NGS based expanded carrier screening for genetic disorders in North Indian population reveals unexpected results – a pilot study

Kanika Singh
Sir Ganga Ram Hospital
Sunita Bijarnia-Mahay (bijarnia@gmail.com)
Sir Ganga Ram Hospital https://orcid.org/0000-0002-5255-8212
Vedam Lakshmi Ramprasad
MedGenome labs ltd.
Ratna Dua Puri
Sir Ganga Ram Hospital
Sandhya Nair
MedGenome labs ltd.
Sheetal Sharda
MedGenome Labs Ltd.
Renu Saxena
Sir Ganga Ram Hospital
Sudha Kohli
Sir Ganga Ram Hospital
Kanwal Gujral
Sir Ganga Ram Hospital
Indrani Ganguli
Sir Ganga Ram Hospital
Ishwar Chander Verma
Sir Ganga Ram Hospital

Research article

Keywords: Genetic Carrier screening, Cystic fibrosis, Hearing loss, Glycogen storage disease type II, Asian Indians

Posted Date: May 6th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-23712/v1

Version of Record: A version of this preprint was published on November 2nd, 2020. See the published version at https://doi.org/10.1186/s12881-020-01153-4.

Abstract

Purpose

To study the carrier frequency and pathogenic variants of common genetic disorders in the north Indian population using next generation sequencing (NGS) in a pilot project of 200 individuals for 88 genes suspected to be common.

Methods

Screening for variants in 88 genes was carried out in 200 individuals, including 88 unrelated couples, by NGS technology, after pre-test counselling. The variants were classified as per ACMG criteria. Pathogenic and likely pathogenic variants were subjected to thorough literature-based curation in addition to the usual filters. Variants of unknown significance (VUS) were not reported. Individuals were counselled explaining the implications of the results, and cascade screening was advised when necessary.

Results

Of the 200 participants, 52 (26%) were found to be carrier of one or more disorders. Twelve individuals were identified to be carriers for congenital deafness, giving a carrier frequency of one in 17 for one of the four genes tested (*SLC26A4, GJB2, TMPRSS3* and *TMC1* in decreasing order). Nine individuals were observed to be carriers for cystic fibrosis, with a frequency of one in 22. Three individuals were detected to be carriers for Pompe disease (frequency one in 67). None of the 88 couples screened was found to be carriers for the same disorder.

Conclusion

Carrier rate for genetic deafness was the highest, followed by cystic fibrosis and Pompe disease. Cystic fibrosis was observed to be common in Indians, contrary to the previous reports in the literature. The pathogenic variants observed in many disorders were different from those observed in the West, emphasizing that population-based carrier screening panels need to be developed and whole gene sequencing strategy should be preferred over targeted genotyping of specific variants.

Introduction

In India and other resource-poor countries as infectious and nutritional disorders having been brought under control, genetic disorders cause a considerable degree of socio-economic burden. The need to reduce this burden is even greater now as the new treatments of genetic disorders are exorbitantly expensive and unaffordable. Carrier screening of pregnant women is the most cost-effective method to achieve this objective. Screening only those families who have a previously affected child is very

inefficient as majority of affected children are born to couples with no previous family history. Similarly screening only those who have an a priori increased risk of being a carrier based on their personal and family history or who are consanguineously married, or in couples who are opting for sperm or egg donation (Assisted Reproduction Technologies) would still be an inadequate strategy to identify the carriers of genetic disorders. It is best to screen all couples for the genetic disorders common in that population.

Carrier screening is performed to identify couples at risk of having a child affected with a serious genetic disorder, and provides them greater reproductive options if both partners are carrying pathogenic variants in the same gene for autosomal recessive disorders, or if the mother is carrier of an X-linked recessive condition, or either of the parents has an autosomal dominant disorder. World-wide carrier screening has evolved from an ancestry-based to pan-ethnic testing, and from single disorders (such as cystic fibrosis or α/β-thalassemia) by traditional Sanger sequencing to multiple disorders through Next Generation Sequencing (NGS). In the West, carrier screening was initially done by targeted genotyping because most of the pathogenic variants were known, and the results were easier to interpret. However, the technology has recently shifted to NGS of full genes to increase the yield. In resource-poor countries targeted sequencing is not suitable as most of the pathogenic variants in different genes have not been identified. Therefore, it was felt that in India carrier screening through sequencing the full gene(s) by NGS would be ideal, taking into consideration only variants that are pathogenic or likely pathogenic. Carrier screening studies in India have chiefly been carried out for β-thalassemia,[1] with isolated studies for p.Phe508del in cystic fibrosis[2] and p.Trp24Ter in *GJB2* related hearing loss.[3] No carrier screening based on NGS with sequencing of whole genes has been reported. The objectives of the present study were to determine the carrier frequency of variants in 88 genes expected to be common in Asian Indians and to identify the pathogenic or likely pathogenic variants.

Material And Methods

Subjects

This study was carried out at Sir Ganga Ram Hospital, a tertiary care multispecialty facility, over a period of 22 months from October 2016 through June 2019. Institutional ethical clearance was obtained prior to commencing the study (Ethical clearance number EC/08/ 16/1066). The molecular analysis was performed at MedGenome Laboratories Ltd, Bangalore. Adults with no significant history of a chronic medical disorder or familial genetic disorder, visiting the Medical Genetics and Obstetrics and/or Gynaecology out-patient clinic for various reasons unrelated to genetic disorders were enrolled, after pre-test counselling. Individuals known to be carriers of β-thalassemia were excluded from the study.

Sample size

Based on the published literature[4] and choosing disorders with a carrier frequency varying from 0.05 to 0.005 the required minimum sample size at a significance level of 5% and with a precision of 2% and

0.2% was calculated to be 457 and 4778, respectively. Due to financial constraints the plan was modified to carry out a pilot study with a smaller sample size of 200 to give an idea of what can be expected from a larger study.

Statistical analysis

Descriptive analysis was done, and outcome reported as proportion of total screened (1/n). Confidence interval was calculated by Wilson formula.

Gene panel and selection

Genes selected were those which cause high impact disorders that have significant effect on lifespan or reduce quality of life; or genes with moderate impact that do not reduce lifespan but impact quality of life; or disorders with significant socioeconomic burden for which couples would consider prenatal diagnosis. Published literature on the prevalence of genetic disorders in India such as the study by Ankala et al,[5] the carrier frequency of genetic disorders observed by Lazarin et al in South Asian population,[4] published papers on the topic, and the genetic register maintained about patients that have been evaluated at our centre were analysed. Eighty eight genes [72 autosomal recessive (AR), 7 X-Linked (XL), 9 Autosomal dominant(AD)/AR] were selected for testing (Table S1). A smaller number of disorders were aimed at to develop a short but efficient panel that could be offered at low cost. The study was planned in coherence with ACMG position statement on prenatal/preconception expanded carrier screening.[6]

Pre and Post test counselling

Prior to the testing, all individuals were counselled about the type of disorders being tested (mostly AR, with significant morbidity or mortality), the implications of being a carrier, the benefits of enrolling with their partner and voluntary nature of testing. Some common disorders were excluded either because these are not detected by NGS technology with great accuracy or the disorder can be screened easily by haematological tests. These included β-thalassemia, deletions in *SMN1* causing SMA (spinal muscular atrophy), FXS (Fragile X syndrome), DMD (Duchenne muscular dystrophy), and CAH (congenital adrenal hyperplasia)) and were offered separately. Relevant personal, family and ethnic data was recorded. All individuals were clinically examined to rule out any chronic disorder. The individuals were counselled about carrier status and its implications, cascade screening of family members and residual risks remaining after the results (unscreened disorders, chromosomal disorders and indels). The study methodology is shown in Fig. 1.

Molecular and Bioinformatic analysis

DNA was extracted from blood using Qiagen kit, and targeted genes were captured by a custom kit. The libraries were sequenced to mean coverage of > 80-100X on Illumina sequencing platform. The sequences obtained were aligned to human reference genome (GRCh37/hg19) using BWA program[7,8] and analysed using Picard and GATK version 3.6[9,10] Gene annotation of the variants was performed using VEP (Variant effect predictor) program against the Ensembl release 87 human gene model.[11]

Clinically relevant pathogenic variants were annotated using published variants in the literature and a set of diseases databases – ClinVar,[12] OMIM (Online mendelian inheritance in man),[13] GWAS catalogue (Genome wide association study in man),[14] HGMD (Human gene pathogenic variant database)[15] and SwissVar.[16]

Common variants were filtered based on allele frequency in 1000Genome Phase 3,[17] GnomAD,[18] dbSNP147,[19] 1000 Japanese Genome[20] and an inhouse database of 100,000 exomes in Indian subjects (Medgenome). Non-synonymous variants effect was calculated using multiple algorithms such as PolyPhen-2 (polymorphism phenotyping v2),[21] SIFT (Sorts intolerant from tolerant),[22] Mutationtaster2,[23] Mutation Assessor[24] and LRT (Likelihood ratio test).[25] Splicing prediction tools used were Mutationtaster2[23], BDGP (Berkeley drosophila genome project)[26] and HSF (human splicing finder)[27]. Various filters applied to variants included Variant quality (pass), 1000 genomes MAF (< 0.05), exonic and canonical splice site and read depth (> 10x). Variants remaining after applying the listed filters were subjected to ACMG classification. Only those variants fulfilling the criteria for pathogenic and likely pathogenic were shortlisted. Literature was reviewed for the filtered variants before assigning carrier status.

Validation of NGS results

All significant variants were manually inspected using IGV (integrative genomics viewer). It was observed that all variants had sequencing depth > 30. No strand biasness was observed. All variants were covered by good MAPQ reads. None of the variants were in highly repetitive regions. All significant variants were further validated using Sanger sequencing, and segregation analysis.

Results

Population demographics:

Among the 200 individuals enrolled, 61.5% belonged to the 31–40 years age group. Eighty eight percent had enrolled with their partner and none of them were consanguineously married. Maximum number of persons belonged to the northern states of India (Delhi (n = 74), Punjab (n = 50), Haryana (n = 44), Uttar Pradesh (n = 18), Himachal Pradesh (n = 5), Jammu and Kashmir(n = 5) and Rajasthan (n = 4) Majority of individuals identified themselves as Hindu Punjabi (20.5%). Some individuals (5%) could not be classified as either they were unsure of their caste and origin or were born of an inter-caste marriage.

Carrier frequency:

Of the 200 participants, 52 (26%) were found to be carrier of one or more disorders (Table 1). Congenital deafness as the most common disorder identified, with a carrier frequency of one in 17, for one of the four genes (*SLC26A4, GJB2, TMPRSS3* and *TMC1* in decreasing order). Cystic fibrosis was the second

most commonly identified disorder with a carrier frequency of one in 22. Three subjects were detected to be carriers for Pompe disease (frequency one in 67).

Table 1
Carrier frequency of the disorders screened

S.no	Disease name (OMIM no.)	N	%	1 in _	Wilson 95% Confidence Interval	
					Lower %	Upper %
	Total no. of carrier individuals	52	26	3.84	19.9	31.9
1	Cystic fibrosis - *CFTR* (219700)	9	4.5	22.22	2.4	8.3
2	Deafness - *SCL26A4* (274600)	5	2.5	40.0	0.78	5
3	Deafness - *GJB2* (220290)	3	1.5	66.67	0.5	4.3
4	Deafness - *TMPRSS3* (601072)	3	1.5	66.67	0.5	4.3
5	GSD type II - *GAA* (232300)	3	1.5	66.67	0.5	4.3
6	Methyl malonicaciduria mut A – *MMAA* (251100)	2	1	100	0.27	3.6
7	AR polycystic kidney – *PKHD1* (263200)	2	1	100	0.27	3.6
8	Galactosemia - *GALT* (230400)	2	1	100	0.27	3.6
9	Smith Lemli Opitz syndrome – *DHCR7* (270400)	2	1	100	0.27	3.6
10	Albinism type II - *OCA2* (203200)	2	1	100	0.27	3.6
11	Megalencephalic leukoencephalopathy with cysts - *MLC1* (604004)	2	1	100	0.27	3.6
12	Gaucher disease - *GBA* (230800)	2	1	100	0.27	3.6
13	Phenylketonuria – *PAH* (261600)	2	1	100	0.27	3.6
14	Epidermolysis bullosa (Junctional) - *LAMC2*(226700, 226650)	2	1	100	0.27	3.6
15	Niemann Pick disease type C1 – *NPC1* (257220)	1	0.5	200	0.088	2.77
16	Deafness - *TMC1* (600974)	1	0.5	200	0.088	2.77
17	Biotinidase deficiency - *BTD* (253260)	1	0.5	200	0.088	2.77
18	Medium chain acyl CoA deficiency -*ACADM* (201450)	1	0.5	200	0.088	2.77
19	Limb girdle muscle dystrophy type 2A -*CAPN3* (253600)	1	0.5	200	0.088	2.77
20	Congenital adrenal hyperplasia -*CYP21A2*(201910)	1	0.5	200	0.088	2.77
21	Primary hyperoxaluria type 1 - *AGXT* (259900)	1	0.5	200	0.088	2.77

S.no	Disease name (OMIM no.)	N	%	1 in _	Wilson 95% Confidence Interval	
					Lower %	Upper %
22	Argininosuccinic aciduria - *ASL* (207900)	1	0.5	200	0.088	2.77
23	Canavan disease - ASPA (271900)	1	0.5	200	0.088	2.77
24	Glutaric aciduria type 1 – *GCDH* (231670)	1	0.5	200	0.088	2.77
25	Krabbe disease - *GALC* (245200)	1	0.5	200	0.088	2.77
26	Congenital ichthyosis - *TGM1* (242300)	1	0.5	200	0.088	2.77
27	Metachromatic leukodystrophy – *ARSA* (250100)	1	0.5	200	0.088	2.77
28	Zellweger syndrome – *PEX1* (214100)	1	0.5	200	0.088	2.77
29	Epidermolysis bullosa dystrophica – *COL7A1* (226600)	1	0.5	200	0.088	2.77
30	Very long chain acyl CoA dehydrogenase deficiency - *ACADVL* (201475)	1	0.5	200	0.088	2.77

None of the 88 couples screened were found where both husband and wife were carriers for the same disorder. No woman was a carrier for the seven X-linked disorders included in the panel (Fabry disease, Ornithine transcarbamylase deficiency, Hemophilia A and B, Hunter syndrome, SCID (Severe combined immunodeficiency) and Adrenoleukodystrophy).

Of the 52 persons (26%) found to be carrier for at least one disorder, majority were carriers for one disorder (n = 47/200 = 23.5%) and five for two disorders (n = 5/200 = 2.5%). No individual was found to be a carrier for three or more disorders.

The average number of significant variants was 0.28 per individual, with a range of 0–2 significant variants and a mode of 1 variant per individual.

Significant variants

Significant variants (pathogenic or likely pathogenic) identified in 200 subjects were 57 (Table 1). Three splice site variants were novel (not reported in the literature or locus specific databases) and fulfilled ACMG criteria[28] for pathogenicity (Table 3). Out of the 47 distinct (every variant counted only once) pathogenic or likely pathogenic variants reported, majority were of the missense type (72.34%). Among the already reported variants, 29.5% (n = 13/47) have been described in patients belonging to the Indian subcontinent (India, Pakistan, Bangladesh) (Table 2&3). The individual variants are listed in Tables 2 & 3 and discussed in more detail later.

Table 2
Pathogenic and likely pathogenic variants observed in the three commonest disorders

Sno	Disorder and Gene	Transcript no.	Variant	ACMG criteria	No of individuals
1	Deafness, AR 4, with enlarged vestibular aqueduct, *SLC26A4*	ENST00000265715	c.1001G > T, p.Gly334Val	PS3 + PM2 + PP2 + PP3 + PP4 + PP5	2
			c.1226G > C, p.Arg409Pro#	PM2 + PM5 + PP2 + PP3 + PP5	1
			c.1468A > C, p.Ile490Leu#	PS1 + PM1 + PP2 + PP3 + PP5	1
			c.1003T > C, p.Phe335Leu	PS1 + PP2 + PP3 + PP5	1
2	Deafness, AR,1A, *GJB2*	ENST00000382844	c.231G > A, p.Trp77Ter#	PVS1 + PS3 + PM1 + PM4 + PP2 + PP3	2
			c.71G > A, p.Trp24Ter#	PVS1 + PS3 + PM1 + PM4 + PP2 + PP3	1
3	Deafness, AR, 8, TMPRSS3	ENST00000291532	c.413C > A, p.Ala138Glu	PM1 + PM2 + PP2 + PP3	1
			c.323-6G > A#	PS3 + PS4 + PM2 + PP3	2
4	Deafness AR,7, *TMC1*	ENST00000297784	c.1165C > T, p.Arg389Ter	PVS1 + PS3 + PM2 + PM4 + PP3	1
5	Cystic fibrosis, *CFTR*	ENST00000003084	c.223C > T, p.Arg75Ter	PVS1 + PS3 + PM2 + PM4 + PP2 + PP3	1

Abbreviations: PVS – pathogenic very strong, PS – pathogenic strong, PM– pathogenic moderate, PP – pathogenic supporting, # - described from the Indian subcontinent

Sno	Disorder and Gene	Transcript no.	Variant	ACMG criteria	No of individuals
			c.1646G > A, p.Ser549Asn[#]	PS3 + PM1 + PM2 + PM5 + PP2 + PP3 + PP5	1
			c.595C > T, p.His199Tyr	PS3 + PM1 + PM2 + PM5 + PP2 + PP3 + PP5	1
			c.3209G > A, p.Arg1070Gln	PS3 + PM1 + PM5 + PP2 + PP3 + PP5	1
			p.Phe508del	PS3 + PM1 + PM4 + PP2 + PP3	1
			c.4096A > T, p.Ile1366Phe	PM1 + PM2 + PP2 + PP3	1
			c.1472G > T, p.Cys491Phe	PM1 + PM2 + PP2 + PP3	1
			c.4009T > G, p.Phe1337Val	PM1 + PM2 + PP2 + PP3	1
			c.1859A > T, p.His620Leu	PM1 + PM2 + PM5 + PP2 + PP3	1
6	Glycogen storage disease II, *GAA*	ENST00000302262	c.1933G > A, p.Asp645Asn	PS4 + PM1 + PM2 + PM5 + PP2 + PP3 + PP5	3

Abbreviations: PVS – pathogenic very strong, PS – pathogenic strong, PM– pathogenic moderate, PP – pathogenic supporting, # - described from the Indian subcontinent

Table 3
Pathogenic and likely pathogenic variants

Sno	Disorder and Gene	Transcript	c.DNA position Protein change	ACMG criteria	No of individuals
1	Methylmalonic aciduria, cbla type, *MMAA*	ENST00000281317	c.433C > T p.Arg145Ter	PVS1 + PS3 + PM4 + PP2 + PP3	2
2	Epidermolysis bullosa dystrophica, AR, *COL7A1*	ENST00000328333	c.5287C > T p.Arg1763Ter	PVS1 + PM1 + PM4 + PP2	1
3	Galactosemia, *GALT*	ENST00000378842	c.563A > G p.Gln188Arg	PS3 + PM1 + PP2 + PP3 + PP4 + PP5	2
4	Smith-Lemli-Opitz syndrome, *DHCR7*	ENST00000355527	c.730G > A p.Gly244Arg	PS1 + PM2 + PP2 + PP3 + PP4 + PP5	1
5			c.862G > A p.Glu288Lys	PM2 + PP1 + PP2 + PP3 + PP4 + PP5	1
6	Biotinidase deficiency, *BTD*	ENST00000303498	c.469C > T p.Arg157Cys	PS3 + PM1 + PM2 + PM5 + PP2 + PP3	1
7	Medium chain ACYL-CoA dehydrogenase deficiency, *ACADM*	ENST00000420607	c.811G > G/A p.Gly271Arg	PS1 + PS3 + PP2 + PP3 + PP5	1

Abbreviations: PVS – pathogenic very strong, PS – pathogenic strong, PM– pathogenic moderate, PP – pathogenic supporting, * - Novel variant, # - described from the Indian subcontinent

Sno	Disorder and Gene	Transcript	c.DNA position Protein change	ACMG criteria	No of individuals
8	Junctional Epidermolysis bullosa, *LAMB3*	ENST00000391911	c.2138-2A > G*	PVS1 + PM2 + PP3	2
9	Metachromatic leukodystrophy, *ARSA*	ENST00000216124	c.1210 + 1G > T*	PVS1 + PM2 + PP3 + PP5	1
10	Peroxisome biogenesis disorder 1A (Zellweger), *PEX1*	ENST00000248633	c.2926 + 2T > C	PVS1 + PM2 + PP3 + PP5	1
11	Polycystic kidney disease, AR, *PKHD1*	ENST00000371117	c.8441-1G > C*	PVS1 + PM2 + PP3	1
12			c.1480C > T, p.Arg494Ter	PVS1 + PS3 + PM2 + PM4 + PP2	1
13	Albinism type 2, *OCA2*	ENST00000354638	c.1580T > G p.Leu527Arg#	PM2 + PP2 + PP3 + PP4 + PP5	2
14	Phenylketonuria, *PAH*	ENST00000553106	c.688G > A p.Val230Ile	PS4 + PM1 + PP2 + PP3 + PP5	2
15	Limb Girdle muscle dystrophy type 2A, *CAPN3*	ENST00000397163	c.1504A > G p.Ile502Val	PM1 + PM2 + PM5 + PP2 + PP3	1
16	Congenital adrenal hyperplasia, *CYP21A2*	ENST00000418967	c.373C > T p.Arg125Cys	PM2 + PP2 + PP3 + PP4 + PP5	1

Abbreviations: PVS – pathogenic very strong, PS – pathogenic strong, PM– pathogenic moderate, PP – pathogenic supporting, * - Novel variant, # - described from the Indian subcontinent

Sno	Disorder and Gene	Transcript	c.DNA position Protein change	ACMG criteria	No of individuals
17	Megalencephalic leukoencephalopathy with subcortical cysts, *MLC1*	ENST00000311597	c.65G > A p.Arg22Gln	PS3 + PP2 + PP3 + PP4	1
18			c.959C > A p.Thr320Lys	PS3 + PM2 + PP2 + PP3 + PP5	1
19	Primary hyperoxaluria type 1, *AGXT*	ENST00000307503	c.302T > C p.Leu101Pro#	PS3 + PM2 + PP2 + PP3	1
20	Gaucher disease, *GBA*	ENST00000327247	c.866G > C p.Gly289Ala#	PM2 + PP2 + PP3 + PP4 + PP5	1
21			c.1448T > C p.Leu483Pro#	PS3 + PM5 + PP2 + PP3 + PP5	1
22	Argininosuccinic aciduria, ASL	ENST00000304874	c.857A > G p.Gln286Arg	PS4 + PP2 + PP3 + PP5	1
23	Canavan disease, *ASPA*	ENST00000263080	c.902T > C p.Leu301Pro#	PM2 + PP2 + PP3 + PP4 + PP5	1
24	Glutaric acidemia type 1, *GCDH*	ENST00000222214	c.281G > A p.Arg94Gln#	PM2 + PM5 + PP2 + PP3 + PP4 + PP5	1

Abbreviations: PVS – pathogenic very strong, PS – pathogenic strong, PM– pathogenic moderate, PP – pathogenic supporting, * - Novel variant, # - described from the Indian subcontinent

Sno	Disorder and Gene	Transcript	c.DNA position Protein change	ACMG criteria	No of individuals
25	Krabbe disease, *GALC*	ENST00000261304	c.956A > G p.Tyr319Cys#	PM1 + PM2 + PM5 + PP2 + PP3	1
26	Congenital ichthyosis, AR 1, *TGM1*	ENST00000206765	c.550C > T p.Pro184Ser	PM2 + PM3 + PP2 + PP3 + PP5	1
27	Very Long chain fatty acid acyl-CoA dehydrogenase deficiency, *ACADVL*	ENST00000543245	c.1480T > C p.Phe494Leu	PM1 + PM2 + PP2 + PP3	1
28	Niemann Pick disease type C1, *NPC1*	ENST00000269228	c.3560C > T p.Ala1187Val	PM5 + PP2 + PP3 + PP4 + PP5	1
Abbreviations: PVS – pathogenic very strong, PS – pathogenic strong, PM– pathogenic moderate, PP – pathogenic supporting, * - Novel variant, # - described from the Indian subcontinent					

Some of the commonly reported pathogenic variants from affected patients from India were also identified: *GJB2* p.Trp24Ter and p.Trp77Ter,[3] *GBA* p.Leu483Pro,[28] *GAA* c.Asp645Asn,[29] *GALT* p.Gln188Arg,[30] *OCA2* p.Leu527Arg,[31] *AGXT* p.Leu101Pro[32] and *MMAA* p.Arg145Ter.[33]

Discussion

This study was designed to determine the carrier frequency of single gene disorders other than β-thalassemia for which the carrier frequency in the Indian population is already known, and screening is usually done through haematological tests. Recessive disorders are considered to be more prevalent in India, owing to the practice of endogamy and consanguinity. β-thalassemia has a carrier frequency of 1–17%, mean of about 3.3%.[1] SMA, Fragile X syndrome and DMD have been noted to be common in all populations including South Asians but are difficult to detect with NGS.[34–5] However, carrier frequency for other single gene recessive disorders is not known and significant differences in prevalence and pathogenic variants have been seen in different populations.[35–6]

CFTR pathogenic and likely pathogenic variants

There were nine significant variants identified in the *CFTR* gene in this cohort. Of these, only one case had the common p.Phe508del pathogenic variant i.e. 11% (n = 1/9).

Two pathogenic variants detected in *CFTR g*ene in this study have been observed before in our laboratory (p.Arg75Ter and p.Ser549Asn). The remaining six pathogenic variants have not been reported in Indians before (Table 2). The variants p.Ser549Asn, p.His199Tyr, p.Arg1070Gln have been described by multiple authors and functional studies have been carried out classifying them as pathogenic as per ACMG criteria. The other four variants p.Ile1366Phe, p.Cys491Phe, p.Phe1337Val, p.His620Leu have been documented associated with disease however lack adequate functional studies and cannot be classified as pathogenic, although they meet the criteria for likely pathogenic variants (Table 2).

Studies on the genetic profile of cystic fibrosis patients in India shows high variability and many rare and new variants have been observed, while only few pathogenic variants (p.Arg1162Ter, p.Met1Thr, c.1161delC, p.Ser549Asp and c.1525-1G > A) are reported more than once.[37–9] This suggests the lack of founder or common mutations in *CFTR* gene and thus the need for full gene sequencing of *CFTR* in suspected cases in the Indian population. In the present study except for p.Phe508del pathogenic variant, no other pathogenic variant was present in the ACMG panel of cystic fibrosis.[40] Mandal et al also suggested that because of heterogeneity in pathogenic variants, a single panel of pathogenic variants cannot be used for diagnosis or carrier testing of CF in India.[41]

CFTR c.3854C > T, p.Ala1285Val variant was identified in three individuals, which although has been reported in literature with the CBAVD (congenital bilateral absence of vas deferens)[42] is more likely to represent a common polymorphism due to its observance in high frequency in the NGS data in Indian population (0.5% minor allele frequency in South Asians in gnomAD exomes). This variant was classified as VUS and not included in the list of significant variants.

A high carrier frequency of cystic fibrosis was noted in this study in a non-Caucasian population. Till date most studies have been done either on a Caucasian population[4] or were targeted genotyping[2] and hence cannot be considered representative of the full gene carrier frequency. The pathogenic variants in cystic fibrosis can vary according to ethnic origin as noted in a recent study by Archibald et al. where an affected fetus had a pathogenic variant outside the listed ACMG panel for cystic fibrosis.[34] Lim et al. reported in ExAC database that the pathogenic variants in the CFTR gene in non-Europeans are different from those in people of European descent. They noted that none of the current genetic screening panels or existing *CFTR* pathogenic variant databases cover a majority of deleterious variants in any geographical region outside of Europe.[43] Among the nine significant variants identified in the *CFTR* gene in the present cohort, only one case had the common p.Phe508del pathogenic variant i.e. 11% (n = 1/9) of all significant *CFTR* variants identified. Kapoor and Kabra et al. studied cord blood samples of 955 new born babies and reported a p.Phe508del carrier frequency of one in 238 (0.42%).[2] This estimated the frequency of homozygous p.Phe508del as 1/228,006. However, this cannot be considered representative of the true prevalence of cystic fibrosis in India as it accounts for only one pathogenic variant. Comparison of p.Phe508del allele frequency with that reported from the West shows that Indians have a

low percentage of the p.Phe508del pathogenic variant frequency (19–44%).[43–5] The lack of hot spots suggests the need for full gene sequencing of *CFTR* in suspected cases in the Indian population. Cystic fibrosis was thought to be extremely rare in India. However, a growing number of publications in the last two decades have suggested a higher prevalence.[41,46] This indicates that CF is much more common in the Indian population with majority of cases being missed or undiagnosed. *CFTR* related pathogenic variants may be rarely recognized in Indians in view of the different phenotypes (including cystic fibrosis and congenital absence of vas deferens), variable clinical severity and lack of awareness regarding diagnostic modalities, and absence of new born screening.

GJB2 c.231G > A, p.Trp77Ter and c.71G > A, p.Trp24Ter

The pathogenic variants identified in GJB2 represent the common Indian pathogenic variants that have been previously reported. Ram Shankar et al studied the pathogenic variants in GJB2 gene in Indian patients with deafness and found p.Trp24Ter to be the most common pathogenic variant in India with a likely founder effect based on haplotype analysis.[3] In addition, they documented two other common pathogenic variants p.Trp77Ter and IVS1 + 1G > A.

They differ from the common pathogenic variants identified in the Western population (c.35delG)[47] and Japanese and Korean populations (c.235delC and p.Val37Ile).[48–9]

SLC26A4 related hearing loss

Hearing loss due to *SLC26A4* has been reported as third most common cause of hearing loss in a study done on pan-ethnic population.[50] In this study, two out of the four significant variants reported have been previously described in individuals belonging to Indian ethnic origin: p.Arg409Pro[51 – 2] and p.Ile490Leu.[53] Other variants found in our study include p.Gly334Val that has been described chiefly in people of Mediterranean origin[54] and the p.Phe335Leu is a common variant reported worldwide.[55]

Carrier screening and prenatal diagnosis for a disorder like hearing loss which impairs quality of life can have differing perceptions among families in different countries. The parental perceptions in Indian culture where resources are scarce towards congenital hearing loss have been pointed out by Nahar et al. previously.[56] While some families are interested in using the information to help in the management, planning and emotional adjustment to the birth of a child with deafness others opt for discontinuing an affected foetus especially if financial resources are scarce.

GBA c.1448T > C, and c.866G > C, p.Gly289Ala

This variant p.Gly289Ala and p.Leu483Pro were observed in one individual in the present cohort. Ankleshwari et al.[28] studied 33 Indian patients with Gaucher disease, and identified p.Leu483Pro as the

most common pathogenic variant 60.60% (n = 20/33). In addition, they reported p.Gly289Ala as a novel pathogenic variant. The variant most commonly observed in Western population (p.Asn370Ser) is observed less commonly in India.

GAA c.1933G > A, p.Asp645Asn variant

We observed three individuals to be carriers for this variant in the GAA gene. This variant was reported for the first time in 1998 by Huie et al. where they demonstrated low enzyme activity with this pathogenic variant in vitro and in vivo.[29] Subsequently this pathogenic variant has been reported in patients affected with infantile onset Pompe disease in several studies.[57–8] This variant lies in exon 14 of the gene, reported to be a hot spot for this gene.[59] An Indian study however reported no hot spots for this gene.[60]

OCA2 c.1580T > G, p.Leu527Arg variant

This variant was observed in heterozygous in two individuals in our cohort and was reported for the first time by Jowerek et al.[31] in a Pakistani albino family with some pigmentation of hair. They reported that this pathogenic variant lies in highly conserved residue of amino acids in the transmembrane 8 domain of the protein and segregated with affected members in the family. They hypothesized that this variant has not been reported in any other ethnic population till date and may be specific to Pakistani albino individuals.

AGXT c.302T > C, p.Leu101Pro variant

We observed one carrier (belonging to Punjabi community) for this variant in our cohort. This variant was reported for the first time by Williams et al.[43] who demonstrated that the mutant gene protein had less than 1% of normal activity in vitro. Subsequently, a study by Chanchlani et al. in three patients with primary hyperoxaluria type 1 showed the p.Leu101Pro variant in homozygous state.[32] All the three patients belonged to north India or Pakistan. They suggested a possibility of this being a founder pathogenic variant in India although larger studies and haplotype analysis are required.

ASPA c. 902T > C, p.Leu301Pro

One individual was found to be carrier for this variant. This variant has been reported in the literature in a patient of Indian ethnicity with classical Canavan disease and raised urine N-acetyl aspartate.[61] While functional studies have not been done, on the basis of the reported literature this variant could be reclassified using ACMG criteria as likely pathogenic. This variant has not been reported elsewhere in the world to the best of our knowledge and could represent an Indian pathogenic variant.

ACADM c.811G > A, p.Gly271Arg

This is a well reported pathogenic variant in the ACADM gene worldwide. It was observed in one individual in this study. The c.985A > G pathogenic variant commonly seen in the West, believed to be a founder pathogenic variant in Caucasians originating from an ancient Germanic tribe was not observed in the present cohort.[62]

Disorders like AR Polycystic Kidney Disease, Methyl Malonic aciduria, Galactosemia, Smith-Lemli Opitz Syndrome, Albinism type II, Cystic megalencephalic leukoencephalopathy, Gaucher disease, Phenylketonuria and Junctional Epidermolysis bullosa can be expected to be common in the Indian population as at least two cases were detected among the 200 individuals screened.

Our group has identified a number of disorders with founder mutations among the Agarwal community. Carriers for only two of these were identified in the current study (Calpainopathy, and Megalencephalic leukodystrophy with cysts). The mutations detected are not the common ones noted in the Agarwal community. However there were only 28 individuals in the cohort belonging to the Agarwal community.

Conclusions

Carrier screening has become widely available in the West and offering it in the preconception period has become the norm in clinical practice. However, in India it is offered in limited circumstances such as when there is a family history or consanguinity. With decreasing costs to NGS panels, carrier screening is likely to become the method of choice to decrease the genetic disease burden in India where treatment is not funded by state agencies and parent resources are scarce. This study also brings out the differences in common pathogenic variants in the West and in Asian Indians, an ethnically distinct population. The variant filtration and interpretation strategies in a healthy population are challenging and literature review is essential before assigning pathogenic status to a variant.

With the availability of NGS based testing in India and growing amount of literature on Indian pathogenic variants and their representation in databases the sensitivity of carrier screening is likely to improve. Targeted genotyping panels like the 23 pathogenic variant panel developed by ACMG for cystic fibrosis are not suitable and likely to miss most of the cases as many of the pathogenic variants identified in cystic fibrosis in India lie outside this panel. A similar point was noted by Archibald et al, where one member of a couple who were carriers for cystic fibrosis had a pathogenic variant outside the ACMG recommended panel. They also emphasized the need for population-based screening to be carried out keeping in mind the ethnicity of the local population.[47] Another point against carrier screening using targeted panels is that once a certain panel is designed, pathogenic variants are not easily added. As new pathogenic variants are identified daily, such a method would be outdated easily. With time as mixing of cultures becomes more wide-spread and societies open to inter caste, inter religion and inter racial marriages ECS is likely to have greater utility than targeted genotyping.

This study also highlights the importance of an Indian database in improving the classification of variants. The high carrier frequency of cystic fibrosis if substantiated in larger population studies would be sufficient ground to initiate new-born screening in the Indian population. As the sample size was small no couple was found to be a carrier for the same disease, however couples were counselled regarding cascade screening for other family members. This study is limited due to the small sample size, inability to detect indels and novel missense pathogenic variations and larger studies would be required to validate the significant observations made in the present study.

Abbreviations

NGS – Next generation sequencing

ACMG – American College of Medical Genetics

VUS – Variants of uncertain significance

AR – Autosomal recessive

XL – X linked

AD – Autosomal Dominant

SMA – Spinal muscular atrophy

FXS – Fragile X syndrome

DMD – Duchenne muscular dystrophy

CAH – Congenital adrenal hyperplasia

BWA – Burrow wheeler aligner

VEP - Variant effect predictor

OMIM - Online mendelian inheritance in man

GWAS catalogue - Genome wide association study in man

HGMD - Human gene pathogenic variant database

SIFT - Sorts intolerant from tolerant

LRT - Likelihood ratio test

BDGP -Berkeley drosophila genome project

HSF - human splicing finder

IGV- integrative genomics viewer

SCID - Severe combined immunodeficiency

CBAVD - congenital bilateral absence of vas deferens

Declarations

• Competing interests:

• The authors declare that they have no competing interests

• Funding:

The authors declare no source of funding

• Authors' contributions:

KS performed the study, including collection of sample, analysis of data and writing of the manuscript. SBM designed and supervised the study including collection, analysis of data and writing of manuscript. VLR, SN and SS carried out next generation sequencing and helped in data analysis. RS and SK carried out sanger confirmation in the lab and helped in data analysis. RDP, KG and IG collected data and supervised in writing the manuscript. ICV conceptualised, supervised in design and flow of study and helped in writing the manuscript. All authors read and approved the final manuscript

• Acknowledgements

The authors would like to thank all the individuals who participated in this study, staff at molecular genetics laboratory at Sir Ganga Ram Hospital, and Medgenome Pvt Ltd, Bengaluru, India for carrying out the molecular analysis of the samples.

Conflict of interest statement:

All authors declare that they have no conflict of interest.

Human rights statements and informed consent:

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all individuals for being included in the study.

Animal Rights

This article does not contain any studies with animal subjects performed by the any of the authors.

Ethics approval and consent to participate:

The study was granted approval from institutional ethics committee, vide letter no. EC/08/ 16/1066.

Consent for publication:

The consent for publication was taken from all the participants who also gave consent for inclusion in the study.

Availability of data and materials:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Data Availability statement

– Data are available on request to protect patient privacy.

References

1. Madan N, Sharma S, Sood SK, Colah R, Bhatia LH. Frequency of β-thalassemia trait and other hemoglobinopathies in northern and western India. Indian J Hum Genet. 2010;16:16–25.
2. Kapoor V, Shastri SS, Kabra M, et al. Carrier frequency of F508del mutation of cystic fibrosis in Indian population. J Cyst Fibros. 2006;5:43–6.
3. RamShankar M, Girirajan S, Dagan O, et al. Contribution of connexin26 (GJB2) mutations and founder effect to non-syndromic hearing loss in India. J Med Genet. 2003;40:e68.
4. Lazarin GA, Haque IS, Nazareth S, et al. An empirical estimate of carrier frequencies for 400 + causal Mendelian variants: results from an ethnically diverse clinical sample of 23,453 individuals. Genet Med. 2013;15:178–86.

5. Ankala A, Tamhankar PM, Valencia CA, Rayam KK, Kumar MM, Hegde MR. Clinical applications and implications of common and founder mutations in Indian subpopulations. Hum Mutat. 2015;36:1–10.
6. Grody WW, Thompson BH, Gregg AR, et al. ACMG position statement on prenatal/preconception expanded carrier screening. Genet Med. 2013;15:482–3.
7. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 2010;26:589–95.
8. Meyer LR, Zweig AS, Hinrichs AS, et al. The UCSC Genome Browser database: extensions and updates 2013. Nucleic Acids Res. 2013;41(Database issue):D64–9.
9. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20:1297–303.
10. Li H, Handsaker B, Wysoker A, et al. 1000 Genome Project Data Processing Subgroup. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25:2078–9.
11. Zerbino DR, Achuthan P, Akanni W, et al. Ensembl 2018. Nucleic Acids Res. 2018;46:D754–61.
12. Landrum MJ, Lee JM, Benson M, et al. ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res*. 2018;46:D1062-D1067. Available at: https://www.ncbi.nlm.nih.gov/clinvar/.
13. Hamosh A, Scott AF, Amberger JS, et al. Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. *Nucleic Acids Res*. 2005;33:D514-517. Available at: https://www.omim.org/.
14. Welter D, MacArthur J, Morales J, et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res*. 2014;42:D1001-1006. Available at: https://www.ebi.ac.uk/gwas/.
15. Stenson PD, Ball EV, Mort M, et al. Human Gene Mutation Database (HGMD): 2003 update. Hum Mutat. 2003;21:577–81. . Available at:.
16. Mottaz A, David FP, Veuthey AL, Yip YL. Easy retrieval of single amino-acid polymorphisms and phenotype information using SwissVar. *Bioinformatics*. 2010;26:851–852. Available at: https://swissvar.expasy.org/.
17. Auton A, Abecasis G, Altshuler D, et al. A global reference for human genetic variation. Nature. 2015;526:68–74. Available at: phase3browser.1000genomes.org/.
18. Lek M, Karczewski KJ, Minikel EV, et al. Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536:285–91. Available at: gnomad.broadinstitute.org/.
19. Sherry ST, Ward M, Sirotkin K. dbSNP-database for single nucleotide polymorphisms and other classes of minor genetic variation. *Genome Res*. 1999;9:677–679. Available at: https://www.ncbi.nlm.nih.gov/projects/SNP/.
20. Nagasaki M, Yasuda J, Katsuoka F, et al. Rare variant discovery by deep whole-genome sequencing of 1,070 Japanese individuals. *Nat Commun*. 2015;6:8018. Available at:

https://ijgvd.megabank.tohoku.ac.jp/.

21. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7:248–249. Available at: http://genetics.bwh.harvard.edu/pph2/.
22. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc*. 2009;4:1073–1081. Available at: http://sift.jcvi.org/www/SIFT_enst_submit.html.
23. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods*. 2014;11:361 – 336. Available at: http://www.mutationtaster.org/.
24. Reva B, Antipin Y, Sander C. Predicting the functional impact of protein mutations: application to cancer genomics. Nucleic Acids Res. 2011;39:e118. Available at: mutationassessor.org/.
25. Chun S, Fay JC. Identification of deleterious mutations within three human genomes. Genome Res. 2009;19:1553–6.
26. Reese MG, Eeckman FH, Kulp D, Haussler D. Improved splice site detection in Genie. J Comput Biol. 1997;4:311–23. . Available at.
27. Desmet FO, Hamroun D, Lalande M, et al. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res. 2009;37:e67. Available at: www.umd.be/HSF3.
28. Ankleshwaria C, Mistri M, Bavdekar A, et al. Novel mutations in the glucocerebrosidase gene of Indian patients with Gaucher disease. J Hum Genet. 2014;59:223–8.
29. Huie ML, Tsujino S, Sklower Brooks S, et al. Glycogen storage disease type II: identification of four novel missense mutations (D645N, G648S, R672W, R672Q) and two insertions/deletions in the acid alpha-glucosidase locus of patients of differing phenotype. Biochem Biophys Res Commun. 1998;244:921–7.
30. Coelho AI, Trabuco M, Ramos R, et al. Functional and structural impact of the most prevalent missense mutations in classic galactosemia. Mol Genet Genomic Med. 2014;2:484–96.
31. Jaworek TJ, Kausar T, Bell SM, et al. Molecular genetic studies and delineation of the oculocutaneous albinism phenotype in the Pakistani population. Orphanet J Rare Dis. 2012;7:44.
32. Williams EL, Acquaviva C, Amoroso A, et al. Primary hyperoxaluria type 1: update and additional mutation analysis of the AGXT gene. Hum Mutat. 2009;30:910–7.
33. Lerner-Ellis JP, Dobson CM, Wai T, et al. Mutations in the MMAA gene in patients with the cblA disorder of vitamin B12 metabolism. Hum Mutat. 2004;24:509–16.
34. Archibald AD, Smith MJ, Burgess T, et al. Reproductive genetic carrier screening for cystic fibrosis, fragile X syndrome, and spinal muscular atrophy in Australia: outcomes of 12,000 tests. Genet Med. 2018;20:513–23.
35. Woodcock IR, Fraser L, Norman P, et al. The prevalence of neuromuscular disease in the paediatric population in Yorkshire, UK; variation by ethnicity and deprivation status. Dev Med Child Neurol. 2016;58:877–83.

36. Gupta D, Bijarnia-Mahay S, Kohli S, et al. Seventeen Novel Mutations in PCCA and PCCB Genes in Indian Propionic Acidemia Patients, and Their Outcomes. Genet Test Mol Biomarkers. 2016;20:373–82.
37. Sachdeva K, Saxena R, Puri R, Bijarnia S, Kohli S, Verma IC. Mutation analysis of the CFTR gene in 225 children: identification of five novel severe and seven reported severe mutations. Genet Test Mol Biomarkers. 2012;16:798–801.
38. Shastri SS, Kabra M, Kabra SK, Pandey RM, Menon PS. Characterisation of mutations and genotype-phenotype correlation in cystic fibrosis: experience from India. J Cyst Fibros. 2008;7:110–5.
39. Sharma N, Singh M, Kaur G, Thapa BR, Prasad R. Identification and characterization of CFTR gene mutations in Indian CF patients. Ann Hum Genet. 2009;73:26–33.
40. Watson MS, Cutting GR, Desnick RJ, et al. Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. Genet Med. 2004;6:387–91.
41. Mandal A, Kabra SK, Lodha R. Cystic fibrosis in India: Past, Present and Future. J Pulm Med Respir Res. 2015;1:002.
42. Sachdeva K, Saxena R, Majumdar A, et al. Mutation studies in the CFTR gene in Asian Indian Subjects with congenital bilateral absence of vas deferens: Report of two novel mutations and four novel variants. *Genetic Testing and Molecular Biomarkers*. 201;15:307–312.
43. Lim RM, Silver AJ, Silver MJ, et al. Targeted mutation screening panels expose systematic population bias in detection of cystic fibrosis risk. Genet Med. 2016;18:174–9.
44. Bowler IM, Estlin EJ, Littlewood JM. Cystic fibrosis in Asians. Arch Dis Child. 1993;68:120–2.
45. Powers CA, Potter EM, Wessel HU, Lloyd-Still JD. Cystic fibrosis in Asian Indians. Arch Pediatr Adolesc Med. 1996;150:554–5.
46. Kabra M, Kabra SK, Ghosh M, et al. Is the spectrum of mutations in Indian patients with cystic fibrosis different? Am J Med Genet. 2000;93:161–3.
47. Green GE, Scott DA, McDonald JM. at al. Carrier rates in the midwestern United States for GJB2 mutations causing inherited deafness. JAMA. 1999;281:2211–6.
48. Taniguchi M, Matsuo H, Shimizu S, et al. Carrier frequency of the GJB2 mutations that cause hereditary hearing loss in the Japanese population. J Hum Genet. 2015;60:613–7.
49. Han SH, Park HJ, Kang EJ, et al. Carrier frequency of GJB2 (connexin-26) mutations causing inherited deafness in the Korean population. J Hum Genet. 2008;53:1022–8.
50. Sloan-Heggen CM, Bierer AO, Shearer AE, et al. Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss. Hum Genet. 2016;135:441–50.
51. Van Hauwe P, Everett LA, Coucke P, et al. Two frequent missense mutations in Pendred syndrome. Hum Mol Genet. 1998;7:1099–104.
52. Madden C, Halsted M, Meinzen-Derr J, et al. The influence of mutations in the SLC26A4 gene on the temporal bone in a population with enlarged vestibular aqueduct. Arch Otolaryngol Head Neck Surg. 2007;133:162–8.

53. Kühnen P, Turan S, Fröhler S, et al. Identification of PENDRIN (SLC26A4) mutations in patients with congenital hypothyroidism and "apparent" thyroid dysgenesis. J Clin Endocrinol Metab. 2014;99:E169–76.
54. Dossena S, Nofziger C, Brownstein Z, Kanaan M, Avraham KB, Paulmichl M. Functional characterization of pendrin mutations found in the Israeli and Palestinian populations. Cell Physiol Biochem. 2011;28:477–84.
55. Pera A, Villamar M, Viñuela A, et al. A mutational analysis of the SLC26A4 gene in Spanish hearing-impaired families provides new insights into the genetic causes of Pendred syndrome and DFNB4 hearing loss. Eur J Hum Genet. 2008;16:888–96.
56. Nahar R, Puri RD, Saxena R, Verma IC. Do parental perceptions and motivations towards genetic testing and prenatal diagnosis for deafness vary in different cultures? Am J Med Genet A. 2013;161A:76–81.
57. Kroos MA, Kirschner J, Gellerich FN, et al. A case of childhood Pompe disease demonstrating phenotypic variability of p.Asp645Asn. Neuromuscul Disord. 2004;14:371–4.
58. Pittis MG, Donnarumma M, Montalvo AL, et al. Molecular and functional characterization of eight novel GAA mutations in Italian infants with Pompe disease. Hum Mutat. 2008;29:E27–36.
59. Ngiwsara L, Wattanasirichaigoon D, Tim-Aroon T, et al. Clinical course, mutations and its functional characteristics of infantile-onset Pompe disease in Thailand. BMC Med Genet. 2019;20:156.
60. Gupta N, Kazi ZB, Nampoothiri S, et al. Clinical and Molecular Disease Spectrum and Outcomes in Patients with Infantile-Onset Pompe Disease. J Pediatr. 2020;216:44–50.e5.
61. Bijarnia S, Kohli S, Puri RD, et al. Molecular characterisation and prenatal diagnosis of Aspartoacylase deficiency (Canavan disease)-report of two novel and two known mutations from the Indian subcontinent. Indian J Pediatr. 2013;80:26–31.
62. Leal J, Ades AE, Wordsworth S, Dezateux C. Regional differences in the frequency of the c.985A > G ACADM mutation: findings from a meta-regression of genotyping and screening studies. Clin Genet. 2014;85:253–9.

Figures

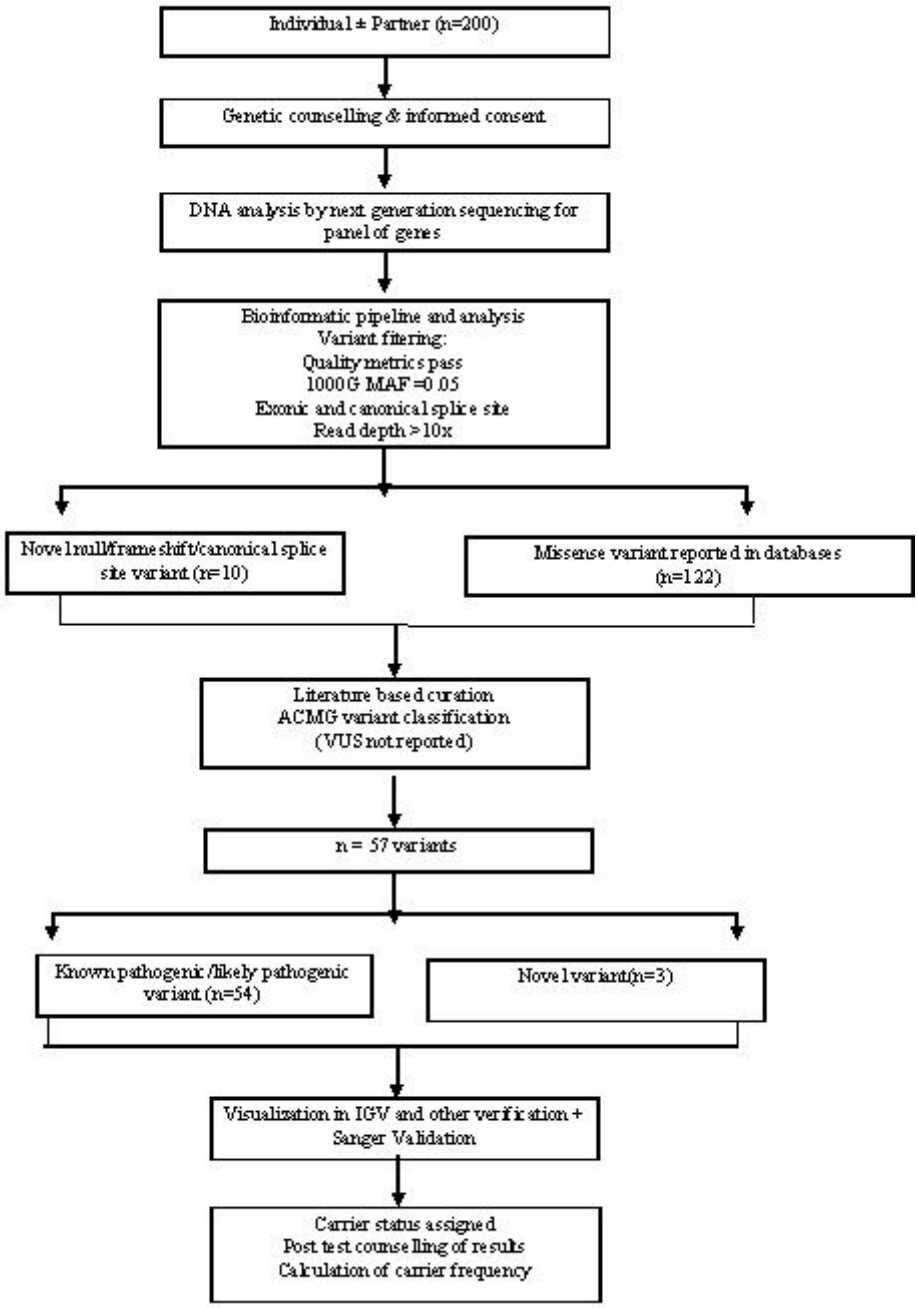

Figure 1

Study flow chart

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryfileS1.docx