

Inbreeding as a cause for deafness: Dadhkai study

Sushil Razdan, Sunil Kumar Raina¹, Kamal K. Pandita², Shiveta Razdan³, Renu Nanda⁴,
Rajni Kaul⁵, Sandeep Dogra⁶

Department of Neurology, ²Internal Medicine and ³Surgery, Acharya Shri Chander College of Medical Sciences, Sidhra, Jammu,
¹Department of Community Medicine, Dr. Rajendra Prasad Government Medical College, Kangra, Himachal Pradesh,
⁴Department of Education, University of Jammu, Jammu,
⁵Gynecology and Obstetrics, and ⁶Microbiology, Govt. Medical College, Jammu, India

BACKGROUND: We report on the higher prevalence of deaf-mutes from a village in Jammu and Kashmir State of India.

MATERIALS AND METHODS: A cross-sectional study among 79 deaf mutes using pedigree analysis, audiometry, imaging and molecular analysis.

RESULTS: A high rate of hereditary deafness with 79 individuals diagnosed to be suffering from non-syndrome deafness in a total population of 2452 individuals residing in the village.

INTERPRETATION: Flourishing of intermarriages led to a population with high prevalence of deafness.

Key words: Dadhkai, deafness, inbreeding

Many deaf individuals and their families want to know the cause of their deafness and particularly whether it is genetic. Proven genetic diagnosis may allow for accurate genetic counseling and family planning, carrier testing for relatives, and may provide essential information about environmental risk factors (e.g., aminoglycoside antibiotics in those with the A1555G mtDNA mutation, or risk of progression of hearing loss in those with dilated vestibular aqueducts).^[2] In addition, precise molecular diagnosis may be important for planning and assessing success of therapies such as cochlear implant^[3] or future gene therapy.

Introduction

Epidemiological surveys of the deaf have consistently shown that about 50% of childhood deafness can be attributed to genetic causes, but all of the surveys have pointed out that the cause cannot be determined in a considerable proportion of individuals.^[1] Recent molecular work has demonstrated that in this group of 'cause unknown' deafness, genetic causes are common and extremely heterogeneous.

Materials and Methods

The study was conducted in 2,452 individuals from Dadhkai village in Gundoh Tehsil of Doda district of Jammu & Kashmir state of India.

Strategically located Jammu and Kashmir State constitutes the northern most extremity of India. Situated between 32.17 degree and 36.58 degree north latitude and 37.26 degree and 80.30 degree east longitude, the total area of the state is 22,22,236 sq. kms including 78,114 sq. Kms under the administration of Pakistan and 42,685 sq kms under that of China.

The state is bounded by Pakistan, Afghanistan and China from the West to the East. It is well connected with rest of the country by air, rail, and road [Figure 1].

The state has four geographical zones of:

1. Sub-mountain and semi-mountain plain known as

Access this article online	
Quick Response Code:	Website: www.ijhg.com
	DOI: 10.4103/0971-6866.96655

Address for correspondence: Dr. Sunil Kumar Raina, Department of Community Medicine, DR. RPGMC, Tanda, Kangra, Himachal Pradesh, India. E-mail: ojasrainasunil@yahoo.co.in

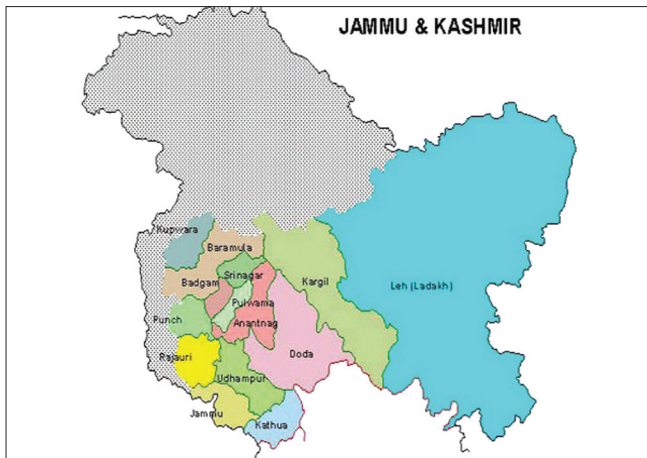


Figure 1: Map of Jammu and Kashmir

1. The Shivalak ranges
2. The Shivalak ranges
3. The high mountain zone constituting the Kashmir Valley, Pir Panchal range and its off-shoots including Doda, Poonch, and Rajouri districts and part of Kathua and Udhampur districts
4. The middle runs of the Indus River comprising Leh and Kargil.

The population (2001) of the State is 10,143,700. The state with its summer and a winter capital at Srinagar and Jammu, respectively, is divided into 20 districts. One fifth of the population in J&K resides in urban areas. 23.83% population has been recorded as urban in the state against the national average of 25.72%.

Doda district is the third largest in terms of area and falls between 32 degree-53' and 34 degree 21' north latitude and 75 degree-1' and 76 degree-47' east longitude.

Spread over in area of 11,691 sq.kms the district has a population of 5.25 lakhs (1991-census) with sex ratio 904 females per 1,000 males and the density of population 36 per sq. kms. The altitude varies from 8,000 ft. to 15,000 ft.

Often called "The Village of Silence," the Dadhkai village has a land area of less than 3 square miles and is located over a hillock connected to mainland by a foot bridge without a road link. Over 300 families with a total population of 2,452 live in 200 houses. The village was established in 1901 by Mir Ali who migrated to this place from somewhere in Jammu district.

The village is exclusively inhabited by descendants

of Mir Ali and his brother's descendents in 1990; the reported number of deaf and dumb in the village was 43. In 2007, it was reported that the number of deaf mutes had gone up to 79.

Often referred to as Latta's in local language, the deaf constitute a sizeable population of Dadhkai. Inheritance was defined with help of construction of a pedigree chart for whole population of 2,452 individuals living in Dadhkai village with the help of Sarpanch of the village and other village elders. The aim of the study was to investigate this high number of deaf mutes in village Dadhkai. The first step of the study involved the clinical examination, including general physical examination, of all deaf individuals. All the patients were examined by a clinical team comprising of neurologist, physician, gynecologist, and surgeon.

An effort was made to monitor the individual and family for known complications and associations of the deafness to rule out syndromic deafness. Although some syndromes may present in an obvious manner to the clinician, others require specialized investigation and a high index of suspicion in order to make the diagnosis.

For these reasons, it was important to examine fully every individual with hearing impairment. Special attention was paid to facial appearance, including the eyes, appearance of the external ears and neck, the skin (its pigmentation and its quality), and examination of the hands for unusual creases, extra or missing fingers, and appearance of the digits. A total of 17 patients were selected randomly from these 79 individuals for audiometry, neuroimaging, and molecular analysis (sequencing study). The patients consisted of 8 males and 9 females ranging in age from 5 years to 47 years with an average age of 19 years.

We performed pure tone audiometry and did CT studies of head and ear in same subgroup of 17 patients. For molecular analysis, DNA was extracted from peripheral blood leukocytes using a commercially available DNA extraction kit (QIAGEN, Hilden, Germany). Sequence analysis of entire gene GJB2 was performed.

The coding exon (Exon2) and flanking intronic regions of GJB2 gene were PCR amplified with forward primer 5'TTGGTGTTCAGCAAGA 3' and reverse primer 5'GGCCTACAGGGTTTCAAAT 3'. The PCR

primers used are forward primer:5'CTCATGGGGGCTCAAAGGAACTAGGAGATCGG3' and reverse primer 5'GGGGCTGGACCAACACACGTCCTTGGG3'. PCR was performed in a total of 25 µl reaction, containing 0.2 mM of each deoxynucleotide, 15 pmol of each forward and reverse primers, 1.0– 1.5 mM MgCl₂, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.75 U of Taq DNA polymerase, and 25 ng of genomic DNA.

PCR conditions were as follows: 35 cycles of denaturation at 95°C for 30 sec; annealing at 55°C or 57°C, depending on the primers for 30 sec; and extension at 72°C for 1 min. The first denaturation step and the last extension step were at 95°C for 2 min and 72°C for 10 min, respectively. Five microliters of the PCR products were separated and visualized on a 2% agarose gel. Fifteen microliters of this PCR product were then treated with 0.3U of shrimp alkaline phosphatase (USB) and 3U of exonuclease I (USB) at 37°C for 1 h, followed by incubation at 80°C for 15 min. This was diluted with an equal volume of dH₂O, and 6 µl was used for the final sequencing reaction. Sequencing reactions were performed in both directions on the PCR products in reactions containing 5 pmol of primer, 0.25 µl of ABI Big Dye Terminator v3.1 Cycle Sequencing Kit, and 1 µl of a dilution buffer (400 mM Tris-HCl, pH 9 and 10 mM gCl₂). Cycling conditions were 95°C for 2 min followed by 35 cycles of 94°C for 20 sec, 55°C for 20 sec, and 60°C for 4 min. Sequencing reaction products were ethanol precipitated, and the pellets were resuspended in 10 µl of formamide loading dye. An ABI 3130XL DNA sequencer was used to resolve the products, and data was analyzed by using ABI sequencing Analysis (v.5.0) and LASERGENE-SeqMan software. The 342 kb deletion of GJB6 gene (GJB6-D13S1830) was assayed by PCR using an internal primer that is located in the deleted segment of GJB6 as previously reported by del Castillo *et al.*, 2002.^[4]

The PCR products were electrophoresed on 2% agarose gel and visualized with ethidium bromide staining.

Results

A high rate of hereditary deafness was documented

in Dadhkai for last 30 years. The aim of the study was to diagnose non-syndromic hearing loss to exclude environmental causes, and to build a picture of the phenotype of the hearing loss, which would be valuable for directing audiometry, imaging, and molecular analysis.

Seventy-nine individuals were diagnosed to be suffering from deaf mutism [Table 1]. The prevalence of deaf mutism was highest in the younger age group of less than 15 years with 48 (61%) individuals belonging to this age group [Table 1]. As far as the sex specification was concerned females predominated in this group and overall [Table 1].

The village deaf heritage could be traced to one common ancestor and is thought to have originated in the Dadhkai only after immigration to this village by that ancestor. Mixed marriages between deaf and hearing spouses comprised 65% of all deaf marriages in the late 20th century. The last deaf person born into the village is 3 years of age, probably the earliest to pick up the deformity non-medically.

Pure tone audiometry in a randomly selected subgroup of 17 patients showed bilateral, severe to profound, sensorineural hearing loss. In the same subgroup computed tomography (CT) studies of head and ear were unremarkable for any structural lesion.

In patient ID No. 3 no amplification for GJB2 gene was observed [Table 2]. Hence DNA quality was checked by setting up PCR for internal control which showed amplification for internal control thus validating the assay run. Therefore, it is likely that the patient may have deletion in GJB2 gene, which explains PCR failure.

The general mutations are not detected in DM7 and DM17 cases, however SNPs or unknown genetic variations are observed [Table 2]. Both these variations are not described earlier and could be novel variations, which need to be confirmed separately.

Table 1: Demographic profile of patients with deaf mutism

Age	Male	Female
0-15 yrs	19	29
16-30 yrs	12	08
31-45 yrs	04	01
46-60 yrs	03	03
Total	38	41

Table 2: Molecular analysis of 17 deaf mutes

ID No.	Gender	Age	Mutations	SNPs	Unknown
DM1	M	22 yrs	G12V (Heterozygous)		L6L (CTG
DM2	M	47 yrs	Not detected		
DM3	F	18 yrs	No amplification		
DM4	M	6 yrs	Not detected		
DM5	M	13 yrs	R165W (heterozygous)	V1531	
DM6	F	13 yrs	Not Detected		
DM7	F	5 yrs	---	-----	Del T at nt 636
DM8	M	20 yrs	Not Detected		
DM9	F	14 yrs	L214P (Heterozygous)		
DM10	F	25 yrs	Not Detected		
DM11	M	27 yrs	Not Detected		
DM12	M	22 yrs	Not Detected		
DM13	F	20 yrs	Not Detected		
DM14	F	16 yrs	Not Detected		
DM15	F	13 yrs	Not Detected		
DM16	F	10 yrs	Not Detected		

Discussion

In population genetics, the founder effect is the loss of genetic variation that occurs when a new population is established by a very small number of individuals from a larger population. It was first fully outlined by Ernst Mayr in 1952,^[5] using existing theoretical work by those such as Sewall Wright.^[6] As a result of the loss of genetic variation, the new population may be distinctively different, both genetically and phenotypically, from the parent population from which it is derived. In extreme cases, the founder effect is thought to lead to the speciation and subsequent evolution of new species.

The founder effect is a special case of genetic drift. In addition to founder effects, the new population is often a very small population and so shows increased sensitivity

to genetic drift, an increase in inbreeding, and relatively low genetic variation. This can be observed in the limited gene pool of Dadhkai. This is also true of Iceland, Easter Islanders, and those native to Pitcairn Island. Another example is the legendarily high deaf population of Martha's Vineyard.^[7] In the late twentieth century, deaf-mutism became a subject of debate and social isolation for Dadhkai villagers. Dadhkai Sign Language (DSL) is commonly used by hearing residents as well as deaf ones till today. This has allowed the deaf residents to smoothly integrate into society. A highlight of Dadhkai is that its surroundings have not deaf-friendly as expected. Consequently, as intermarriage flourished, the village community increasingly resembles each other.

References

1. Morton NE. Genetic epidemiology of hearing impairment. *Ann N Y Acad Sci* 1991;630:16-31.
2. Glindzics MB. Hereditary deafness and phenotyping in humans. *Br Med Bull* 2002;63:73-94.
3. Green GE, Scott DA, McDonald JM, Teagle HF, Tomblin BJ, Spencer LJ, *et al.* Performance of cochlear implant recipients with GJB2 related deafness. *Am J Med Genet* 2002;109:167-70.
4. Del Castillo I, Villamar M, Moreno-Pelayo MA, del Castillo FJ, Alvarez A, Tellería D, *et al.* A deletion involving the connexin 30 gene in nonsyndromic hearing impairment. *N Engl J Med* 2002;346:243-9.
5. Provine WB. Ernst Mayr: Genetics and speciation. *Genetics* 2004;167:1041-6.
6. Charles Edward Banks. *The History of Martha's Vineyard*. Vol. 1. Boston: George H. Dean; 1911. p. 73.
7. Templeton AR. The theory of speciation via the founder principle. *Genetics* 1980;94:1011-38.

Cite this article as: Razdan S, Raina SK, Pandita KK, Razdan S, Nanda R, Kaul R, *et al.* Inbreeding as a cause for deafness: Dadhkai study. *Indian J Hum Genet* 2012;18:71-4.

Source of Support: Nil, **Conflict of Interest:** None declared.