TROPICAL GENETICS

Volume 4, No 1, May 2024
https://ojs.genetikawan-muda.com/index.php/tg

Original Research

First record of characterization C-KIT mutation in buccal swab DNA of piebaldism suspect balo from balo community, Pujananting South Sulawesi

Cut Muthiadin[1*], Tuty Arisuryanti[2], Isna Rasdianah Aziz[3], Hajrah[4], Khusnul Khatimah[5], Muhammad Tasri Hidayat[6]

[1]Department of Biology, Universitas Islam Negeri Alauddin Makassar, Indonesia
[2]tuty-arisuryanti@ugm.ac.id,Department of Biology, Gadjah Mada University, Indonesia
[3]Department of Biology, Universitas Islam Negeri Alauddin Makassar, Indonesia
[4]hajrah.sukri@uin-alauddin.ac.id,Department of Biology, Universitas Islam Negeri Alauddin Makassar, Indonesia
[5]60300116010@uin-alauddin.ac.id Department of Biology, Universitas Islam Negeri Alauddin Makassar, Indonesia
[6]tasrihidayat24@gmail.com,Department of Biology, Universitas Islam Negeri Alauddin Makassar, Indonesia
[*]**Corresponding author:** e-mail address: cutmuthiadin@uin-alauddin.ac.id

Article Info

Article history:
Received 22 Maret 2024
Accepted 20 April 2024
Available online 31 May 2024

Keywords:
KIT-C gene, Piebaldism, To Balo, Buccal Swab

How to cite:

Abstract

Piebaldism is a disorder in the area of the body caused by an autosomal genetic predominance of the development of melanocytes.The method used in this study is a qualitative method which aims to determine the variation of the KIT-c gene present in the To Balo mucosa samples.The results of this study indicate that the change is caused by a point mutation process where in patients with piabeldism the nucleotide bases of sequence 1391 and 1392 undergo changes, where the normal nucleotide base adenine (A) changes to cytosine (C) and cytosine (C) changes to adenine (A). So it can be concluded that the To Balo community in Bulo-Bulo village, Pujananting District, Barru Regency, South Sulawesi suffers from an autosomal dominant hereditary disease or piebaldism.

Copyright © 2024. The Authors. This is an open access article under the CC BY-NC-SA license (http://creativecommons.org/licenses/by-nc-sa/4.0/).

Introduction

In South Sulawesi, Barru Regency, there is a community that has its own characteristics called the *To Balo*. The local people call it *To Balo*, because "*To*" in Bugis language means Humans or People while "Balo" is white spots. *To Balo* are people who live in land, to be precise, in the Bulu Pao mountains. These mountains stretch long enough to cross the border of two districts, namely Pangkep and Barru Regencies, to be precise in the village of Bulo-Bulo, Pujananting District, Barru District (Longi, 2003) The *To Balo* is characterized by its striped skin color in almost all parts of its body. The people of *To Balo* believe that they got a curse from the God, but in the medical world it is a genetic disease that has almost the same characteristics as piebaldism.

Piebaldism is an autosomal dominant genetic disorder caused by loss of melanocytes in the skin and hair follicles because of mutations in the proto-oncogene kit gene. The presence of white patches on the hair and skin has been present since birth or is permanent. People affected by piebaldism will experience skin depigmentation on the hair and forehead that has a white forelock shape, also

experience skin depigmentation found on the thighs to the ankles and arms (Biologi et al., 2018). Meanwhile according to (Yin et al., 2009) Piebaldism is an autosomal dominant skin disorder in which white patches can be seen on the skin and hair as well as on the forehead, chest, abdomen and extremities, this occurs due to incomplete melanocyte processes from birth. This disease occurs due to mutations in the KIT gene located on chromosome 4q12. This gene functions in encoding the cell surface transmembrane tyrosine kinase which is a cell growth receptor and is an important factor in melanoblast migration and proliferase. Mutations of the KIT gene have been identified in about 40 mutations in piebaldism patients.

DNA is indispensable in gene manipulation research, DNA is the genetic material passed down from generation to generation. In a study of isolation, DNA can be isolated from various places of origin, in all tissues and body fluids there is DNA, so that genomic DNA can be isolated from all biological materials that have cell nuclei such as blood, cement, roots, hair, bones and others (Siswanto et al., 2017).

DNA analysis can use PCR techniques with blood samples, but this requires invasive procedures and causes discomfort for the individual being examined and is relatively expensive (Dea Emanuela & Dhanardhono, 2017). So there is another method, namely by using the buccal swab method with samples in the form of the oral mucosa found on the cheeks (Siswanto et al., 2017). This study aims to find the mutations in the KIT-C gene using the mucosal swab method.

Methods

This research is qualitative research with a descriptive approach which aims to provide an overview of the object of research which will then be drawn a conclusion, with the following stages:

Sampling

The sample used is a sample of the oral mucosa taken from a member of the To Balo community in Bulo-Bulo Village, Pujananting District, Barru Regency using a cotton bud by rubbing the inner cheek of the probandus starting from the back up towards the front with a one-way swab.

DNA extraction

Genomic DNA extraction was carried out following the DNA extraction protocol contained in The Gsync TM DNA Extraction Kit.

DNA amplification and electrophoresis

The isolation results were then amplified using a Biorad PCR machine in 30 ul of a solution consisting of 15 µl consisting of PCR Master Mix Nexpro, 2.5 µl DNA Template samples (100 ng/µl), 7.5 µl Water, 2.5 µl primer (10 pmol each forward and reverse primer). The primers used were KIT genes.

Forward: 5'TGTGAACATCATTCAAGGCG3' and
Reverse: 5'TGACTGCTAAAATGTGTGATATCCC3', which were obtained from exon 17 which was cut with the Web3 Primer program.

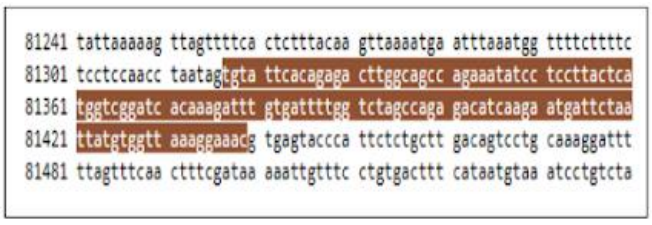

Figure 1. Exon 17 fragment in Human KIT Protein

DNA sequencing

PCR results from samples showing positive electrophoretic results were then sequenced using the sequencing service of 1stBASE Laboratories Sdn Bhd. Next, an analysis of the sequencing results was carried out by performing a BLAST of the nucleotide sequences from the sequencing results with the data base available on the site.www.ncbi.nlm.nih.gov which is used to look for the similarity of a nucleotide or protein sequence (query sequence) with a database sequence (subject sequence). Sequence alignment was performed using the Clustal W program.

Results and Discussion

DNA Extraction

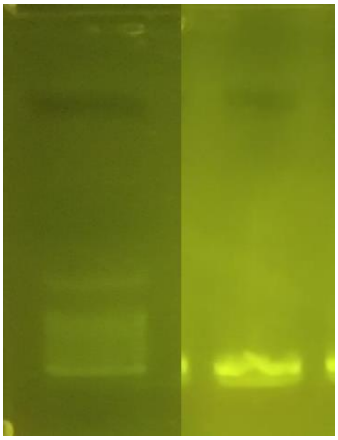

Figure 2. Visualization of DNA extraction results

DNA extraction analysis is the separation of the genome into smaller fragments. In the DNA extraction stage, there are several stages, namely the sample preparation stage using a buffer which aims to separate or break the leukocytes in the sample then to facilitate the lysis stage which has been set at high temperatures to accelerate lysis. The next stage is DNA binding or DNA enhancement, namely DNA collection from the addition of absolute ethanol, at this stage the addition of high ethanol cannot damage DNA. The resulting DNA is then transferred to the GD Column which will bind the DNA from the GD Column matrix while the contaminants will be suspended. The next stage is washing using a Wash Buffer which will remove the contaminants but the matrix remains bound to the DNA. The final stage, namely the DNA rehydration process aims to dilute the DNA with the addition of an Elution Buffer solution, (Biologi et al., 2018).

In this study the results of DNA extraction were taken from a person suffering from piebaldism in the To Balo community using the mucosal swab method in the mouth and from the electrophoresis results obtained good quality test results indicated by the appearance of DNA bands in the test sample (Figure 2). After the DNA extraction process was carried out, the amplification process was continued using PCR (Polymerase Chain Reaction).

PCR is a technique for amplifying DNA targets using a pair of primers so that they can detect DNA variations in genes (Supriyatna & Ukit, 2016). The amplification in this study used the primers FOR, 5'TGTGAACATCATTCAAGGCG3' and REV 5'TGACTGCTAAAATGTGTGATATCCC3' with 14 cycles of steps consisting of denaturation at 95°C for 35 seconds, annealing at 63°C for 45 seconds, and extension at

72°C for 75 seconds. And followed by 27 cycles consisting of denaturation at 95°C for 30 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 75 seconds. Then the post elongation process was carried out at 72ºC for 5 minutes. At this stage there are several cycles that are passed, each cycle has a different function. Denaturation serves to separate the template DNA chain, at the annealing stage there will be a primer attachment process with regions that are comlementary to the primer sequence.

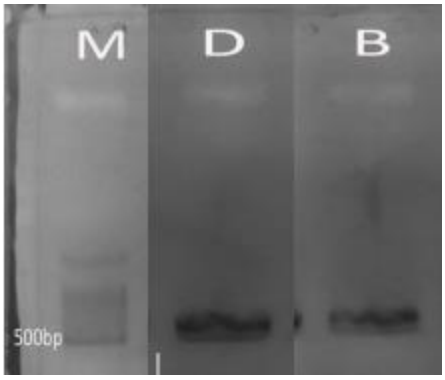

Figure 3. PCR electrophoresis results

From the results of electrophoretic observations (Figure 3), the same band was obtained which was clearly visible and stable at 1200 bp. Further analysis of the electrophoresis results was carried out using the sequencing method to search for and align the tested DNA to the database available at NCBI in order to obtain accurate data related to the sample DNA.

DNA sequencing

The results of the BLAST analysis obtained (Table 1), namely from the study sample, it was found that genes that have similarities with Homo sapiens KIT proto-oncogen receptor tyrosine kinase (KIT) with accession number NG007456.1 have a max score of 725 each. The total score, from max score and the observed total score can be seen that both of them have the same database sequence (Hilir et al., 2014). Max score indicates that the highest score for each segment is parallel to the sample sequence and database, while the total score shows the total score of all segments parallel to the sample sequence and database. According to Claverie (2012), the higher the similarity of the sample sequence to the database sequence and a score <50 indicates that the sample sequence has no similarities. From the similarity of the max score and total score, it can be seen that the sample sequence and database are similar (Prabhakar et al., 2012). It can be concluded that the research results obtained from the sample sequence and the database have similarities in the results of the max score and total score with a value of 725.

In the cover query the value obtained is 98% which indicates that there is a suitability of the length of the sample sequence and the database. From a high value indicates that the higher the level of homology of the sample with the database (Hilir et al., 2014). The value obtained from the E-value (Expectation value) is 0.0 which indicates that the sample sequence has homology with the database, because the lower the value obtained, the higher the level of homology (Claverie, 2012). Then the value of 100.00% obtained from the percentage of identity shows that the sample sequence and database data have similarities.

Alignment analysis of a nucleotide sequence aims to show that the location of the sequence changes, does not change, varies or develops so that it is different from its ancestor. This can be seen if

the nucleotide sequence of a protein from two organisms when compared is similar then it can be believed that the two organisms came from an ancestor. This alignment analysis is widely used in various applications, including identifying phylogenetic trees (Hilir et al., 2014).

Table 1. Sequencing results on the BLAST application for piabeldism sufferers and normal people

Description	Max. scores	total score	Cover queries	E value	Per. indents	Acc. Len	Accession
*Homo sapiens*KIT proto-oncogene receptor tyrosine kinase (KIT)	725	725	98%	0.0	100.00%	89695	NG00746.1
Human KIT protein and alternatively splicet KIT protein (KIT) gene	725	725	98%	0.0	100.00%	89603	U6383.1

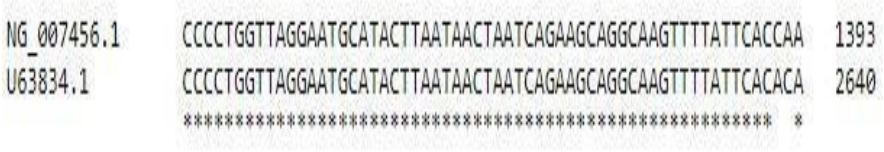

Figure 4. Nucleuotide alignment results with the W cluster program

The process of aligning nucleotide sequences with the application of W clusters (Figure 4) shows the result of mutations. At codons 1391 and 1392, the type of mutation is a point mutation. This point mutation is a mutation caused by a change in the chemical compound of one or more base pairs in a single gene which will then cause a change in phenotypic properties (Warmadewi, 2017). This mutation causes a change in the nucleotide base arrangement from adenine base (A) to cytosine (C) as well as a change in cytosine base (C) to adenine base (A) from this mutation it is found that this type of mutation is classified as a type of silent mutation or silent mutation, namely changes that occur the base in the gene that will cause a change in the genetic code but will not result in a change in the encoded amino acid.

In 1991 Lutz Giebel and R. Spritz conducted a study and found mutations in the KIT gene in exon 13 codon 664 which is the cause of piebaldism. Mutations occur in the KIT gene that cause piebaldism, and in 2004 Murakami et al discovered 6 new point mutations in the KIT gene in piebaldism patients (Sabran et al., 2018).

Conclusion

Mucosal swab is a method that can be used in DNA isolation. Using the mucosal swab method, it can be seen that there are mutations in the KIT-C gene. These mutations cause changes in the nucleotide arrangement at codons 1391 and 1392. Thus, mutation can be the cause of the appearance of white patches which characterizes piebaldism which is considered to be a genetic disease that afflicts the *To Balo* community.

Acknowledgments

Contributors who are not mentioned as authors should be acknowledged, and their particular contribution should be described. All sources of funding for the work must be acknowledged, both the research funder and the grant number (if applicable) should be given for each source of fund.

References

Biology, D., Unhas, F., Lolodatu, H., Johannes, E., & Agus and Arfan Sabran, R. (2018). *DNA Profile of Follicle Stimulating Hormone Receptor (Fshr) Gene In Women Acne By Using Pcr Technique And Dna Sequencing* (Vol. 3, Issue 1).

Claverie, JM and Notredame, C. (2003). *Bioinformatics for Dummies*. Indianapolis (USA): Wiley Publishing.

Dea Emanuela, D., & Dhanardhono, T. (2017). *Difference of DNA Quantity Extracted from Buccal Swab with Different Amount of Swab.* Tuntas Dhanardhono, Saebani JKD, 6(2), 443–450.

Hilir, I., Nugraha, F., Indriyani Roslim, D., Putri Ardilla, Y., & Mei, D. (2014). *Bioscientific* 6 (2) (2014) Partial Analysis of Ferritin2 Gene Sequences in Rice (Oryza sativa L.) Analysis of Partial Gene Sequence Ferritin2 on Rice Plants (Oryza sativa L.) Indragiri Hilir, Riau Info Article Abstract. https://doi.org/10.15294/biosaintifika.v6i2.3102

Longi. (2003). *Exercising in the Cultural Village.* Makassar: Erlangga Press.

Prabhakar, V., Shivendra, A., Rajni, C., & Saurabh, S. (2012). Fast dissolving tablets: An overview. *International Journal of Pharmaceutical Sciences Review and Research*, 16(1), 17–24.

Sabran, AA, Agus, R., & Hatta, M. (2018). GENETIC ASPECTS OF SKIN WARRANT OF THE TO BALO COMMUNITY GROUP IN SOUTH SULAWESI. *BIOMA : JOURNAL OF BIOLOGI MAKASSAR.* https://doi.org/10.20956/bioma.v3i1.5561

Siswanto, JE, Berlian, T., Putricahya, E., Panggalo, L. V, & Yuniani, L. (2017). Isolation of DNA from Peripheral Blood Samples and Buccal Swabs in Infants with ROP: Comparison of Test Results for Concentration and Purity Index. *Sari Pediatrics,* 18(4), 270. https://doi.org/10.14238/sp18.4.2016.270-7

Supriyatna, A., & Ukit, U. (2016). Screening and Isolation of Cellulolytic Bacteria from Gut of Black Soldier Flays Larvae (Hermetia illucens) Feeding with Rice Straw. *Bioscientific: Journal of Biology & Biology Education,* 8(3), 314. https://doi.org/10.15294/biosaintifika.v8i3.6762

Warmadewi, DA (2017). *Textbook of Genetic Mutations*. Denpasar: Faculty of Animal Husbandry, Udayana University.

Yin, XY, Ren, YQ, Yang, S., Xu, SX, Zhou, FS, Du, WH, Lin, D., Wang, PG, Zhang, SM, & Zhang, XJ (2009). A novel KIT missense mutation in one Chinese family with piebaldism. *Archives of Dermatological Research*, 301(5), 387–389.