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Research Article

The Relationship of the Gene Allele Apolipoprotein E with Height in Down Syndrome 21

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ABSTRACT

Background: Apolipoprotein E (APOE) is a form of polymorphic protein located on the long arm of chromosome 19 at position 13.2 (19q13.2), translated into three alleles of the gene, namely normal allele 3 and dysfunctional allele 2 and 4. Patients with Down's Syndrome Trisomy 21 may have different allele frequencies and combinations of APOE gene genotypes with controls, which can result in decreased cognitive function and inhibition of bone growth. This study aims to analyze the relationship of the Apolipoprotein E Gene Allele with the height of patients with Down Syndrome Trisomy 21. **Methods:** This research is a cross sectional study with a comparative cross-sectional study design. **Results:** The sample used was the result of DNA extraction of patients with Down's Syndrome Trisomy 21 as many as 33 samples and 33 controls stored in the Biomedical Laboratory, Faculty of Medicine, Andalas University, Padang, Indonesia. The next step is to examine the APOE gene polymorphisms using PCR and sequencing techniques. The results showed that there was a significant relationship between the APOE gene allele and height (p=0.009). **Conclusion:** This study concluded that there was a significant relationship between the APOE gene allele and the height of patients with Down Syndrome Trisomy 21.
Key words: Down's Syndrome, Apolipoprotein E, Height.

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INTRODUCTION

Down syndrome is a chromosomal abnormality that is often found in Indonesia and is a health problem that requires serious attention because people with Down syndrome have a very complex phenotype with various clinical problems. Down syndrome is the most common cause of mental retardation and is characterized by short stature and less height than the normal population.[1,2]

Down syndrome can occur in all ethnic groups and among all socioeconomic levels with an estimated incidence of between 1/1000 to 1/1,100 live births worldwide. Every year about 3,000 to 5,000 children are born with this chromosomal abnormality and it is believed there are about 250,000 families in the United States who are affected by Down's Syndrome.[3]

In China the overall incidence of Down's Syndrome was 2/1000 in 2012. The incidence of Down's Syndrome in Hong Kong was 0.3 per 1,000 population in 2010 which is much lower than other areas in China. The incidence in South Western Nigeria was 1 in 865 live births in 1982 and 1 in 500 live births in South Africa during the 20 years between 1974 and 1993. The incidence of Down's Syndrome in continental Europe is 1-3/1000 live births. The prevalence rate of Down's Syndrome in Australia is much lower than the worldwide rate of about 1 in 700 dues to the high termination rate in Australia. In 2010; the overall population rate of people with Down syndrome is around 1:1,700.[4]

Apolipoprotein E (APOE) is a form of polymorphic protein encoded by a gene located on chromosome 19q13.2. In the brain, APOE is a major protein, its production and accumulation are increased in central nervous system disorders and peripheral nerve injuries. APOE has the ability to interact with LDL receptors associated with proteins so that APOE has a central role in plasma lipoprotein metabolism and cholesterol homeostasis.[5]

The APOE gene generally has three alleles, namely the 2, 3, and 4 alleles. The APOE gene polymorphism is associated with an increase in the frequency of the 4 allele which results in inhibition of neuronal branching and growth, this can cause many neurons to degenerate. In addition, in the APOE gene polymorphism, there is an increase in the 2 allele which causes an increase and uncontrolled branching and growth of neurons Which results in hyperlipoproteinemia and leads to atherosclerosis.[6]

Short stature is one of the hallmarks of Down's syndrome sufferers, even from the time of the fetus, it was reported that fetuses with a femur length ratio <0.91 of normal showed a high risk of suffering from trisomy 21. Patients with Down's syndrome will also experience growth retardation where the growth rate is very high. Decreases during the growth period between the ages of 6 months to 3 years and in adolescence.[7,8]

People with Down's Syndrome also tend to have a lower height than the normal population. The average birth length of people with Down's Syndrome 1 Standard Deviation is lower than that of the general population, this indicates that children with Down's Syndrome have shown stunted growth during pregnancy. In addition, during the first three years of life people with Down's Syndrome experience slower growth than the general population, after which they will experience a relatively constant lag during the age interval of 3-12 years. During puberty, Down's syndrome sufferers will experience a growth rate that is higher than the growth rate during childhood, but still lags behind when compared to the normal

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population. While in adulthood, people with Down syndrome are more prone to suffer from osteoporosis than the normal population.[2,9]

APOE indirectly enhances bone formation by protecting osteoblasts from apoptosis via the p53 cascade mechanism and by promoting osteoblasts uptake of lipoprotein-produced vitamin K. Physiological levels of ApoE3 and ApoE4 stimulate osteoblast differentiation by down-regulating ERK1/2 phosphorylation. The ERK1/2 pathway inhibits the expression of asterisks and RUNX2, which are master transcription factors in osteoblast differentiation. Thus, ApoE increased bone formation not only indirectly, but also directly by stimulating osteoblastogenesis, and ApoE3 showed greater induction of osteoblastogenesis compared to ApoE4. Moreover, given that mature osteoblasts produce ApoE not only systemic circulating ApoE but also locally produced ApoE from osteoblasts may play an important role in preserving bone mass.[10]

Regarding the effect of ApoE on osteoclasts, both ApoE3 and ApoE4 inhibited osteoclastogenesis through suppression of the c-Fos, NFATc1 and NF-kB pathways. ApoE4 inhibits RANKL-induced osteoblast differentiation through regulation of c-Fos, NFATc1 and NF-kB. ApoE3 showed inhibition of c-Fos, DC-STAMP, and Cathepsin K gene expression compared to ApoE4. ApoE3 has a stronger effect on osteoblastogenesis and the expression of osteoblast-related genes compared with ApoE4, which may account for the different association between APOE polymorphisms and bone metabolism.[11]

MATERIAL AND METHODS

This research is a cross sectional study with a comparative cross-sectional study design. The sample used was the result of DNA extraction patients with Down's Syndrome Trisomy 21 as many as 33 samples and 33 controls stored in the Biomedical Laboratory, Faculty of Medicine, Andalas University, Padang, Indonesia. The next step is to examine the APOE gene polymorphisms using PCR and sequencing techniques.

PCR and electrophoresis

APOE PCR using primer pair APOE-F and Primer APOE-R was carried out with the initial denaturation temperature at 98°C for 5 minutes, then followed by 35 cycles of a series of processes consisting of further denaturation at 98°C for 30 seconds, annealing at a temperature of 58°C for 15 seconds, and elongation at 72°C for 50 seconds. The PCR process ended with the final elongation step at 72°C for 5 minutes.

Research ethics

This research has passed the ethical review and has received registration number No: 276/UN.16.2/KEP-FK/2021 from the Research Ethics Commission of the Faculty of Medicine, Andalas University.

RESULTS

Height data for both Down's syndrome and controls had abnormal data distribution with the Kolmogorov-Smirnov p-value on the normality test of 0.000. The data has been normalized, but the distribution of height data is still not normal. Therefore, to test the relationship of the APOE gene allele with height, the Mann – Whitney test was used. The p value shown in Table 1.

DISCUSSION

The APOE gene is located on the long arm (q) of chromosome 19 at position 13.2 (19q13.2). The APOE gene consists of four exons and three introns, totaling 3597 base pairs. In melanocyte cells APOE gene expression can be regulated by MITF.[12] APOE is a polymorphic form, which translates into three alleles of the gene: normal: 3 allele and dysfunctional: 2 allele and 4 allele. The alleles are distinguished

Table 1: The relationship of the APOE gene allele with height.

Variable	Allele Classification	n	Mean	p
Height	Dysfunctional alleles ε2 and ε4	26	25.85	0.009
	Normal allele ε3	40	38.48	
	Total	66		

From the table above, it can be concluded that "there is a significant relationship between the APOE gene allele and height", p = 0.009.

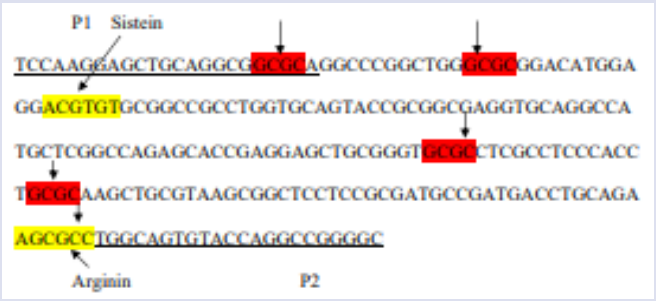

Figure 1: The sequence of the gene APOE allele ε3[14]

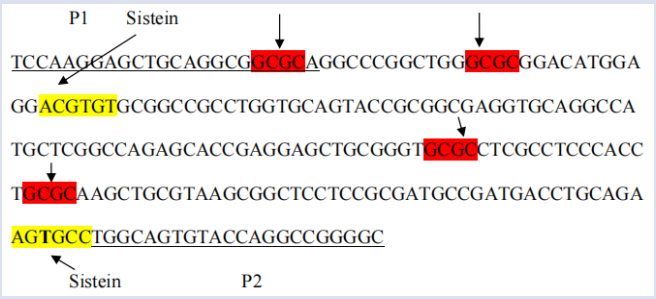

Figure 2: The sequence of the gene APOE allele ε2[14]

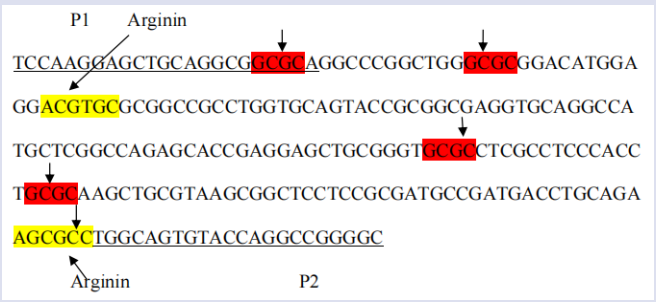

Figure 3: The sequence of the gene APOE allele ε4[14]

from one another only by amino acid substitution at positions 112 and 158. The 2 allele has cysteine at positions 112 and 158 in the receptor binding region, the 3 allele has cysteine at position 112 and arginine at position 158 while the 4 allele has arginine on both sides. APOE gene polymorphism has a strong effect on the level of allele production, high concentration of APOE indicates increased production of 4 allele and low concentration of APOE is associated with production of 2 allele. So, it can be concluded that if an individual has a high level of 2, then the level of VLDLs in charge of transporting excess cholesterol from the blood decreases.[13] The APOE gene sequence of the 3 allele is shown as follows.[14]

The 3 allele is the predominant isoform because the 3 allele is found in the majority of the population, which is around 70–80%. The 4 allele is found in about 10-15% of the population, the 4 allele is associated

with an increase in serum total cholesterol and has a large contribution to coronary heart disease, and is a major risk factor for Alzheimer's disease. The 2 allele is found in about 5–10% of the population. The 2 allele has a protective effect against Alzheimer's disease and is associated with longer survival in Alzheimer's patients. In addition, the APOE gene has a genotype combination which is the result of a cross from the combination of 2, 3, 4 alleles. Humans have three combinations of homozygous APOE genotypes, namely 2/ε2, 3/ε3 and 4/ε4, three heterozygous combination sets, namely 3/ε2, 4/ε3 and 4/ε2. The 3/ε3 phenotype is the most common phenotype, which is about 50–70% of the entire population.[15]

Patients with Down's Syndrome have an average birth weight and length between 0.5-1.0 SD lower than the normal population. At the age of 3 years, 30% of children with Down syndrome have a body length less than the third percentile, 60% are between the third and tenth percentiles, and the remaining 10% are normal growth.[16]

The weight gain of children with Down's Syndrome is faster than the increase in their height so that they are often overweight at the age of 36 months. Slow growth and deficiency of gonadal hormones are the hallmarks of Down's Syndrome. The average maximum height gain is 8.5 cm per year for men and 7.3 cm per year for women. The mean age at peak growth spurt was 12.3 years for boys and 10.8 years for girls, lower than healthy children. Short stature is characteristic of most children with Down's Syndrome. The average height for most ages is around the second percentile of the general population.[17]

In Down's syndrome growth pattern, there is a decrease in growth rate from birth to adolescence, especially between the ages of 6 months and 3 years and at puberty. Children with Down's Syndrome reach their maximum height at a relatively young age, which is 16 years for boys and 15 years for girls.[18]

ApoE expression is regulated by RANKL during osteoclast differentiation. Induction of c-Fos and NFATc1 have been shown to be important for their ability to stimulate osteoclast differentiation at an early stage. ApoE overexpression suppresses the RANKL-dependent induction of c-Fos, NFATc1 and TRAP. Cell-cell fusion events occur in the conversion of pre-osteoclast trap-negative to TRAP-positive precursors. ApoE also inhibits induction by RANKL DC-STAMP and ATP6v0d2, genes associated with cell fusion during osteoclastogenesis. Collectively, these results suggest that ApoE regulates osteoclast differentiation at several levels.[19]

ApoE is a secreted molecule and a putative autocrine and paracrine factor. Consistent with ApoE gene overexpression data, exogenous recombinant ApoE significantly inhibited RANKL-mediated osteoclastogenesis. In contrast, knock-Down ApoE increased mRNA and protein levels of markers of osteoclast differentiation and enhanced osteoclastogenesis. This evidence suggests that ApoE has an inhibitory role in osteoclast differentiation.[20]

Activation of mapk and NF-kB pathways is a well-characterized feature of the RANK signal. ApoE did not alter p38, ERK, and JNK-stimulated RANKL activation. On the other hand, our results reveal that ApoE overexpression suppresses the signaling activity of the NF-kB pathway. ApoE-mimetic peptide COG112, containing amino acid residues 133–149 located in the ApoE holoprotein receptor-binding region, inhibits the NF-kB signaling pathway in murine colitis models or in colonic epithelial cells. Upon RANKL stimulation, IKK induces p65 phosphorylation at serine 536 and the subsequent translocation of p65 nuclei are key steps for NF-kB activation.[21]

ApoE has an inhibitory effect on the phosphorylation and core translocation of the p65 component of NF-kB. NF-kB regulates the expression of c-Fos and NFATc1 in RANKL-induced osteoclastogenesis. Thus, it is possible that ApoE could inhibit c-Fos and NFATc1 induction in osteoclasts by preventing NF-kB activation. ApoE might suppress osteoclast differentiation through inhibition of NF-B, a detailed mechanism for this effect of ApoE will be a topic of future study.[22]

NFATc1 induction by RANKL stimulation was inhibited by ApoE overexpression. ApoE knockout mice fed a high-fat diet showed increased aortic OSCAR expression associated with increased NFATc1 expression. Oscar was initially characterized as a co-stimulatory regulator of osteoclast and dendritic cell function and as a novel protein expressed by vascular endothelial cells and regulated by oxLDL in a calcium/NFAT-dependent manner. ApoE can reduce the binding of transcription factors to the NFATc1-binding regulatory site in the OSCAR promoter.[23]

In osteoclasts, NFATc1 also induces OSCAR expression by forming a complex with PU.1 and the microphthalmia-associated transcription factor. The suppression of NFATc1-mediated OSCAR expression by ApoE may account for the antiosteoclastogenic function of ApoE. In summary, ApoE efficiently prevents RANKL-induced osteoclast differentiation.[24]

CONCLUSION

This study concluded that there was a significant relationship between the APOE gene allele and the height of patients with Down Syndrome Trisomy 21.

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