Cholanaickan tribes are prone to sickle cell disease in near future

Sickle cell disease (SCD) is an autosomal recessive genetic blood disorder due to single nucleotide mutation (GAG > GTG) in the beta globin gene. In SCD patients, the red blood cells (RBCs) become sickle-shaped in the deoxygenated state. The sickle haemoglobin (Hbs) has an amino acid substitution at the sixth position of the \( \beta \)-globin chain (p.Glu6Val). Among the wide spectrum of illnesses observed in sickle cell anaemia patients, who are homozygous for the mutant gene, the most important is chronic anaemia with an extremely low haemoglobin concentration and frequent episodes of sickle cell crisis. SCD was detected for the first time in India in 1952 in an Irula boy of Nilgiris, Tamil Nadu\(^1\) and then in eastern India\(^2\). Subsequently, it has been detected in many other regions of the country\(^3\). Incidences of sickle cell anaemia have been reported more among the tribal communities compared to scheduled castes or other classes. Of the 36 tribal communities in Kerala, the highest rate of SCD has been reported in the Irula tribe (14.2%), while it was only 4.1% in Mulakurumba, 3% in Paniya and 1.6% in Kattunaickans\(^4\). Cholanaickans, one of the particularly vulnerable tribal groups in Kerala, are a numerically small (124 numbers in 42 households), semi-nomadic hunter-gatherer community forming only 0.08% of the total tribal population in the state\(^3\). Consanguineous marriages occurring frequently among this demographically small and diminishing tribal community may augment the risk factor associated with genetic disorders. SCD had been reported previously among the Kattunaickans\(^6\), from whom the Cholanaickans are reported to have originated\(^7\). Till date, no published information is available regarding the genetic status of this community with respect to the sickle cell haemoglobin disorder.

In this study, the genetic status of SCD was investigated through restriction enzyme analysis and DNA sequencing of PCR-amplified beta globin gene. Blood samples were collected from 33 individuals of the Cholanaickan community as part of the SCD project of the Government of Kerala by a medical team of Kozhikode Medical College, Kerala. Solubility test was carried out in all the analysed individuals as the screening test. DNA was isolated from dried blood spot on filter paper disc purified by FTA agent (GE Healthcare, UK). Direct PCR was performed using beta globin-specific primers to amplify 376 bp beta globin gene fragment. The PCR-amplified beta globin fragment was subjected to Dde1 restriction enzyme digestion, as the presence of sickle cell mutation eliminates the Dde1 restriction site. Among the 33 analysed samples, 1 sample had restriction bands at three positions, viz. 376, 201 and 175 bp respectively (Figure 1), indicating the presence of a mutated allele leading to a heterozygous carrier state. The rest of the 32 samples were normal for the beta globin gene which produced 2 restricted bands of 201 and 175 bp. None of the samples was positive for the homozygous state of SCD, caused due to mutations in both the beta globin alleles resulting in an undigested single band at 376 bp. To confirm the presence of sickle cell mutation, the 376 bp PCR product of the heterozygous carrier was subjected to TA cloning using pTZ57R/T vector backbone (InsTA clone PCR cloning kit, ThermoFisher Scientific) and transformation with competent Escherichia coli (JM 109) strain in our laboratory. The recombinant plasmid was outsourced for Sanger sequencing (SciGenom, Cochin). DNA sequencing confirmed the presence of GAG > GTG (p.Glu6Val) mutation in the beta globin allele (GenBank accession no. MF 150546).

The study could detect one sickle cell carrier trait which is heterozygous for the mutant sickle globin gene. The sickle cell trait is not regarded as a disease state, because it is generally asymptomatic. However, it is important to be aware of the presence of carrier gene as it is more prone to complications at high altitudes or during exhaustive labour. As the sickle cell carrier detected in this community is an unmarried person aged 70 years, there is least chance for the transfer of this mutant beta globin allele to the next generation. However, inter-marriages among the Kattunaickans and the Cholanaickans is a common practice of late, which can lead to more incidences of SCD within this most vulnerable community in near future.

Unlike in the past, today healthcare facilities are reaching the far-fledged settlements of Cholanaickans. Medical camps are being conducted on a monthly basis in these settlements by medical practitioners of the public health centres to take care of health issues. In view of the probable risk of occurrence of SCD within the community, preventive strategies based on creating awareness about the disease, genetic literacy and access to primary healthcare facilities are important. Further strengthening of the existing healthcare system through additional facilities for proper screening, diagnosis and management of SCD can contribute to enhanced lifespan, survival and overall welfare of this particularly vulnerable tribal community.

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**Figure 1.** Dde1-based RFLP of beta globin gene. Lanes A1–A12, normal; lane A13, Heterozygous carrier; lane AS, Heterozygous carrier (control sample); lane SS, Homozygous sickle diseased (control sample).
Candidate molecular markers for monoecy in dioecious tree spice nutmeg (*Myristica fragrans* Houttt.) and analysis of genetic variability in a core collection

Nutmeg (*Myristica fragrans* Houttt.) is a major tree spice valued for its diverse uses in flavouring and pharmaceutical industry. Dioecious sex nature is the greatest bottleneck in its cultivation, and to avoid the male plants commercial orchards, propagation is necessitated through budding and grafting\(^1\). Previous attempts to develop molecular markers linked with the female sex form were differentially successful\(^2-4\). For higher yields, planting should be done using monoecious plants or at the sex ratio of one male plant for 10 female plants. Thus, the development of a marker will enable identification of monoecy at seedling phase itself and hence the selection of seedlings for planting.

The southern part of India has considerable genetic variability in this cross-pollinated crop, especially for growth, sex forms, yield and traits of fruits, mace and nuts\(^5-7\). A systematic genetic diversity analysis will enable determination of population structure\(^8\) and to develop conservation strategies. The molecular marker-based analysis of genetic variability and population structure is an approved strategy and in plants with little genomic information, random primer-based systems are more reliable\(^9\). Hence, in the present study we used random amplified polymorphic DNA (RAPD) marker system\(^10\). The study performed using *Myristica fragrans* core collection from

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Figure 1. Amplification pattern in select nutmeg accessions with RAPD primers – a, OPE15 and b, OPE16. Lanes M, Ladder (EcoRI/HindIII, 1000 bp); lane 1, Acc.1; lane 2, Acc.5; lane 3, Acc.8; lane 4, Acc.9; lane 5, Acc.11; lane 6, Acc.14; lane 7, Acc.18; lane 8, Acc.21; lane 9, Acc.23; lane 10, Acc.24; lane 11, Acc.30; lane 12, Acc.35; lane 13, Acc.36; lane 14, Acc.37; lane 15, Acc.38; lane 16, Acc(H).1 and lane 17, Acc(H).4.