

## GENETIC DIVERSITY OF HILL-BANDA TRIBAL POPULATION OF ODISHA WITH REFERENCE TO ADH AND ALDH2 GENE VARIANTS

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### ABSTRACT

Hill-Banda tribal population (also commonly known as Bondo, Bondo-Highlanders) is one particularly vulnerable tribal group of Odish settled in Banda Hill of Khairput Block of Malkanagiri district. The hills lie between 18<sup>o</sup>.15' to 18<sup>o</sup>.30' North latitude and 82<sup>o</sup>.15' to 82<sup>o</sup>.30' East longitudes. The entire area is a hilly and mountainous terrain covering about 130 square kilometres with altitudes approximately ranging from 1,500 feet to 3,700 feet from the mean sea level. Though this population is now isolated and endogamous in nature, still genetically they are not homogeneous in nature. Primarily this study was designed for studying the protective aspect of ADH and ALDH2\*2 gene variants against alcoholism, but researcher try to understand the genetic distance with other populations and genetic diversity of Hill-Banda tribal population with reference to ADH and ALDH2 gene variants. In this present study the alcohol drinking habit of hill-Banda tribe was studied and for knowing the status of alcoholism problem, modified Michigan Alcoholism Screening Test schedule was tested for 714 households of 1411 individuals (618 male + 793 female). Out of these, 110 unrelated individuals (18 years and above) blood samples were collected in 6ml K2EDTA vacutainer for DNA analysis taken with consent for the analysis of protecting nature of ADH and ALDH2\*2 gene. PCR standardised with MJ Research (PTC-200) gradient cyler PCR system and regular PCR activities done by ABI GeneAmp 9700 thermalcycler. PCR amplicons (70ng) were directly sequenced using the ABI Big-Dye Terminator Cycle Sequencing Kit. Extended products were purified by alcohol precipitation followed by washing with 70% alcohol. Purified products were analysed in ABI 3730 automated DNA analyser using 10% Hi-Dye Formamide. Sequencing results were checked by sequencing analysis v5.2. Genetic distance analysis and dendogram are made with the help of DISPAN software. From these analysis, it is observed that in ADH variant, hill-Banda population are close to the Gond and the Sahariya population, whereas ALDH2\*2 variant wise they are close to mongoloid groups.

### INTRODUCTION

Food and drink have been basic to our existence. In fact, food and drink convey many meanings, and help us

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in meeting a number of personal, social, economic, and cultural needs. Anthropologists have been known for their expertise in the study of societies, in which preparation and drinking of alcoholic beverages constitute an important and inalienable component of their socio-cultural traditions and



customs. Primarily this study was designed for studying against alcoholism, but researcher try to understand the genetic distance with other populations and genetic diversity of Hill-Banda tribal population with reference to ADH and ALDH2 gene variants.

Hill-Banda tribal population (also commonly known as Bondo, Bondo-Highlanders) is one particularly vulnerable tribal group of Odish settled in Banda Hill of Khairput Block of Malkanagiri district. The hills lie between 18<sup>0</sup>.15' to 18<sup>0</sup>.30' North latitude and 82<sup>0</sup>.15' to 82<sup>0</sup>.30' East longitudes. The entire area is a hilly and mountainous terrain covering about 130 square kilometres with altitudes approximately ranging from 1,500 feet to 3,700 feet from the mean sea level. In this paper the used synonyms name Hill-Banda, Bondo, and Bondo-Highlanders may treat as same population.

Hill-Banda speaks a dialect of Mundari group, which is affiliated to Austro-Asiatic language family. They are known among themselves as 'Remo' means "Man". The Hill-Banda are linguistically placed in the "South Munda" language group with the Bado-Gadaba and the Didayi as affiliating members. Hill-Banda exhibits a great difference in appearance, customs, and character from other groups belonging to the same language group. They have linguistic and cultural affinities with the neighbouring tribes namely the Gadaba, the Paranga, and the Didayi. The origin and affiliation of the Hill-Banda are contradictory. Some early ethnographers tended to regard the Hill-Banda as an offshoot of the Gadaba - a neighbouring but much larger tribe of similar racial and cultural affiliation. Henderson describes the Hill-Banda as one of the three tribes into which the Gadaba are divided [1]. Elwin (1950) [2] mentioned that "the Bondo-Highlanders could not be mistaken; the Mongoloid cast of countenance is very marked, ---- among the women rather than the men and among the least among the younger men". Furer-Haimendorf (1945) [3] opines that though the dialect of the Bondo and the Gadaba belong to one group, they are not mutually understandable. He says, "In spite of many divergences in material possessions, social customs and individual, religious beliefs and a certain difference in appearance accentuated by dress and ornaments, there seems to persist among Gadabas and Bondo-Highlanders a similar cultural atmosphere and we can hardly doubt that they are both representatives of ancient Austro-Asiatic culture."

## METHODOLOGY

In this present study the alcohol drinking habit of Hill-Banda tribe was studied and for knowing the status of alcoholism problem, modified Michigan Alcoholism Screening Test schedule was tested for 714 households of 1411 individuals (618 male + 793 female). Out of these, 110 unrelated individuals (18 years and above) blood samples were collected in 6ml K2EDTA vacutainer for DNA analysis taken with consent for the analysis of protecting nature of ADH and ALDH2\*2 gene. PCR

the protective aspect of ADH and ALDH2\*2 gene variants standardised with MJ Research (PTC-200) gradient cyler PCR system and regular PCR activities done by ABI GeneAmp 9700 thermalcycler. PCR amplicons (70ng) were directly sequenced using the ABI Big-Dye Terminator Cycle Sequencing Kit. Extended products were purified by alcohol precipitation followed by washing with 70% alcohol. Purified products were analysed in ABI 3730 automated DNA analyser using 10% Hi-Dye Formamide. Sequencing results were checked by sequencing analysis v5.2. Genetic distance analysis and dendrogram are made with the help of DISPAN software.

## RESULTS AND DISCUSSION

ADH1C\*349Ile is seen registering same kinds of protection against alcohol dependency in several populations of world especially observed among the Black and Oriental populations. The Hill-Banda are characterised by high incidence of ADH1C\*349Ile (A allele) (0.657) along with other caste and tribal populations of India (Table-1).

What is most glaring observation that emerged from the above result is that the Hill-Banda population is endowed with the presence of both protective alleles and normal alleles. The dendrogram based on genetic distances, which shown closer genetic affinity of the Hill-Banda with the Maria Gond in ADH gene cluster family, is corroborated by ethno historical evidence states that the Hill-Banda are originally from Baster, the adjoining Gond tribal area [4-6]. However, linguistically both speak different dialects: the Hill-Banda speak Mundari, where as the Maria Gond speak Dravidians.

Two probable explanations may be offered here with respect to occurrence of ADH protective allele. Firstly, the gene might have come from the ancestral Gond population through movement of population in recent past. Secondly, the independent mutations in ADH gene have come about as an outcome of exhaustions genetic system facilitating protection against alcoholism.

No clear-cut differentiation is noticed between caste and tribal populations in the distribution of ADH SNP minor alleles, the Hill-Banda stand close to the Gond and Sahariya of Madhya Pradesh as compared to Korku, Patapu, Bhil. The Onge population is singularly not clustered with any one of the tribal populations of central India, showing its different origin (fig. 1). The standard genetic distances and respective standard errors of Standard genetic distances (x1000) calculated for seven Indian populations are presented in Table-2 and with population wise average heterozygosity and standard error presented in Table-3.

From the above two tables (Table 2&3), close genetic affinity of the Hill-Banda with the Gond, and the Sahariya is exhibited, despite their cultural and linguistic divergence.

Gene-geography studies revealed that Oriental populations of Mongoloid origins have higher frequencies



of ALDH2\*2 allele than the Caucasoid [7-13]. The Hill-ALDH2\*2 allele (0.12%) in their population, which is totally absent in the other Indian tribal populations so far studied. It is observed from the Table-4 that the average highest frequency of the gene variant ALDH2\*2 is reported from Japanese, followed by Chinese and Koreans and the gene is totally absent in Caucasians and other studied Indian tribal populations except the Hill-Banda. The Hill-Banda in heterozygous state for ALDH2\*2 are found protected against alcoholism, who have the distinction of perpetual alcohol drinkers. Two explanations may be offered to explain possible occurrence of the gene. First, like ADH gene variant, the ALDH2\*2 gene variant might have come in to the Hill Banda as a result of independent mutation, in the ancestral population in the remote part to serve as an inhibitory factor to alcoholic dependence. Secondly, the gene might have entered

Banda interestingly revealed the presence of into the Hill-Banda population as an offshoot of the ancestral mongoloid population of East-Asian origin during Neolithic period. Morphologically some of the Hill-Banda are found with presence epicanthic eye folds. When the distribution of the allele was made clan wise (Table-5), it is observed that the allele is comparatively greater among the individuals belonging to *Sisa clan*.

From the Table-5, it is seen that 50% of individuals with ALDH2\*2 allele are from *Sisa clan*. Even the *Kirsani clan* members' possessing the allele has inherited it from their parents (mothers) who belonged to *Sisa clan* prior to marriage. On enquiry, it was ascertained from the respondents of *Sisa clan* that they had gone to Manipur and Northeast provinces to work as tea garden labour and brought with them women from Northeast area as wives.

Population	Gene frequency of ADH1B*47 (ADH2*2)		Gene frequency of ADH1C*349(ADH3*349)		Reference
	A allele	G allele	A allele	G allele	
Onge	0.000	1.000	1.000	0.000	[5]
Pattapu	0.045	0.955	0.750	0.250	[5]
Gond	0.000	1.000	0.5714	0.4286	[5]
Korku	0.000	1.000	0.5791	0.4209	[5]
Bhil	0.000	1.000	0.6250	0.3750	[5]
Sahariya	0.020	0.980	0.5918	0.4082	[5]
Brahmin	0.000	1.000	0.556	0.444	[6]
Kshatriya	0.000	1.000	0.692	0.308	[6]
Vysya	0.000	1.000	0.583	0.417	[6]
Akuthota	0.000	1.000	0.697	0.321	[6]
Kamma	0.000	1.000	0.767	0.233	[6]
Kapu	0.000	1.000	0.667	0.333	[6]
Pokanati	0.000	1.000	0.603	0.397	[6]
Panta	0.000	1.000	0.541	0.459	[6]
Vanne	0.000	1.000	0.683	0.317	[6]
Baliya	0.000	1.000	0.79	0.21	[6]
Ekila	0.000	1.000	0.667	0.333	[6]
Kurava	0.000	1.000	0.645	0.355	[6]
Thogata	0.000	1.000	0.688	0.313	[6]
Yadava	0.000	1.000	0.683	0.317	[6]
Ediga	0.000	1.000	0.533	0.467	[6]
Gandla	0.000	1.000	0.583	0.417	[6]
Jangam	0.000	1.000	0.5	0.5	[6]
Devangapattur	0.000	1.000	0.559	0.441	[6]
Chakli	0.000	1.000	0.673	0.327	[6]
Mangali	0.000	1.000	0.577	0.423	[6]
Vadde	0.000	1.000	0.619	0.381	[6]
Madiga	0.000	1.000	0.672	0.328	[6]
Mala	0.000	1.000	0.663	0.337	[6]
Erukala	0.000	1.000	0.56	0.44	[6]
Sugali	0.000	1.000	0.703	0.297	[6]
Yanadi	0.000	1.000	0.652	0.348	[6]
Dudekula	0.000	1.000	0.7	0.3	[6]

**Table 1. Comparison of gene frequencies of ADH1B\*47His and ADH1C\*349Ile among some Indian populations**



Sheik	0.000	1.000	0.81	0.19	[6]
Hill-Banda	0.054	0.946	0.657	0.343	Present Study

**Table 2. Standard genetic distances and standard error of Standard genetic distances (x1000) among seven Indian populations, below and above diagonal respectively**

	Onge	Pattapu	Gond	Korku	Bhil	Sahariya	Hill-Banda
Onge		13.2	40.4	38.5	25.7	40.8	45.3
Pattapu	24.1		15	8	2.3	14.1	17.2
Gond	92	32		2.8	12.8	1.3	1.4
Korku	72.4	21.8	3.3		3.6	1.9	2.6
Bhil	37.7	3.2	21.8	5.8		8.6	14.1
Sahariya	82.4	23.2	-1.9	0.7	11.7		1.4
Hill-Banda	96.3	36.3	-0.4	6.4	27.3	1	

**Table 3. Population wise average heterozygosity and standard error**

	Average Heterozygosity	Standard error
Onge	0.260505	0.076096
Pattapu	0.330916	0.070034
Gond	0.325731	0.071249
Korku	0.351876	0.070783
Bhil	0.35639	0.071931
Sahariya	0.334286	0.06936
Hill-Banda	0.328595	0.062247

**Table 4. Distribution of ALDH2 genotype and gene frequency among various populations of Mongoloid and Caucasoid origin**

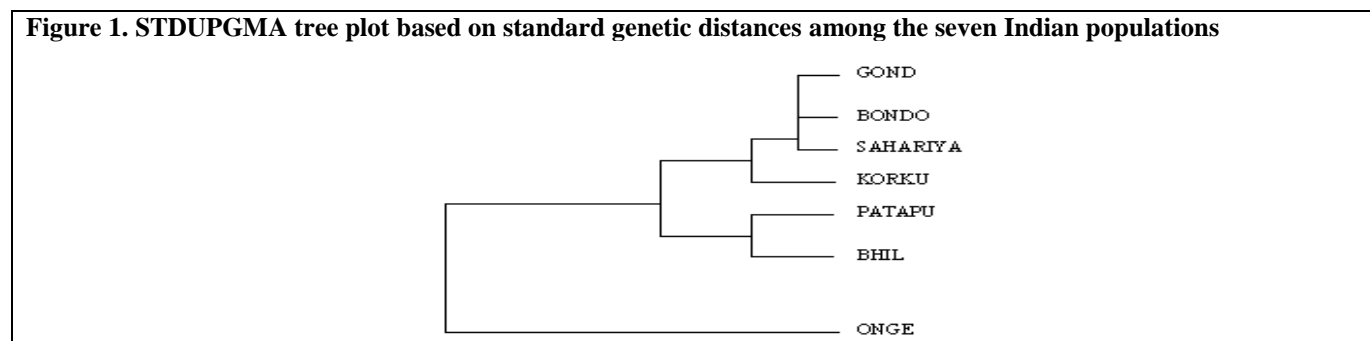
Subjects	n	Genotype frequency			Gene frequency		Reference
		ALDH2 (2*12/*1)	ALDH2 (2*1/2*2)	ALDH2 (2*2/2*2)	ALDH2*1	ALDH2*2	
Mongoloids							
Thais (Northeast)	124	113	11	0	0.956	0.044	[7]
Thais(North)	111	100	11	0	0.95	0.05	[8]
Fillipinos	86	85	1	0	0.994	0.006	[8]
Malays	73	68	5	0	0.966	0.034	[8]
Koreans	218	156	58	4	0.849	0.151	[8]
Chinese	132	92	38	2	0.841	0.159	[8]
Chinese	50	26	18	6	0.7	0.3	[9]
Taiwanese aborigine	58	56	2	0	0.98	0.02	[10]
Japanese	53	29	23	1	0.764	0.236	[8]
Japanese	58	32	21	5	0.73	0.27	[11]
Japanese	424	235	160	29	0.743	0.257	[12]
Japanese	129	70	48	11	0.729	0.271	[13]
Caucasoids							
Germans	193	193	0	0	1	0	[8]
Swedes	99	99	0	0	1	0	[8]
Hungarians	117	114	3	0	0.987	0.013	[8]
<b>INDIANS</b>	179	173	5	1	0.98	0.02	
Onge	40	40	0	0	1	0	[14]
Pattapu	95	95	0	0	1	0	[14]
Gond	47	47	0	0	1	0	[14]
Korku	133	133	0	0	1	0	[14]
Bhil	34	34	0	0	1	0	[14]



Sahariya	48	48	0	0	1	0	[14]
Hill-Banda	110	88	22	0	0.88	0.12	Present Study

**Table 5. Distribution of ALDH2\*2 (rs 671) protective allele among different clan groups of the Hill-Banda**

Clan Group	Male	Female	Total
Sisa	10 (45.5%)	1 (4.5%)	11 (50.0%)
Kirsani	1 (4.5%)	6 (27.4%)	7 (31.9%)
Batri	2 (9.1%)	1 (4.5%)	3 (13.6%)
Challan		1 (4.5%)	1 (4.5%)
Total	13 (59.1%)	9 (40.9%)	22 (100%)

**Figure 1. STDUPGMA tree plot based on standard genetic distances among the seven Indian populations**

## CONCLUSIONS

When the distribution of both ADH and ALDH2 alleles in the Hill-Banda population are taken together, one observes that protection of Hill-Banda individuals from adverse effect of alcoholism has been naturally enforced on the population due to dual action of functional variants of ADH and ALDH2\*2 alleles.

Though this population is now isolated and endogamous in nature, still genetically they are not homogeneous in nature. From these analysis, it is observed that in ADH variant, hill-Banda population are close to the Gond and the Sahariya population, whereas ALDH2\*2 variant wise they are close to mongoloid groups.

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