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Analysis of Allele Frequency Distribution Apoliprotein E Gene in Patients With Down Syndrome Trisomy 21

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A B S T R A C T

Background: Down syndrome is a global health problem of particular concern because people with Down syndrome have a wide variety of clinical disorders and are one of the causes of mental retardation and serious physical growth disorders.Down syndrome can occur at all socioeconomic, ethnic and demographic levels. The incidence of Down Syndrome will increase as the mother's age increases at the time of pregnancy and the incidence varies in different populations: 1 in 319 to 1 in 1000 live births. Each year, an estimated 3,000 to 5,000 children are born with Down syndrome. The APOE gene is located in the long arm (q) of chromosome 19 at position 13.2 (19q13.2). The APOE gene consists of four exons and three introns, a total of 3597 base pairs. In melanocyte cells APOE gene expression can be regulated by MITF. APOE is a form of polymorphic, which translates into three gene alleles: normal: allele ε3 and dysfunctional: allele ε2 and allele ε4. The polymorphism of the APOE gene had a strong effect on the level of allele production, a high concentration of APOE indicated that the production of ε4 allele was increased and a low concentration of APOE was associated with the production of ε2 allele

Purpose: To Analyze of Allele Frequency Distribution Apoliprotein E Gene in Patients With Down Syndrome Trisomy 21

Methods: This research is an analitic observasional study with a comparative 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.

Results: Samples of Down syndrome patients had variations in the distribution of alleles, where alleles ε4 and ε2 were found even though the allele frequency ε3 was still the highest allele frequency. Meanwhile, in the samples representing the normal control population, ε3 and ε2 alleles were found and no ε4 allele was found. Although the allele is not associated with Down Syndrome, Down Syndrome sufferers have a 2.26 times greater risk for e2 and e4 alleles than e3.

Conclusion: There was a difference in the frequency distribution pattern of the APOE gene allele in patients with Down Trisomy Syndrome 21 compared to the control.

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INTRODUCTION

Down syndrome research has gone through a very long phase. In the 19th century, this condition was clinically described earlier by Jean Etienne Dominique Esquirol in 1838 and Edouard Seguin in 1844. But Down syndrome was first described clearly and in detail in 1866 by John Langdon Down, an English physician. Nearly a century later Down syndrome was identified as a trisomy of chromosome 21 by Dr. Jérôme Lejeune in 1959.[1]

Down syndrome is a set of symptoms caused by chromosomal abnormalities, namely the occurrence of partial or complete triplication of chromosome 21, where normal individuals only have a pair of chromosome 21. Down syndrome is a global health problem of particular concern because people with Down syndrome have a wide variety of clinical disorders and are one of the causes of mental retardation and serious physical growth disorders.[2]

Down syndrome can occur at all socioeconomic, ethnic and demographic levels. The incidence of Down Syndrome will increase as the mother's age increases at the time of pregnancy and the incidence varies in different populations: 1 in 319 to 1 in 1000 live births.[3] Each year, an estimated 3,000 to 5,000 children are born with Down syndrome.[4]

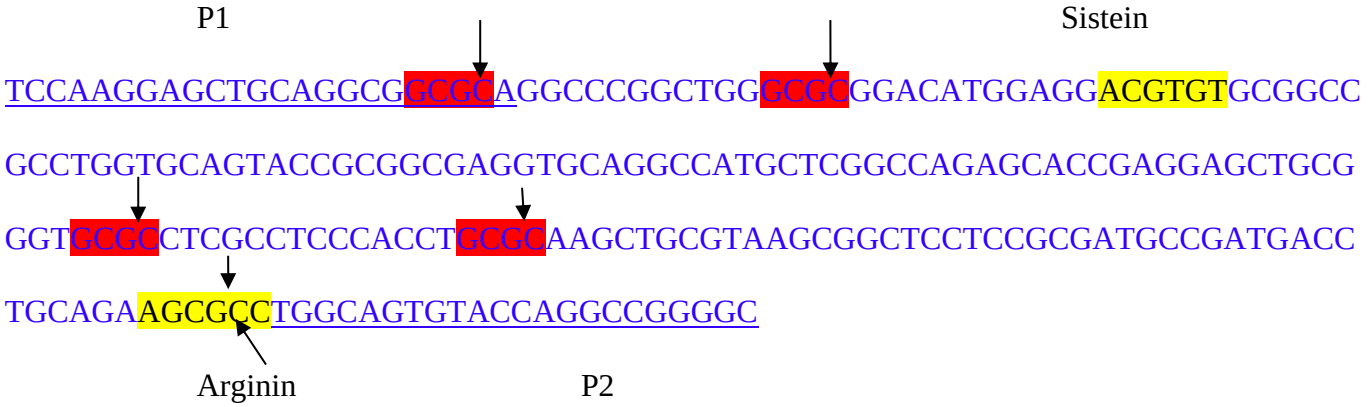
The incidence of Down Syndrome in continental Europe is 1-3 in 1000 live births. The prevalence rate of Down Syndrome in Australia is much lower than the worldwide prevalence rate where in 2010; the overall population rate of people with Down syndrome is about 1:1,700. The incident in Southwest Nigeria 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.In China, the overall incidence of Down Syndrome was 2/1000 in 2012. The incidence of Down Syndrome in Hong Kong was 0.3 per 1,000 population in 2010 which is much lower than any other in China.[5]

According to data from Basic Health Research (Riskesdas) conducted by the Ministry of Health (Kemenkes) in 2010, the prevalence of Down syndrome was 0.12 percent, this value increased to 0.13 percent in 2013. In other words, there are 0.13 percent of children aged 24-59 months in Indonesia who suffer from Resitation syndrome increased in the 2018 Riskesdas data to 0.21 percent.[6]

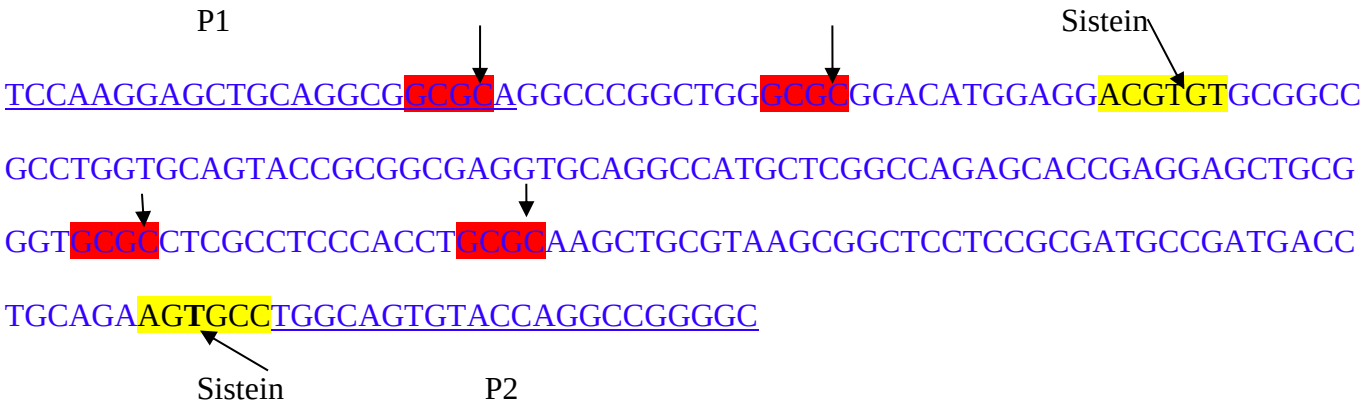
The APOE gene is located in the long arm (q) of chromosome 19 at position 13.2 (19q13.2). The APOE gene consists of four exons and three introns, a total of 3597 base pairs. In melanocyte cells APOE gene expression can be regulated by MITF.[7]

APOE is a form of polymorphic, which translates into three gene alleles: normal: allele ε3 and dysfunctional: allele ε2 and allele ε4. These alleles are distinguished from each other only by amino acid substitution at positions 112 and 158. The ε2 allele has cysteine at positions 112 and 158 in the receptor binding region, the ε3 allele has cysteine at the 112 position and arginine at the 158 position while the ε4 allele has arginine on both sides. The polymorphism of the APOE gene had a strong effect on the level of allele production, a high concentration of APOE indicated that the production of ε4 allele was increased and a low concentration of APOE was associated with the production of ε2 allele. So it can be concluded that if an individual has a high ε2 level, then the level of VLDLs that are responsible for transporting excess cholesterol from the blood decreases.[8]

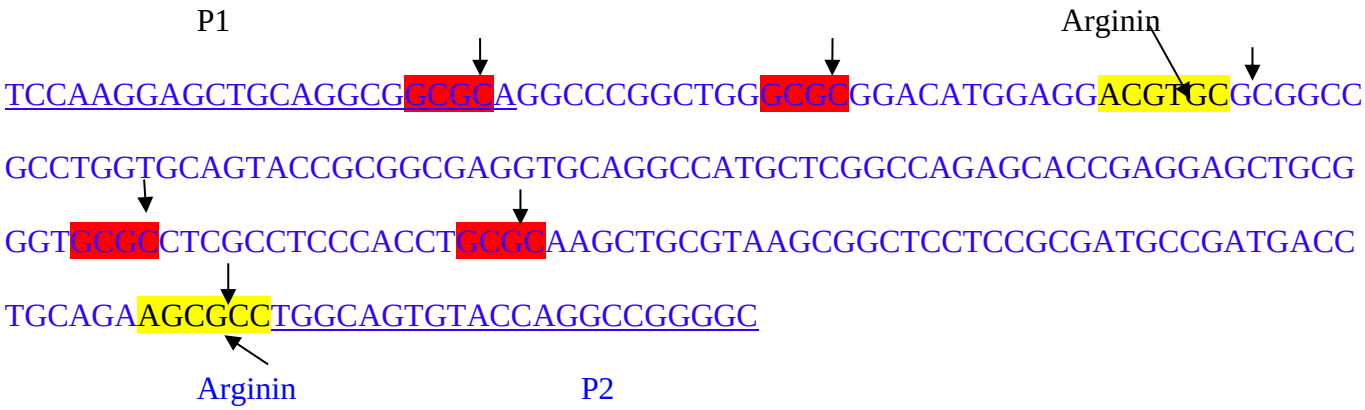
The sequence of the APOE gene of the ε3 allele is seen as follows [9]



The sequence of the APOE gene of the ε3 allele is seen as follows [9]



The sequence of the APOE gene of the ε3 allele is seen as follows [9]



APOE has three alleles namely ε2, ε3 and ε4 which are distinguished by a single amino acid substitution. The ε3 allele is the predominant isoform because this ε3 allele is found in the majority of the population, which is around 70–80%. Found in about 10–15% of the population, ε4 allele is associated with an increase in total serum cholesterol and has a large contribution to coronary heart disease, as well as being a major risk factor for Alzheimer's disease. The ε2 allele is found in about 5–10% of the population. The ε2 allele has a protective effect against Alzheimer's disease and is associated with longer survival in people with Alzheimer's. In addition, the APOE gene has a genotype combination which is the result of crossing from a combination of ε2, ε3, ε4 alleles. Humans have three combinations of APOE homozygous genotypes, namely ε2/ε2, ε3/ε3 and ε4/ε4, three sets of heterozygous combinations, namely ε3/ε2, ε4/ε3 and ε4/ε2. The ε3/ε3 phenotype is the most commonly found phenotype, which is about 50–70% of the entire population [10]

METHOD

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

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.

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RESULT

Samples of Down syndrome patients had variations in the distribution of alleles, where alleles ε4 and ε2 were found even though the allele frequency ε3 was still the highest allele frequency. Meanwhile, in the samples representing the normal control population, ε3 and ε2 alleles were found and no ε4 allele was found. The frequency distribution of APOE gene alleles as seen in table 1

Table 1 APOE gene allele frequency

Allele	Down syndrome		Control		Total		
	n	%	n	%	n	%	p
ε2	4	3	10	7,6	14	10,6	0,012
ε3	51	38,6	54	40,9	105	79,5	
ε4	11	8,3	2	1,5	13	9,8	
Total	66	50	66	50	132	100	

From the table above, it can be seen that "there is a difference between the distribution of APOE gene allele frequencies in Down syndrome patients and controls", p=0.012.

To see the Odd Ratio value of the table above is not qualified, so it is simplified to a table of 2 x 2 by combining the alleles ε2 and ε4 into dysfunctional alleles and the allele ε3 into normal alleles, so that the result is obtained in table 2 where the OR value is 2.265

Tabel 2 APOE Gene Allele Ratio Odds

Allele	Down syndrome		Control		Total		p	OR (95% CI)
	n	%	n	%	n	%		
ε2 dan ε4	25	18,9	14	10,6	23	29,5	0.056	2,265
ε3	41	31,1	52	39,4	43	70,5		1,047-4,900
Total	66	50	66	50	132	100		

Although the allele is not associated with Down Syndrome, Down Syndrome sufferers have a 2.26 times greater risk for e2 and e4 alleles than e3.

DISCUSSION

Overall, there was a difference between the distribution of APOE gene allele frequencies in Down syndrome patients and controls. This can be seen in table 1 where the value of p=0.012 is obtained. Both the samples of Down Syndrome patients and the control sample had the highest frequencies of the ε3 allele compared to the frequencies of ε2 and ε4 alleles. This can be seen from table 1 which shows that in patients with Down Syndrome the frequency of the ε3 APOE gene is 51 samples (38.6%). Meanwhile, the control had the ε3 allele of 54 samples (40.9%).

The ε3 allele is a predominant isoform found in the majority of the population, which is around 70–80%. The ε3 allele is a normal allele and has not undergone amino acid substitution at positions 112 and 158. The ε3 allele in humans functions to maintain and maintain the growth of neurons, especially in the dorsalis ganglion, so that the ε3 allele in humans with β-Very Low Density Lipoprotein (β-VLDL) is protective against neurodegenerative events and behavioral disorders.[9]

To see the risk factors for Down syndrome having dysfunctional alleles, the OR value in table 2 is 2.265 which means that Down syndrome patients have a 2x greater risk of having dysfunctional alleles ε2 and ε4 compared to the control group that does not suffer from Down syndrome. More ε4 allele was found in samples of Down syndrome patients. This can be seen from table 2 which shows the frequency distribution of the APOE gene allele ε4 has a frequency of 11 samples (8.3%). Meanwhile, in the control group, ε4 allele was found in 2 samples (1.5%).

The ε4 allele is found in about 10–15% of the population. The ε4 allele is an APOE gene allele that has undergone amino acid substitution at position 112 which initially had a series of ACGTGT bases into ACGTGC, so that the amino acid that was originally cysteine at that position turned into arginine. Meanwhile, at the 158th position, the base series remains AGCGCC as in allele ε3. The increase in ε4 allele is very closely related to premature brain aging of people with Down syndrome which is also a triggering factor for the emergence of Alzheimer's disease. Almost all people with Down syndrome after the age of 35 have amylod plaques and neurofibril kinks in their brains as seen in people with Alzheimer's dementia. In addition, ε4 allele has a large contribution to the increase in total serum cholesterol and in coronary heart disease. This is because the ε4 allele which has a high content of β-VLDL can inhibit the branching and growth of neurons which then affects the stability of microtubules mediated by cell surface lipoprotein receptors, especially in the Low Density Lipoprotein (LDL) receptor pathway[11]

The carrier of the ε4 allele has higher plasma cholesterol, which is strongly associated with atherosclerosis and cardiovascular risk. Atherosclerosis in the microvessels around the mature follicles in the ovaries can cause an oxygen deficit in the follicle which, according to the hypothesis of microcirculation, can reduce the size of the spindle, resulting in chromosomal nondisjunction. This hypothesis would predict an increased incidence of Down syndrome in families with hypercholesterolonemia. An increase in the frequency of the e4 allele was observed in meiosis II errors in young mothers and was not observed in meiosis I errors.[12]

APOE is produced in most organs including the ovaries, where it may be involved in chromosomal segregation. Meiosis II spindle dysfunction can be explained both by the specific binding of the APOE isoform to microtubule-related proteins, as seen in AD, and by possible disruption of APOE with microtubule stability and function. This hypothesis would allow the prediction of a higher number of meiosis II errors in Down syndrome populations that have a higher frequency of the e4 allele. Many factors may be involved in non-chromosomal disjunction, and meiosis II errors occur in about 25% of cases of mother-related Down Syndrome.[12]

In addition, as seen in table 2 that distinguishes the distribution of APOE gene alleles between Down Syndrome patients and the normal population represented by the control is in Down syndrome patients ε2 allele has a frequency of 4 samples (3%). Meanwhile, in the control population, the ε2 allele had a frequency of 10 samples (7.6%).

The ε2 allele is found in about 5–10% of the population. The ε2 allele is an APOE gene allele that has undergone amino acid substitution at the 158th position which initially had a series of bases AGCGCC to AGTGCC, so that the amino acid that was originally arginine at that position turned into cysteine. Meanwhile, in the 112th position, the base series remains ACGTGT as in allele ε3. The ε2 allele can increase the branching and growth of neurons so that it has a protective effect against Alzheimer's disease and is associated with longer survival in Alzheimer's patients. However, individuals with ε2 allele are particularly susceptible to developing the genetic disorder hyperlipoproteinemia type III thereby increasing the risk for atherosclerosis.[10]

Knowledge of the Ɛ2 allele is comparatively rarer because the e2 allele is slower to accumulate. In some studies, the Ɛ2 allele is associated with a reduced risk of DA but it is difficult to determine whether Ɛ2 protects against cognitive decline especially in episodic memory.[13]

In a cohort study involving parents who were examined annually for five years, having one or more copies of the e2 allele was associated with the rate of episodic memory changes but not with changes in other cognitive systems. Episodic memory performance is slightly improved in those who have at least one e2 allele. In contrast, episodic memory decreased slightly in those with the e3/3 genotype and a sharper decline in those with at least one e4 allele. The results showed that the apoE Ɛ2 allele protected against episodic memory decline in the elderly.[14]

Progressive episodic memory loss is a hallmark of Alzheimer's disease. The e2 allele appears to have a relatively selective effect on episodic memory, consistent with the APOE genotype influencing the risk of Alzheimer's Disease primarily by multiplying or slowing down the biological processes that cause the disease rather than through other mechanisms. Several previous longitudinal studies have assessed the independent contribution of e2 and e4 alleles to altering cognitive function. The effect of e2 on cognitive decline is almost the same as that of e4, or slightly less, but in the opposite direction. These findings underline the limitations of binary APOE size that distinguish people with and without specific alleles and suggest that an ordinal approach to scaling the overall impact of apoE may be possible. The incidence of Alzheimer's Disease was reduced among those with the e2 allele but not significantly, probably due to limited statistical power and lack of effects of e2 on forms of cognition other than episodic memory.[14]

CONCLUSION

There was a difference in the frequency distribution pattern of the APOE gene allele in patients with Down Trisomy Syndrome 21 compared to the control.

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