

# Linkage between bronchial responsiveness to methacholine and gene markers of IL-4 cytokine gene cluster and T-cell receptor $\alpha/\delta$ gene complex in Korean nuclear families

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## Summary

**Background** Several candidate genes have been reported to be linked to intermediate phenotypes of asthma in Caucasian populations.

**Objective** To evaluate linkage between phenotypes of asthma and gene markers of high affinity IgE receptor- $\beta$  gene (D11S97), IL-4 cytokine gene cluster (IL-4R1), and T-cell receptor  $\alpha/\delta$  gene complex (D14S50) in Korean nuclear families.

**Methods** Nuclear families (127 probands and their 130 siblings) for the linkage analysis were ascertained through asthmatic children. Linkages between total serum IgE response, skin responses to common aeroallergens, and bronchial responsiveness to methacholine were performed using a sib-pair approach.

**Results** The square difference of the slope of the dose–response curve (DRS) between sib-pairs with two IL-4R1 identical alleles was smaller than with one or with neither IL-4R1 identical allele ( $P = 0.004$ ). As for D14S50, the differences of DRS between sib-pairs with two identical alleles and with one identical allele were smaller than with neither identical alleles ( $P = 0.01$ ). As for D11S97, no significant differences were observed among the groups with identical alleles of two, one or zero. With regard to total serum IgE levels, no significant linkage was found between this phenotype and the above three gene markers. As for skin responses to common aeroallergens, significant evidence was obtained to establish a linkage between this phenotype and the marker IL-4R1 ( $P = 0.01$ ). However, no significant linkage was found between this phenotype and the markers D11S97 and D14S50.

**Conclusion** The expression of bronchial responsiveness to methacholine may be influenced by genetic factors in the IL-4 cytokine gene cluster and/or T-cell receptor  $\alpha/\delta$  gene complex, but the genetic influence of the Fc $\epsilon$ RI- $\beta$  gene may be minimal in the expression of bronchial responsiveness in Korean nuclear families.

**Keywords:** asthma, bronchial responsiveness, total IgE, skin response, chromosome 11q13, chromosome 5q31-q33, chromosome 14q11, linkage

*Clinical and Experimental Allergy*, Vol. 31, pp. 103–109. Submitted 18 November 1999; revised 12 March 2000; accepted 18 May 2000.

## Introduction

Asthma is an obstructive airway disease that develops

because of the presence of a genetic predisposition and exposure to inciting agents such as allergens, respiratory infections and environmental pollutants. In addition, asthma is closely related to atopy, especially in younger patients. Family studies of allergy and asthma have found evidence of genetic factors but have been unable to clearly

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delineate the number, or the mode of inheritance, of the major genes responsible [1–4].

In 1989, Cookson and co-workers [5] provided evidence of a genetic localization for atopy, which was defined as high total serum IgE, positive RAST or a positive skin test, to chromosome 11q13. It appears that in the population examined, the inheritance of atopy at this locus occurs through the maternal line [6]. The  $\beta$  subunit of the high-affinity receptor of IgE (Fc $\epsilon$ RI- $\beta$ ) is also positioned on chromosome 11q13, and it appears to be in close linkage with the gene for atopy in the population examined [7,8]. However, attempts to independently replicate the linkage to chromosome 11q13 have produced conflicting results [9–13].

The chromosome 5q31–q33 region of the genome contains several candidate genes which have been implicated in the pathogenesis of asthma. This included the interleukin (IL)-4 cytokine cluster (IL-3, IL-4, IL-5, IL-9, IL-13, and the granulocyte/macrophage colony-stimulating factor) and the  $\beta_2$ -adrenergic receptor. This makes chromosome 5q a very attractive candidate region, and it has been reported to be linked to both total serum IgE [14,15] and to bronchial hyperresponsiveness (BHR) [16].

Studies on T-cell receptor (TCR) genetic variants [17] and their relationship to atopy are less well advanced. One genetic linkage study, examining IgE responsiveness to common aeroallergens and a highly polymorphic microsatellite marker related to the TCR  $\alpha/\delta$  gene complex on chromosome 14 and the TCR- $\beta$  gene complex on chromosome 7, suggested a genetic linkage to the  $\alpha/\delta$  complex [18].

The aim of this study was to determine the linkage between the asthma phenotypes, such as total serum IgE levels, skin responses to common aeroallergens and bronchial hyperresponsiveness to methacholine, and the gene makers of the above candidate genes.

## Subjects and methods

### Subjects

Probands of the families studied were among asthmatic children visiting the Paediatric Clinic of the Seoul National University Hospital. Families for the study were ascertained between January 1996 and December 1997 from children between 4 and 15 years of age. A full verbal and written explanation of the study was given to all of the family members interviewed, and nuclear families (127 probands and 130 siblings) that were so inclined gave informed consent and were included in this study. Informed consent for subjects younger than school age was given by their parents. The study was approved by the Ethics Committee of the Seoul National University Hospital.

Asthmatic children had a history of episodic wheezing or breathlessness and showed reversible airway obstruction on treatment with a bronchodilator (more than 15% of predictive value of FEV<sub>1</sub>). Probands and their siblings gave blood specimens for the measurement of total serum IgE levels and the genotyping of candidate genes using microsatellite markers. They also underwent bronchial provocation tests to methacholine and skin prick tests to common aeroallergens.

### Clinical phenotyping

None of the subjects had received oral or inhaled bronchodilators for 3 days preceding the methacholine bronchial provocation test. Subjects with viral upper respiratory tract infections during the last 2 weeks were excluded from the methacholine challenge. The methacholine bronchial provocation test was carried out in 108 probands and 98 sibs, using the methods of Chai *et al.* [19]. Concentrations of 0.125, 0.625, 1.25, 2.5, 6.25, 12.5 and 25 mg/mL of methacholine were prepared by dilution with buffered saline. A Rosenthal–French dosimeter (Laboratory for Applied Immunology, Baltimore, USA) was used to deliver the aerosol generated by a nebulizer (DeVilbiss, Baltimore, USA). The slope of the dose–response curve (DRS), defined as the percentage fall in FEV<sub>1</sub> at the last dose divided by the log of the last dose (mg/mL) administered, was calculated for a continuous index of bronchial responsiveness to methacholine [20].

Skin prick tests were performed using a panel of 10 common aeroallergens (Bencard Co., UK). None of the subjects had received antihistamines orally during the 5 days preceding the study. The panel included *Dermatophagoides pteronyssinus*, *D. farinae*, cat fur, *Aspergillus fumigatus*, *Alternaria alternata*, tree pollen mixture, grass pollen mixture, mugwort pollen, ragweed pollen and cockroach. A positive control of histamine (1 mg/mL) along with a negative diluent control was included in all tests. After 15 min of the prick test, the mean diameter of a wheal formed by the allergen was compared with that formed by histamine. If the former was the same, or larger than the latter (allergen/histamine ratio  $\geq 1.0$ ), the reaction was defined as positive. Total serum IgE levels were determined in 127 probands and 122 sibs using the enzyme-linked immunosorbent assay (ELISA), as previously described [21].

### Molecular methods

Genomic DNA was obtained from 5 ml peripheral blood using a DNA extraction kit (Guachem, Glasgow, UK), as recommended by the supplier. Polymerase chain reaction (PCR) was performed, using primers for the microsatellite markers on chromosomes 11q13 (D11S97), 5q31–q33

(IL-4R1) and 14q11 (D14S50). The primer sequences were as follows:

D11S97 sense: 5'-GGCAGGGTCCCTTTCAGCGTCT-CATCCACAGT-3'

antisense: 5'-TTTTTCAGGAGGTCAGGCTGTGGCTGTAGGTCG-3'

IL-4R1 sense: 5'-GTCTCCACCCACAGCAGG-3'

antisense: 5'-TATGTGCCAGGCATTACGCT-3'

D14S50 sense: 5'-AACACCCCTAATTCACCACT-3'

antisense: 5'-ATGATTCCACAAGATGGCAG-3'

Each 35 µL reaction mixture contained 2.5 µL human genomic DNA (200 ng), 0.5 µL Taq polymerase (5 U/µL, Boehringer Mannheim, Mannheim, Germany), 1 µL of 10 pmol of each primer, with the buffer recommended by the supplier (final concentration 1.5 mmol/L MgCl<sub>2</sub>). PCR was performed in a 96-well plate, using a programmable thermal cycler (Perkin-Elmer, Foster City, CA, USA). Amplification conditions for D11S97 were: 30 cycles of 94 °C for 30 s, 68 °C for 7 min after an initial denaturation at 94 °C for 7 min. The reactions were terminated at 4 °C after a final extension step at 68 °C for 7 min. As for the marker IL-4R1, the amplification conditions were: 30 cycles of 94 °C for 30 s, 57 °C for 45 s, and 72 °C for 40 s after an initial denaturation at 94 °C for 2 min. The reactions were terminated at 4 °C after a final extension step at 72 °C for 7 min. In the case of marker D14S50, the amplification conditions were: 29 cycles of 94 °C for 50 s, 58 °C for 50 s, and 72 °C for 50 s after an initial denaturation at 94 °C for 3 min. The reactions were terminated at 4 °C after a final extension step at 72 °C for 5 min. Amplified products were resolved on 1% agarose gels with ethidium bromide. Sizes of the alleles were determined by the electrophoresis of the products and size marker, and genotypes were independently assigned by two investigators.

#### Statistical analysis

Results were expressed as mean  $\pm$  SD unless otherwise indicated. Affected sib-pairs analysis was used to determine

linkage between skin reactivity to common aeroallergens and the gene markers of candidate genes. Sib-pairs were included in the linkage analyses only when marker genotypes were known. The significance of the deviation from the expected degree of sharing was assessed with the chi-square test. A *P*-value of 0.05 or less was regarded as significant.

Because intermediate phenotypes of asthma, such as total serum IgE levels and bronchial responsiveness to methacholine, are quantitative phenotypes which display a continuous distribution, a quantitative trait locus (QTL) sib-pair approach was used to determine linkage between the phenotypes and gene markers of candidate genes. The square difference between siblings for the quantitative traits was calculated, and analysis of variance (ANOVA) was employed to make comparisons between the three groups with neither, one or two identical alleles. A *P*-value of 0.05 or less was regarded as significant.

#### Results

One hundred and twenty-seven probands and their 130 siblings participated in the study. Total serum IgE level was also measured in 31 age-matched non-atopic non-asthmatic controls. Table 1 summarizes the clinical characteristics of the probands, siblings and controls. The mean value of log (total serum IgE, IU/mL) was  $2.36 \pm 0.6$  in probands,  $2.22 \pm 0.7$  in siblings and  $1.66 \pm 0.9$  in controls. With respect to skin responses to common aeroallergens, 68.0% of the probands and 48.7% of the siblings showed positive skin responses to one or more of the 10 common aeroallergens. The mean value of DRS (%/mg/mL) was  $10.9 \pm 12.6$  in probands and  $31.3 \pm 25.3$  in siblings.

In 128 sib-pairs whose genotypes of FcεRI-β were determined, 54 sib-pairs (41.8%) shared two D11S97 alleles, 58 (45.0%) shared one allele and 17 (13.2%) shared neither allele. In 125 sib-pairs whose genotypes of IL-4 cytokine gene cluster were determined, 58 sib-pairs (46.4%) shared two IL-4R1 alleles, 51 (40.8%) shared one

**Table 1.** Characteristics of study subjects

|                         | Probands<br>( <i>n</i> = 127) | Siblings<br>( <i>n</i> = 130) | Controls<br>( <i>n</i> = 31) |
|-------------------------|-------------------------------|-------------------------------|------------------------------|
| Male/female             | 93/34                         | 57/73                         | 20/11                        |
| Age, mean (range), year | 9.0 (4–15)                    | 9.8 (5–16)                    | 9.0 (5–15)                   |
| LogIgE*                 | $2.36 \pm 0.6$                | $2.22 \pm 0.7$                | $1.66 \pm 0.9$               |
| SPT                     | 68.0% (83/39)                 | 48.7% (57/60)                 | 0%                           |
| DRS*                    | $10.9 \pm 12.6$               | $31.3 \pm 25.3$               | ND                           |

LogIgE: log (total serum IgE, IU/mL); SPT: positive skin-prick test response (allergen/histamine ratio  $\geq 1.0$ ) to one or more common aeroallergens; DRS: slope of dose–response curve (%/mg/mL); ND: not done. \* Data are expressed as mean  $\pm$  SD.

**Table 2.** Affected sib-pair analysis between skin responses to common allergens and gene markers of candidate genes

|                   | 11q13 (D11S97)   |            | 5q31–33 (IL-4R1) |            | 14q11 (D14S50)   |            |
|-------------------|------------------|------------|------------------|------------|------------------|------------|
|                   | No. of sib-pairs | <i>P</i> * | No. of sib-pairs | <i>P</i> * | No. of sib-pairs | <i>P</i> * |
| Identical alleles |                  | 0.2        |                  | 0.01       |                  | 0.49       |
| Two               | 18               |            | 24               |            | 12               |            |
| One               | 22               |            | 18               |            | 27               |            |
| None              | 6                |            | 4                |            | 7                |            |

\*Chi-square test.

allele and 16 (12.8%) shared neither allele. In 121 sib-pairs whose genotypes of the T-cell receptor  $\alpha/\delta$  gene complex were determined, 39 sib-pairs (32.2%) shared two D14S50 alleles, 70 (57.9%) shared one allele and 12 (9.9%) shared neither allele.

Figure 1 shows the square difference of log (total serum IgE, IU/mL) between each proband and sibling, respectively. For the gene marker of Fc $\epsilon$ RI- $\beta$  (D11S97), no significant differences were found among the groups with two ( $n = 52$ ,  $0.70 \pm 0.29$ ), one ( $n = 47$ ,  $0.81 \pm 0.28$ ) or zero ( $n = 15$ ,  $0.80 \pm 0.29$ ) identical alleles. Also, for the gene marker of the IL-4 cytokine gene cluster (IL-4R1), no significant differences were found among the groups with two ( $n = 49$ ,  $0.72 \pm 0.31$ ), one ( $n = 51$ ,  $0.75 \pm 0.29$ ) or zero ( $n = 17$ ,  $0.81 \pm 0.31$ ) identical alleles, or for the gene marker of the T-cell receptor  $\alpha/\delta$  complex (D14S50) with two ( $n = 34$ ,  $0.75 \pm 0.38$ ), one ( $n = 66$ ,  $0.76 \pm 0.27$ ) or zero ( $n = 11$ ,  $0.81 \pm 0.28$ ) identical alleles.

Table 2 shows the results of the affected sib-pair analysis of skin responses to common aeroallergens as qualitative

traits, with the gene marker on the candidate genes. There were significant linkages between the positive skin responses to one or more aeroallergens and the marker IL-4R1 ( $P = 0.01$ ). However, there was no significant evidence for a linkage between the positive skin responses to allergens and the markers D11S97 and D14S50 ( $P = 0.2$  and  $P = 0.49$ , respectively).

Figure 2 shows the square difference of DRS /1,000 (%/mg/mL) between each proband and sibling, respectively. For IL-4R1, the square difference between sib-pairs with two identical alleles ( $n = 41$ ) was smaller than with one identical allele ( $n = 38$ ) or no identical allele ( $n = 13$ ), and

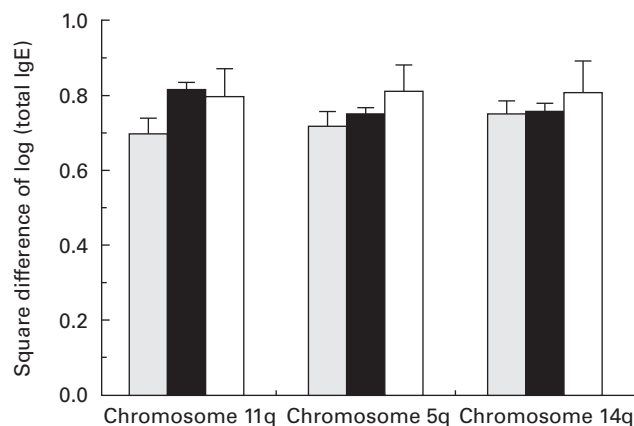


**Fig. 1.** Difference in total serum IgE levels between each proband and sibling according to the number of identical alleles. Data are expressed as mean  $\pm$  SD (IU/mL). (■), Two identical alleles; (■), one identical allele; (□), no identical alleles.

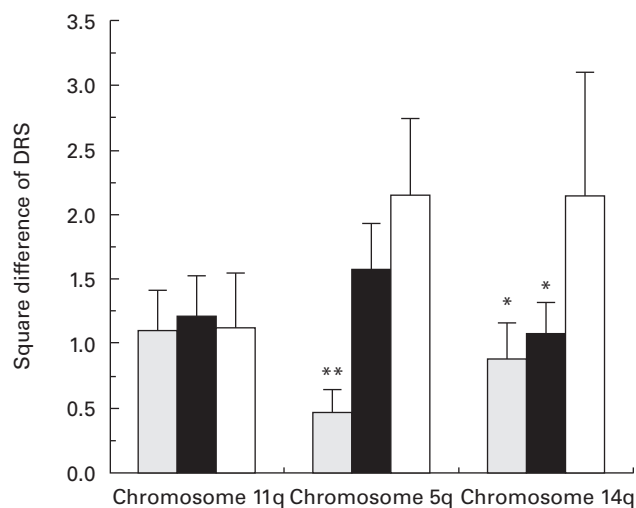


**Fig. 2.** Difference in bronchial responsiveness to methacholine between each proband and sibling according to the number of identical alleles. Data are expressed as mean  $\pm$  SD /1,000 (%/mg/mL). DRS: slope of dose–response curve, defined as the percentage fall in FEV<sub>1</sub> at the last dose divided by the log of the last dose (mg/mL) administered. \* $P < 0.05$  compared with the difference between sib-pairs with no identical allele. \*\* $P < 0.05$  compared with the difference between sib-pairs with one and no identical allele. (■), Two identical alleles; (■), one identical allele; (□), no identical alleles.

there was no difference between sib-pairs with one identical allele and with no identical allele ( $0.46 \pm 1.19$  vs  $1.58 \pm 2.29$  vs  $2.16 \pm 2.14$ ,  $P = 0.004$ ). For D14S50, the differences between sib-pairs with two identical alleles ( $n = 29$ ) and one identical allele ( $n = 52$ ) were smaller than with no identical alleles ( $n = 10$ ), but no significant difference was observed between sib-pairs with two and one identical allele ( $0.88 \pm 1.52$  vs  $1.07 \pm 1.86$  vs  $2.16 \pm 2.89$ ,  $P = 0.01$ ). For D11S97, no significant differences were observed among the groups with two ( $n = 36$ ), one ( $n = 43$ ) or no identical alleles ( $n = 14$ ) ( $1.09 \pm 1.93$  vs  $1.21 \pm 2.07$  vs  $1.12 \pm 1.62$ ).

## Discussion

In this study, it was found by the quantitative trait locus sib-pair approach that bronchial responsiveness to methacholine was linked to a gene marker on chromosome 5q31–q33 (IL-4 cytokine gene cluster) and on chromosome 14q11 (T-cell receptor  $\alpha/\delta$  gene complex), but not linked to a gene marker on 11q13 (Fc $\epsilon$ RI- $\beta$  gene). However, total serum IgE levels were not linked to the above candidate gene markers, although skin responses to common aeroallergens were linked to only the gene marker of the IL-4 cytokine gene cluster.

Asthma is a respiratory disease that is characterized by variable airway obstruction, airway inflammation and bronchial hyperresponsiveness (BHR) [22]. Significant familial aggregation of the phenotypes associated with asthma has been described in numerous studies [23–25]. Although the many studies published on the genetics of asthma support the concept that asthma has a significant familial predisposition, there is little or conflicting information on the mode of inheritance, and even less on the physical mapping and sequencing of the disease genes. While genes for Mendelian disorders such as cystic fibrosis have been localized and cloned, complex genetic disorders such as asthma and allergic diseases are far more complicated. In contrast to cystic fibrosis, a disease which has a specific history and can be identified by laboratory testing [26], asthma is a multifactorial disease with varied phenotypic expressions. Thus, defining the asthma phenotypes is a central issue with respect to detecting the genetic components of these conditions [27]. In this study, intermediate phenotypes associated with asthma were defined as total serum IgE, skin responses to common aeroallergens and BHR.

Parametric linkage analysis was considered inappropriate because of the incomplete penetration of the phenotypes of asthma and the unknown mode of transmission. In this study, linkage was analysed using the non-parametric method due to controversy about the mode of transmission of the intermediate phenotypes of asthma. When the

non-parametric sib-pair approach is applied to a large number of nuclear families, this method offers an advantage because they are probably more representative of the population than a small number of large families [28].

Cookson and co-workers [5] have suggested that a gene for atopy is located on chromosome 11q13, by observing evidence for linkage to markers located in this region. Other studies support this linkage, but there are investigators who have been unable to replicate these findings [9–13]. A previous study [21] revealed a significant linkage between IgE receptor-mediated histamine release from basophils and the gene marker of chromosome 11q13 with the same populations as this study. In this study, however, no significant linkage was found between intermediate phenotypes of asthma, such as total serum IgE levels, skin responses to common aeroallergens and bronchial responsiveness to methacholine, and the gene marker of chromosome 11q13. Meanwhile, Marsh and co-workers [14] reported a significant linkage between total serum IgE and IL-4 or a nearby gene, in chromosome 5q31.1 of Caucasian Amish families. Studies in Dutch families showed a linkage between total serum IgE levels and the markers on chromosome 5q.32 [29]. It was also reported that BHR is inherited with atopy in the same genomic region [16]. On the other hand, studies on Australian and British random populations [30] and on large atopic families in Minnesota, USA [31], failed to find a linkage between IgE and/or BHR and chromosome 5q31–q33. In this study on Korean nuclear families selected through a proband with asthmatic children, significant linkages were found between bronchial responsiveness to methacholine and skin responses to common allergens, and the gene marker on chromosome 5q31–q33, but no linkage was found between total serum IgE levels and this gene marker. These inconsistent findings may be attributable to differences in the populations (such as ethnicity) and ascertainment of the families studied, and/or to differences in the definitions of phenotypes or statistical methods. No definitive data on the cause(s) of these inconsistencies have been presented.

The chromosome 5q31–q33 region contains the genes for IL-3, IL-4, IL-5, IL-9, IL-13, GM-CSF and acidic fibroblast growth factor. IL-4 is a key cytokine involved in both the regulation of IgE synthesis, and in the provision of a signal for immunoglobulin switching [32]. Cytokines such as IL-3, IL-5 and GM-CSF are known to prolong eosinophil survival and to enhance their activity [33], which results in an increase in asthmatic airway inflammation. The genetic linkage between intermediate phenotypes of asthma, such as skin responses to aeroallergens and bronchial responsiveness, and the gene marker of the IL-4 cytokine gene cluster in the present study suggests that airway allergic inflammation caused by generalized IgE

responses to aeroallergens, which are determined by genetic factors on the chromosome 5q31–q33, plays an important role in the expression of bronchial responsiveness.

T-cell receptor (TCR) is usually made up of  $\alpha$ - and  $\beta$ -chains, although 5% of the receptors consist of  $\gamma$ - and  $\delta$ -chains. The  $\beta$ -chain locus is on chromosome 7, and the  $\alpha$ -chain locus is on chromosome 14. The  $\delta$ -chain genes are found within the  $\alpha$ -chain locus. An enormous potential for TCR variety follows from the presence of many variable (V) and junctional (J) segments within the TCR loci. However, the usage of the TCR V $\alpha$  and V $\beta$  segments by lymphocytes is not random, and may be under genetic control [17,34]. An Oxford group [18] studied the genetic linkage between IgE response and microsatellite marker from the TCR  $\alpha/\delta$  and TCR  $\beta$  regions. No linkage was detected between the IgE response and TCR- $\beta$ , but significant linkages were found between the IgE response and the TCR  $\alpha/\delta$  region. Unfortunately, this study has not been replicated independently by other investigators. In the present study, total serum IgE levels and skin responses to common allergens were not linked to the gene marker at the TCR- $\alpha/\delta$  locus, but bronchial responsiveness to methacholine was weakly linked to this gene marker. This finding suggests that generalized IgE responses to allergens may not be genetically determined by the TCR  $\alpha/\delta$  gene complex, but allergen-specific immune responses genetically determined by TCR may influence the expression of bronchial responsiveness.

In conclusion, the expression of bronchial responsiveness to methacholine may be influenced by genetic factors in chromosome regions 5q31–q33 and/or 14q11, but the genetic influence of the Fc $\epsilon$ RI- $\beta$  gene may be minimal in the expression of bronchial responsiveness in Korean nuclear families.

## Acknowledgements

This study was supported by a research grant from the 1996 National R & D Program (MOST), Republic of Korea.

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