通过组织病理切片反映了白内障小鼠的晶状体上皮细胞在晶状体赤道部有过度增殖现象，并向晶状体前、后极延伸。通过突变检测，证明了 γS 晶体蛋白是此隐性白内障小鼠的致病基因，并提示 γS 晶体蛋白可能具有调节晶体上皮细胞生长、分化的功能。

先天性白内障有数十种不同的晶体形态上的表现。不同的晶体表型不仅可以出现在不同的先天性白内障家系中，而且在同一家系，甚至同一患者两个眼都可表现为不同的晶体混浊类型。另一方面，同一种形态表现也可出现在不同定位的先天性白内障家系中。正因为其明显的遗传异质性给先天性白内障的遗传学研究带来了一定的困难。目前认为，找到一个有明确家族遗传史的大家系，进行深入的遗传学分析研究，是探明其遗传机制的有效方法。我国有 500 多万白内障患者，其中先天性白内障在我国发病率为 0.05%，近 1/2 的先天性白内障是遗传因素所控制的，具有家族遗传史白内障家系众多。我们围绕一个连续五代的板层状白内障大

家系和两个小家系进行展开工作，通过全基因组扫描，将板层状白内障基因定位于 16q22 上 5.11 cM 内。著名的 Marner 类型白内障丹麦大家系是一个位于哥本哈根南部的先天性白内障大家系，早在 1878 年首次报道，1949 年该家系发展至 542 人并由 Marner 跟踪报道并作了白内障形态学分析，1988 年 Eibreg 等对此家系共 965 人进行了遗传分析，发现此白内障基因位于 16q22 并与结合珠蛋白（HP）连锁，但致病基因在一百多年的时间内未找到。我们通过遗传研究，参照家系白内障表现，推测 Marner 类型白内障可能位于我们定位的白内障候选区域并且致病基因可能相同。通过突变检测，发现在这四个家系中位于候选区域的热休克蛋白转录因子 HSF4 基因上发生了四个分别不同的核苷酸错义突变。本实验在国际上，首次证明了热休克蛋白转录因子 HSF4 是白内障致病基因。

关键词：白内障、连锁分析、定位克隆、基因突变

Abstract

Cataract is a common eye disease. Hereditary congenital cataract is one of the most common causes of childhood blindness and poor vision. It includes autosomal dominant, autosomal recessive, and X-linked forms. Among them, nonsyndromal autosomal dominant cataract (ADC) is the most common form. Up to now, at least 23 loci of nonsyndromal cataract have been identified on the basis of linkage analyses and gene mutations. However, the cataract loci which have been mapped to date account for only a limited fraction of cataract. The identification of new disease loci is thus underway. The mechanism of cataract is still unclear.

We found a recessive cataract mutation arose spontaneously in a KUNMING outbred mouse strain. After sib mating for ten years, the mutant mice were crossed to B10/Sn mice to generate F1 hybrids, which were further backcrossed to the mutant mice. Genomic DNA was extracted from N2 mice. Fluorescent amplified fragment length polymorphism analysis (AFLP) was performed to map the cataract locus in the whole genome. Genomic DNA was digested with EcoRI combined with either MseI or TaqI. Then, two adapters were added to the ends of the digested fragments respectively. After a pre-amplification and a selective amplification, the PCR products were separated on denaturing polyacrylamide gel on a LI-COR IR2 Automated Sequencer. A fragment linked to the cataract phenotype was identified. This fragment was isolated and purified from the polyacrylamide gel in an additional experiment in which 33P-dATP was used to label the PCR products. After the fragment was sequenced, the primers specific for the fragment were designed. The T31 Mouse Radiation Hybrid Panel (Research Genetics Inc.) was typed by PCR with the specific primers. Five microsatellite markers were then used to confirm and map the recessive cataract gene into a 23.8 cM region on the mouse chromosome 16. Histological analysis shows a severe degeneration of the epithelial cells underneath the anterior lens capsule, while those cells in the equatorial region display an excessive proliferation and migration. After mutation analysis, we found a homozygous G to A nucleotide conversion at position 489 of Crygs in mutant mice, leading to a truncated gene product (Trp163Stop). This finding suggests that Crygs is not only a lens structural protein but also likely to be involved in epithelial cell proliferation, apoptosis and migration.

In spite of the high incidence of ADC in the Chinese population, there are, however, no reports about linkage analyses of ADC in Chinese families. Here, we identified and analysed a five-generation family affected with lamellar cataract. Among the offspring of the affected parents, 30 were affected, while only 17 were unaffected, suggesting biased transmission of disease allele from the affected individuals to their offspring (P=0.02). We used a whole genome scan strategy to map the disease locus and found a linkage between markers on 16q22 and the cataract phenotype. According to previous reports, another congenital cataract locus, Marner cataract (CAM) is located near this region. In 1949, Marner described the CAM phenotype in a large Danish family, with 542 members in 8 generations. In 1988, Eiberg found that CAM shows strong linkage to haptoglobin with lod score=8.33 at theta=0.05 for males and females combined. Mutation screening of the heat shock transcription factor 4 gene (HSF4) in this candidate region identified a T to C transition in exon 3, resulting in a Leu115Pro substitution within the

DNA-binding domain in the Chinese five-generation family. Furthermore, we identified a C to T transition at nucleotide 362 in affected family members in the Danish family with the Marner's cataract phenotype and two further missense mutations in sporadic cataract cases, resulting in an Ala20Asp and an Ile87Val substitution. These mutations are all within the DNA-binding domain. This work demonstrates that HSF4 is very important in lens development. It is the first report of mutations in a transcription factor regulating heat shock proteins associated with cataract.

Keyword： cataract、positional cloning、linkage analysis、mutation