

dom sampling from provincial capitals and from rural areas according to demonstration were used. Among a total screened population of 3 837 597 persons, there were 37 808 cases of migraine. The morbidity and the annual incidence rate were 985.2/100 000 and 79.7/100 000, respectively. The male/female ratio was 1:4. Inland plateaus were higher morbidity areas (more than 1 500/100 000) and coastal provinces and cities were lower morbidity areas (less than 400/100 000). The incidence rate was higher in spring in the south, while it was higher in summer in the north. The incidence rate was higher in hot and damp climate. The morbidity under 14 years old in China was significantly lower than those in overseas reports.

Key words Migraine [Sampling studies]

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用PCR法筛选基因重组的阳性克隆

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从转化克隆中筛选和鉴定阳性克隆的方法有酶切分析法和基因探针杂交法两种。我们用PCR法对重组的克隆进行了筛选, 得到了阳性克隆。我们用编码落矶山斑点热立克次体17-KDa抗原基因的DNA序列(J Bacteriol. 1989; 169, 2385)合成引物, 上游引物: 5'-GGAATT CATGAACTATTAT CT, 下游引物: 5'-CGGGATCCCTCAATTCAACATTG。以普氏立克次体Brelm株DNA为模板, 用这对引物通过PCR扩增出DNA片段。然后, 用BamHI/EcoRI双酶消化后, 再和用BamHI EcoRI双酶消化的载体质粒pUC19片段连接, 转化到大肠杆菌JM101中, 经过夜培养, 得到转化菌落。用接种针从每一个菌落中挑取少量材料, 分别接种于对应号码的试管内, 管内装有3mlLB液体培养基(含有终浓度为60μg/ml的氨苄青霉素)。培养4~6小时后, 离心, 取少量菌体材料直接用于PCR扩增。PCR

反应的各组分如下: 1×缓冲液40μl(含有0.5%Nonidet P40, 0.5%吐温200); dNTPs4μl(终浓度各为200μmol/L); 引物各加1μl(终浓度各为1μmol/L); 待检菌落材料4μl。反应程序与我们以前的报道〔疾病监测, 1992, 7(5): 121〕相同。反应中, 我们用pUC19空载体作阴性对照, 用提纯的普氏立克次体DNA作阳性对照。用PCR法对50个转化的克隆菌落进行扩增, 结果得到5个阳性克隆, 这5个克隆均扩出496个碱基的片段, 与预期的结果一致。

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