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Zygosity Test of Twin Pairs Using 26 STR Loci in the Indonesian Population

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Abstract

Background: Twin research can play an important role in understanding the interplay between genes and environment. Classifying twins as monozygotic (identical) or dizygotic (fraternal) is an essential first step in conducting research that will yield valid and replicable findings. Identical twins (theoretically) share 100% of their genes, while fraternal twins share, on average, 50% of their genes.1 This study aims to determine zygosity in 12 twins with Non-Syndromic Cleft Lip and Palate (NSCLP) discordant, concordant, and non-NSCLP.

Material and Methods: Blood samples from 12 twin pairs, which include of 8 twins Non-syndromic Cleft Lip and Palate (NSCLP) discordant, 2 twins NSCLP concordant, and 2 twins non-cleft (normal). DNA extraction was using Promega Kit and quantified was using Nano drops. DNA amplification was by PCR with 26 loci, which was packaged in Powerplex Fusion 6C Kit. PCR products were sequenced using ABI3500 and the visualization can be seen using Genemapper TM ID-X 1.6 software.

Results: The results show that each pair of 11 twins have a similar alleles in 26 STR loci, which means they are monozygotic twins. Only 1 twin has a different alleles in 10 STR loci, which means they are dizygote.

Conclusion: Zygosity tests on MZ twins using STR loci has high similarity and sensitivity. This test would be more accurate by analyzing a higher number of STR loci.

Keywords: Monozygote; twins; STR; Loci.

Introduction

Zygosity test is usually used to identify the etiology of congenital disease. Monozygotic twins (MZ) or identical twins generated from a single egg cells, fertilized by one sperm cell, while dizygotic twins (DZ) generated from two different egg cells

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and fertilized by two different sperm cells. Dizygotic twins come from two zygotes and usually share half of their genome, while MZ share 100% of their genomic information, since they came from the same zygote. However, additional genetic component, such as epigenetic factors and somatic post-zygotic mutations, can explain their different expression in MZ. Another proof of MZ genetic differences has been reported in specific and clinical development. Twin study showed consistent genetic component in Non-Syndromic Cleft Lip and Palate (NSCLP) etiology, with higher compatibility in MZ (25-50%) compared to DZ (3-6%). Molecular analysis of MZ discordance has been tried to identify NSCLP genetic factors. De novo nonsense mutation in IRF6 was detected in twins that affected from discordance twins with clefting Van der Woude syndrome. However, another research using different technical approaches did not succeed in identifying genetic distinction in NSCLP discordance twin's pair. This twin could become the source of phenotype expression variation, epigenetic, and postzygotic mutagenesis, which can be an alternative method to identify gene of a congenital abnormality. There was a hypothesis that de novo postzygotic mutation could cause discordance MZ twins with NSCLP, which in contrast genetically identical. To test this hypothesis, a research on two pairs of MZ twins with high density of SNP genotypes has been done which was in accordance with the analysis of de novo postzygotic copy number variation (CNV).[2] Identification of molecular genetic distinction between cleft MZ twins indicated that the usage of twins studies could be improved through heritability study to gen discovery.[3]

Besides observing a congenital abnormality and mutation, zygosity test has a function as a forensic identification process zygosity test using STR has very high discrimination. Thirteen STR loci have been found to 1998 and determined by the Global Forensic Group in collaboration with FBI (Federal Bureau of Investigation). To date, STR loci have been improved, which means the examined areas in the chromosome are higher, thus increasing the accuracy during identification process.[4-10]

Materials and Methods

Study participants

This zygosity test study has been approved by Health research ethics committee, Universitas Airlangga School of Medicine No. 290/EC/KEPK/FKUA/2020. The total sample were 12 Indonesian twin pairs, which consist of 8 twins Non-syndromic Cleft Lip and Palate (NSCLP) discordant, 2 twins NSCLP concordant, and 2 twins non-cleft (normal).

Specimens Collection

The sample was whole blood which was taken into EDTA tube and kept at 4°C.

DNA extraction and quantification.

Promega Maxwell^{TM} 16 purification Kit (promega) was used for DNA extraction according to the manual guide. The concentration and purity were checked by using Nanodrop at 260/280 absorbance. The extracted DNA was kept at -20°C.[11,12,13]

DNA Amplification

DNA was amplified using PCR with the powerplex fusion 6C kit according to the manufacturer protocol (Promega, 2018). The kit contained 26 STR locus including D3S1358, D1S1656, D2S441, D10S1248, D13S317, PENTA E, D16S539, D18S51, D2S1338, CSF1P0, PENTA D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, SE33, D22S1045, DYS391 , FGA, DYS576, DYS570.[14-16]

Sequence Process

Sequencing was done using ABI3500 machine and the result was analyzed using Genemapper^{TM}ID-x 1.6 software.[15]

Result

The result of 11 twins indicated that each pair has a similar allele on the 26 STR locuss. Figures 1 to 5 showed the allele from ZT7 samples using Genemapper^{TM}ID-x 1.6 software. Figures 6 to 10 displayed ZT8 sample which was the twins' a pair of ZT7. One pair has a different alleles in 10 loci, which means they dizygotic twin.

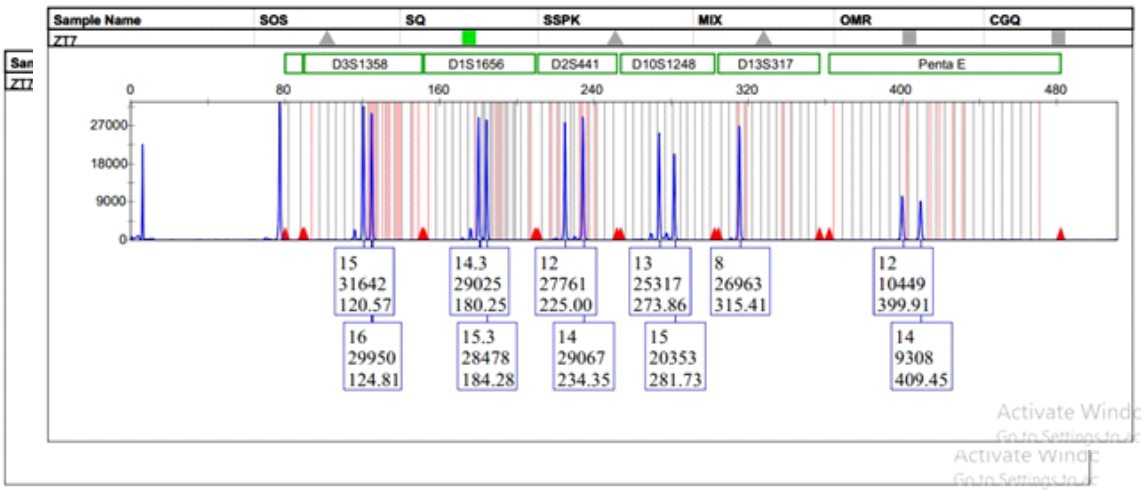

Figure 1: Electropherograms sample ZT7 with locus D3S1358, D1S1656, D2S441, D10S1248, D13S317, PENTA E

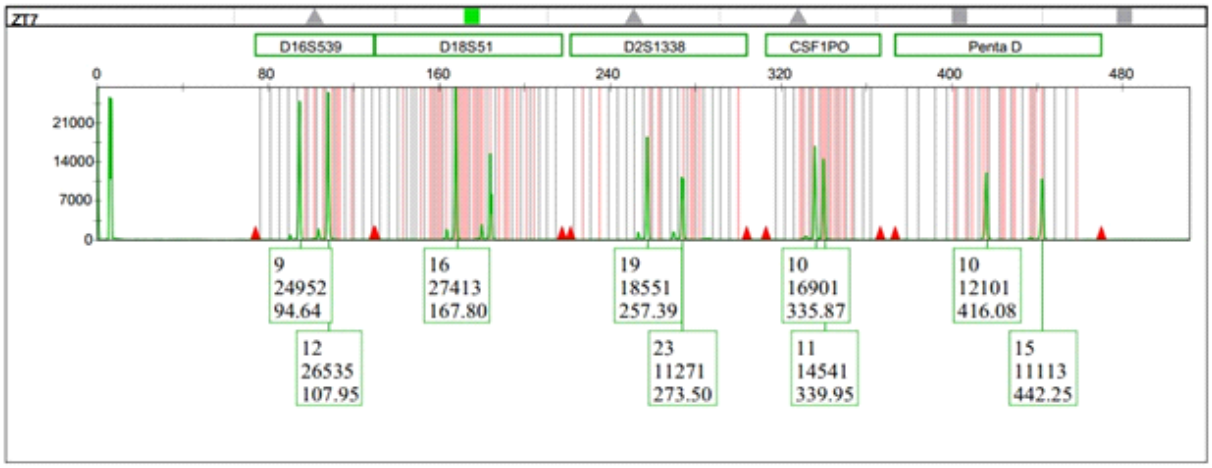

Figure 2: Electropherograms sample ZT7 with locus D16S539, D18S51, D2S1338, CSF1P0, PENTA D

Discussion

The previous study revealed that >80% of progeny from one of the twins would carry at least one germline mutation which would be detected in their father sperm. Researchers suggested to perform paternity test on the MZ twins with whole genome sequencing.[17] Furthermore, the research conducted by Bruder et. al. differed twins genetically but not specifically for forensic tests. To improve zygosity test accuracy, it is suggested to analyze DNA from Y chromosome to observe the distinction between closely related males.[18]

The usage of STR in zygosity test is so far the gold standard due to the high sensitivity and the wide range of loci detection. The zygosity test of umbilical cord from MZ twins examining 15 STR loci had high sensitivity results.[19] The study conducted by Dziennik et.found that the zygosity test can be performed using cell-free DNA (cfDNA) samples taken from mother's plasma.[20] However, this is different from one of the studies which reported that the method to differentiate an individual MZ twins cannot be observed by using STR, but employing mitochondrial DNA and finding a heterogenic SNP.[21] Another factor influencing MZ twin's distinction epigenetically was the source of DNA samples and biological characters. In addition, DNA methylation could also performed to observe the CpG island difference on MZ twins sample.[22]

Conclusion

Based on the results of this research, this supports that the zygosity tests on MZ twins using STR loci

has high similarity and sensitivity. This test would be more accurate by analyzing the higher number of STR loci.

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