strand
New Generation Healthcare

Diagnosis of Rare Diseases using NGS Based Multi-gene Testing

A Story of >1000 Indian Patients

A study by
Strand Life Sciences

Index

NGS Based Multi-Gene Testing for Rare Diseases: Findings from an Analysis of a 1000+ Indian Patients

Overview: Rare Diseases

- Rare disease worldwide - ~7000-8000 (350 million people)
- Indian scenario - >50 million with Rare disorder.
- NO major treatment options.
- Conclusive DIAGNOSIS – a major challenge!

Why multi-gene testing?

Rare disorders in India

High infant mortality rate (61-69/1000)
(Global: 32/1000)

Third most common cause of mortality
(Genetic Disorders)

High burden on healthcare system

Impact of multi-gene testing

80% rare are genetic in origin

Multiple genes responsible for same disorder

Differential diagnosis – impact on management

Overview: Rare Diseases

- About 7000-8000 rare diseases are known worldwide. Around the globe, it is estimated that the Rare disorder community includes about 350 million people.
- In India, it is estimated that about >50 million people are affected with Rare disorder.
- A majority of these patients lack proper treatment options.
- A key challenge existing globally is the inability to properly diagnose these diseases in a timely manner delaying medical management and therapy.

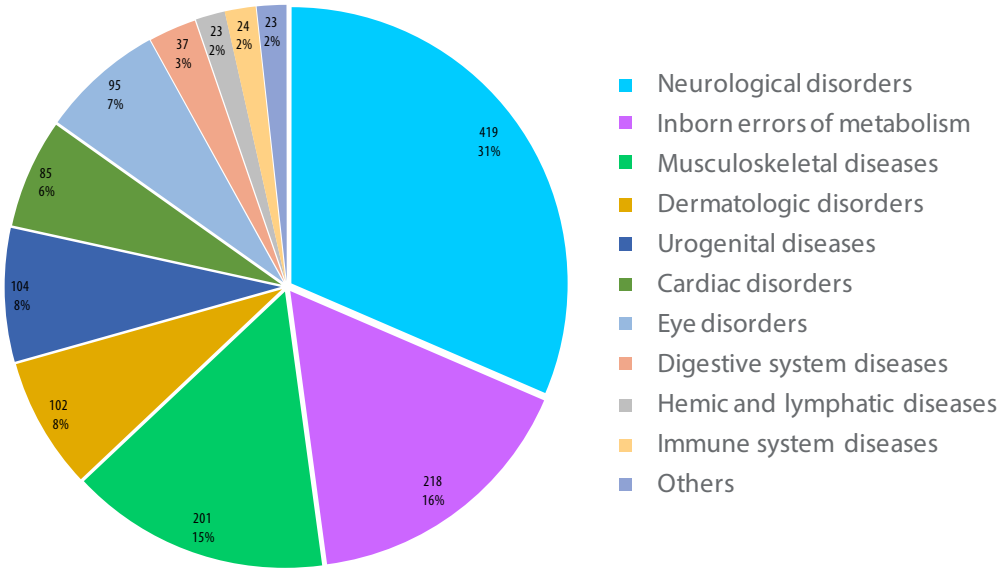
Rare Diseases – Indian scenario

- In India, the infant mortality rate (IMR) is 61-69/1000 live births, far above the global average of 32/1000 (WHO, 2015).
- 1 out of every 20 newborns admitted to the hospital, carries a genetic disease that eventually account for nearly 1 out of 10 infant mortality (Rao and Ghosh, 2005).
- In India's urban areas, congenital malformations and genetic disorders are the third most common cause of mortality in newborns (Pradhan et al., 2011)
- Huge cost of treatment on the Indian Health Care System
- 70% of birth defects are shown to be preventable if community genetic services are used for diagnosis, care and prevention of genetic diseases at the community level.

Why multi-gene testing?

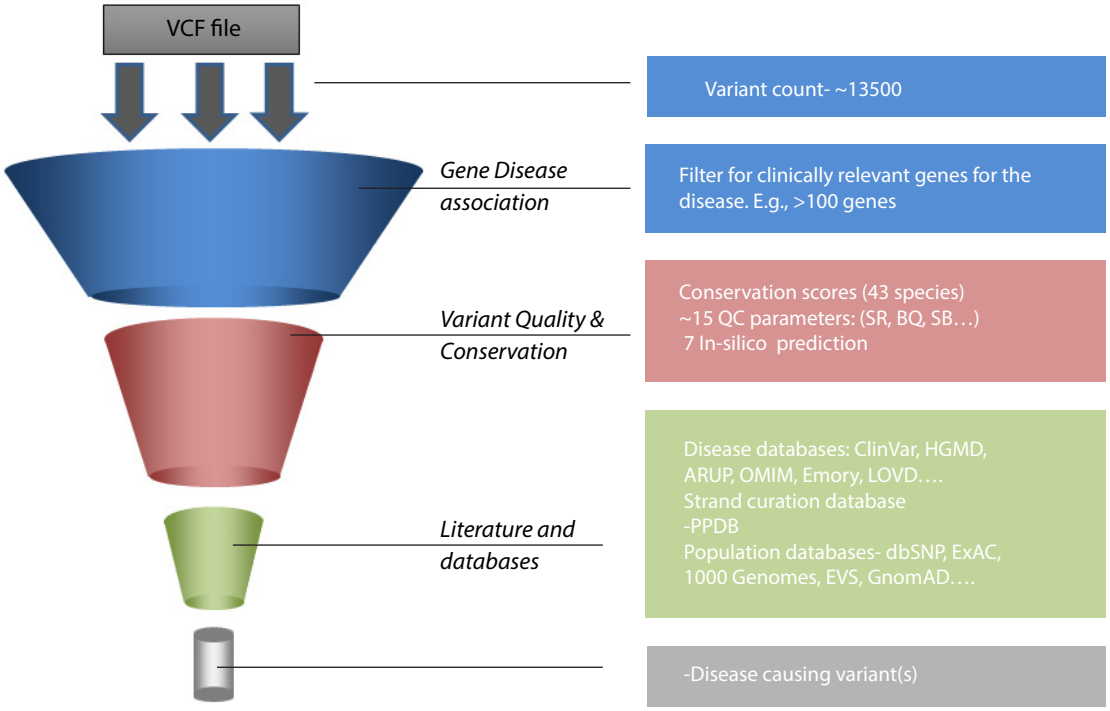
- 80% of the rare diseases are genetic in origin (NORD, 2007; NIH, 2008). Many if not all are caused by defects in a single gene.
- There are considerable genetic heterogeneity and also due to overlapping phenotypes, differential diagnosis are necessary.
- Multiple different mutations in a single gene may result in disease of varying features or severity.
- For some rare conditions, multiple genes may contribute collectively to manifestations of the disorder.
- Disease may also arise as a result of sporadic or chance (de novo mutations)
- Arriving at Clinical Diagnosis/ Differential Diagnosis: Limitations on access to the most up-to-date information about rare diseases (including diagnostic criteria) and other diagnostic resources.

Inherited diseases tested by NGS Total Cases ~1350

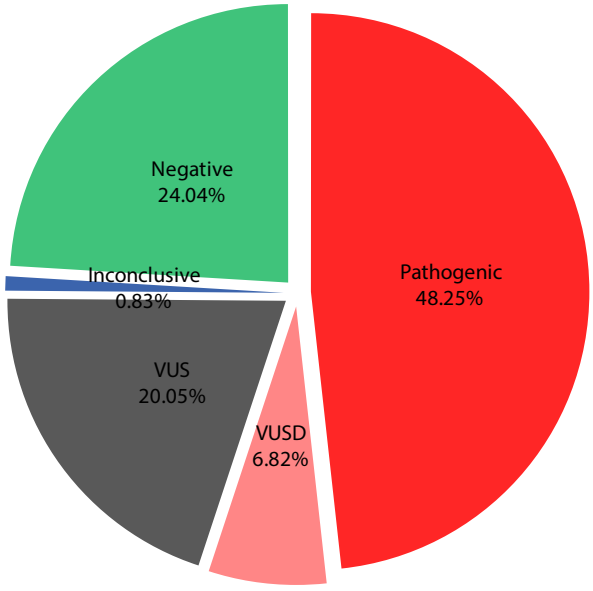


Interpretation Work flow

Strand Clinical Exome Test Multi-Gene Test (based on NGS) >4500 genes



Detection Rate of NGS based testing of Inherited diseases



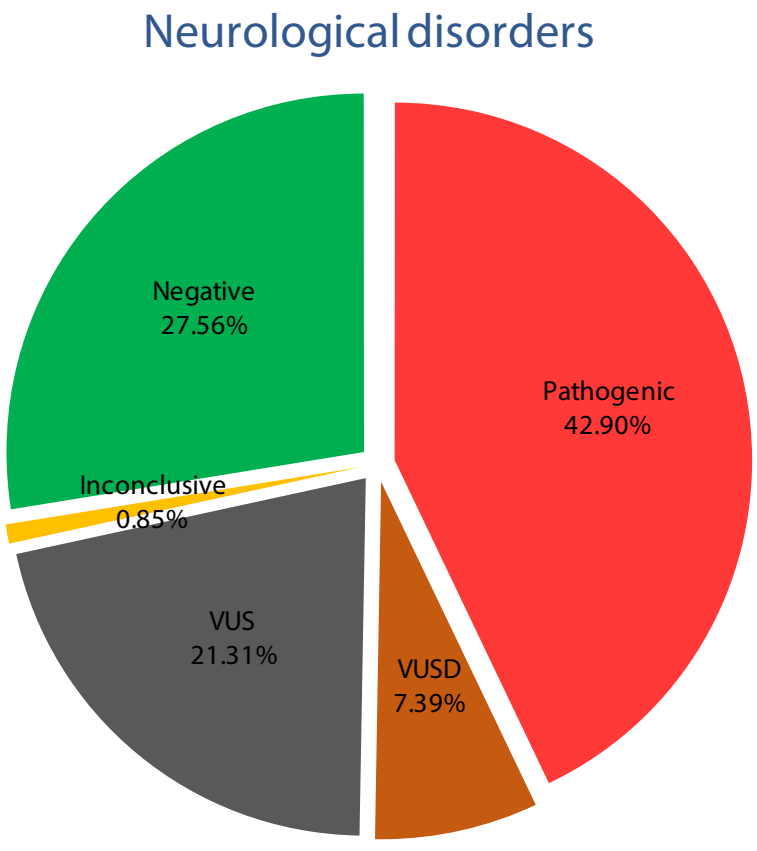
VUS: Variant of uncertain significance

VUSD: Variant of uncertain significance with probable damaging effect (Potentially pathogenic)

Rare Disease Tests - Globally

Lab	Test	Success Rate	Link
Baylor	WES	25%	http://www.irdirc.org/wp-content/uploads/2013/06/Christine-Eng.pdf
GeneDx	WES	24% - 31%	https://www.genedx.com/genedx-blog/exome-sequencing-at-genedx-8-things-you-didnt-know/
CCHMC	WES	30%	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4522872/
UCLA	WES	26%	http://jamanetwork.com/journals/jama/fullarticle/1918775
Sick Kids,Toronto	WGS	34%	http://www.nature.com/articles/npjgenmed201512

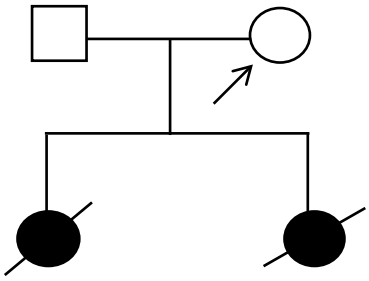
Neurological disorders (>400 cases)



VUS: Variant of uncertain significance

VUSD: Variant of uncertain significance with probable damaging effect (Potentially pathogenic)

Case study - 1



Clinical indications: Carrier testing

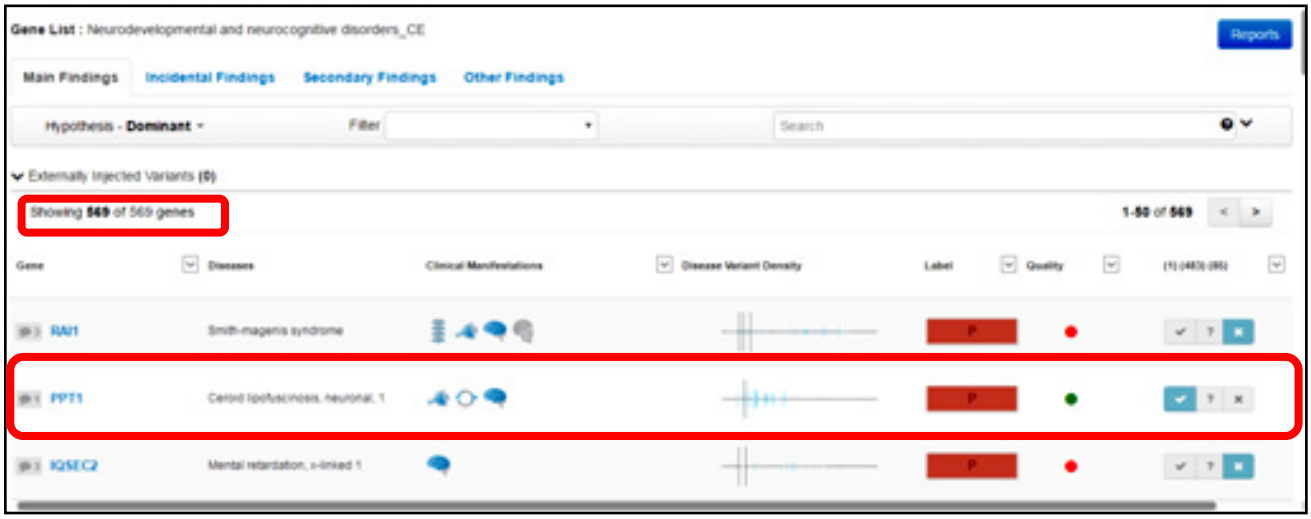
Family history : Children born manifested myoclonic jerks, regression of milestones, loss of speech and eye contact, leading to death.

The first child showed diffuse cortical atrophy.

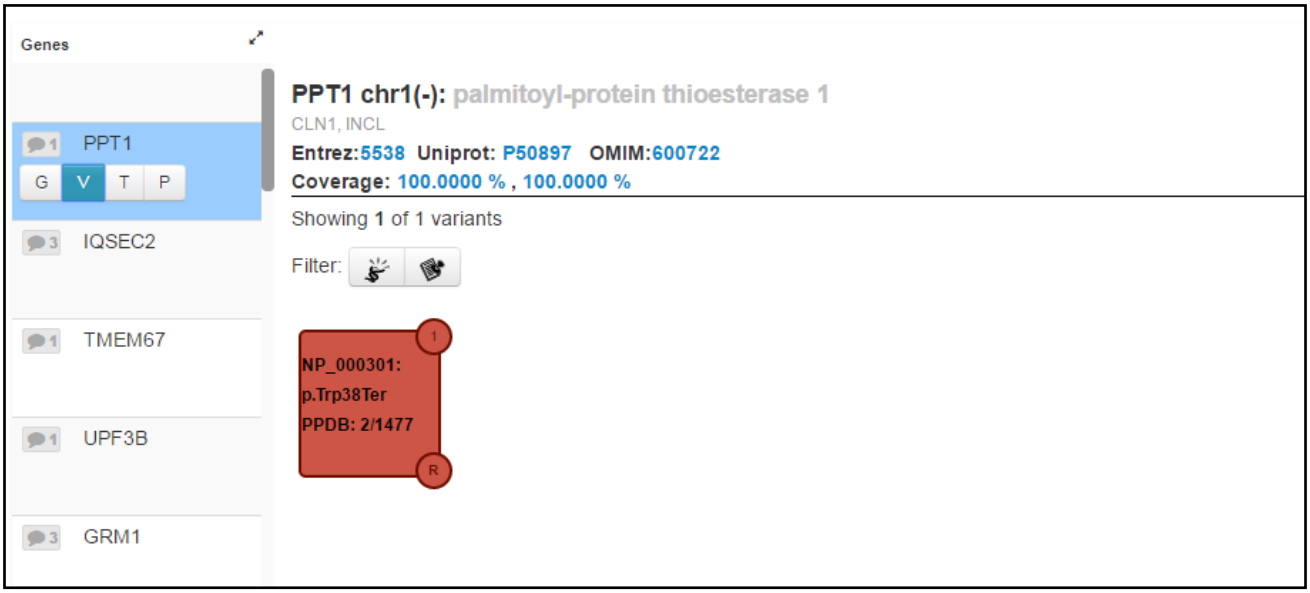
Key genes assessed (Neurocognitive and neurodevelopmental disorders)

569 genes

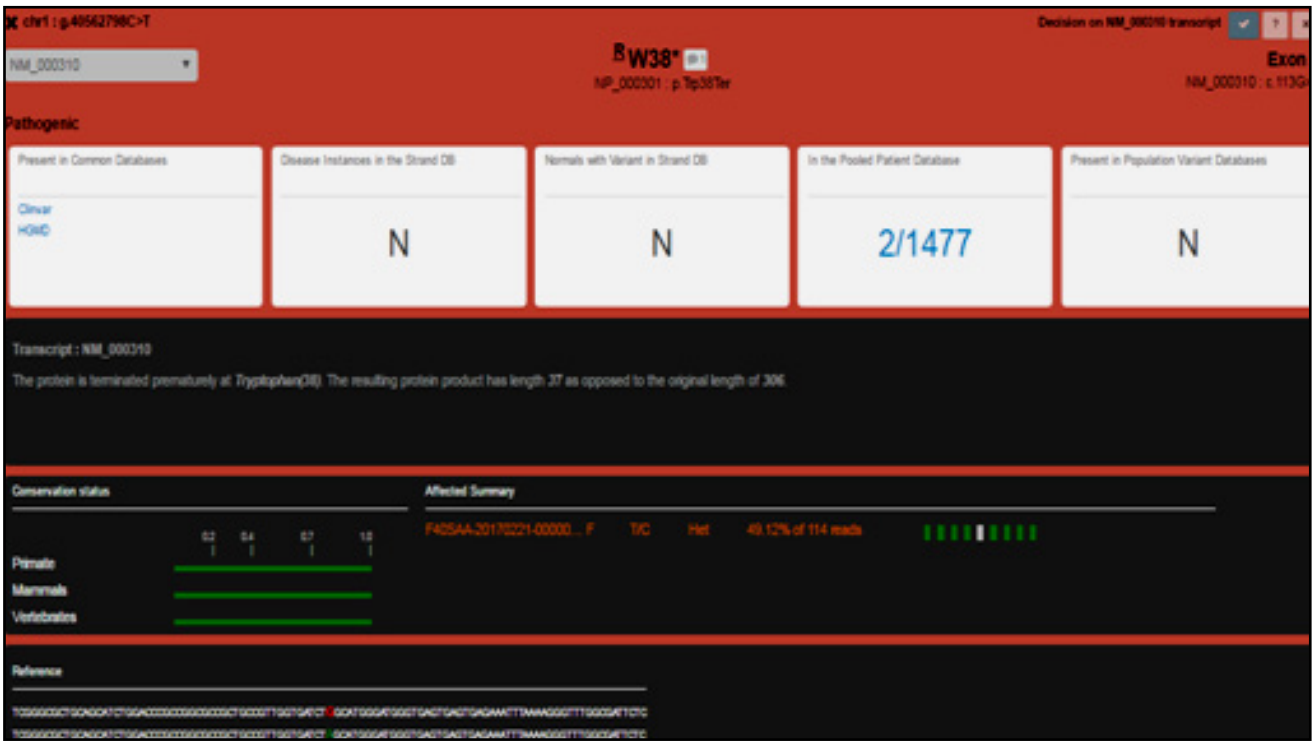
StrandOmics – Overview



Variant cards



Variant view



Transcript view

Results – Key findings

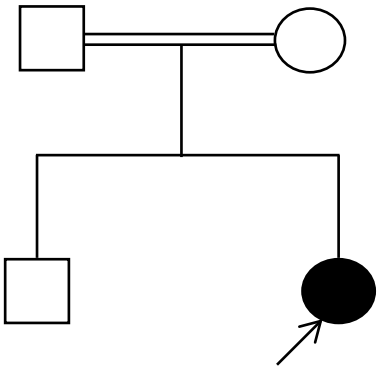
RESULT ⊕ Postiive for a heterozygous **'pathogenic'** variant, which was detected in exon 1 of the *PPT1*

Key Findings

Gene	Variation	Zygosity	Inheritance	Clinical significance
PPT1	chr1:40562798c>T c.113G>A p.Trp38Ter	Helerozygous	Recessive	Pathogenic

Diagnosis neuronal ceroid lipofuscinoses (NCL).

Case study - 2



Clinical indications: 1 year - Female

Normal perinatal history
- Jitteriness since birth.
Normal till 4 months, developed tonic posturing and lost head control and social smile
- Microcephaly, Spastic quadriplegia, Seizures

Tested for **Microcephaly, epilepsy and spastic paraplegia**

Key genes : 277 (Microcephaly, epilepsy and spastic paraplegia)

Total No. of variants in the panel: 15334

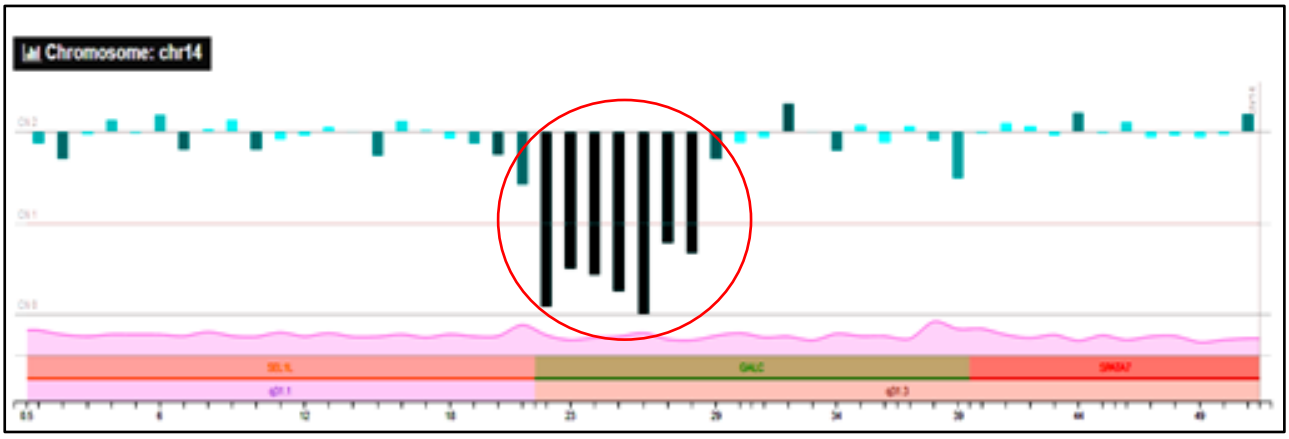
Total No. of variants in Key genes: 904

Total No. of variants in Secondary genes: 14430

No disease-causing variant was detected

STRAN-000004491

Copy number variation analysis



Deletion of Exons 11-17 in the GALC gene (Krabbe disease)

STRAN-000004491

Validated by PCR

Case study -2: Final Report

RESULT (uninformative) for disease-causing or likely disease-causing variants in the genes (as mentioned above) tested in this sample

Secondary Findings :

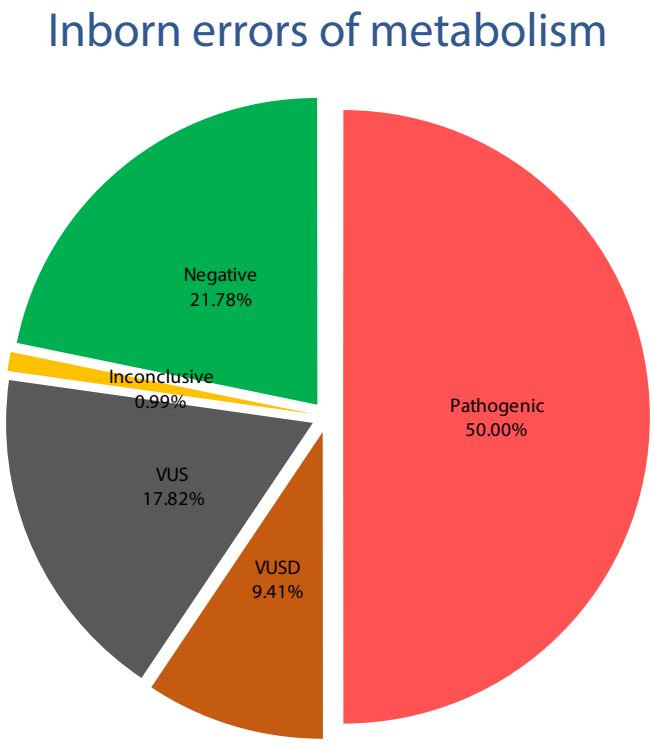
A homozygous 'pathogenic'variant was identified in the GALC gene, which causes deletion of exon 11-17 of the GALC gene.

Secondary Findings :

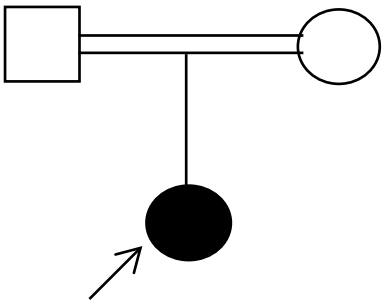
Gene	Variation	Zygosity	Clinical significance
GALC	chr14:88401028-?_88417092+del c.(1161+1_1162-1)_(*31_?)del (Exon 11-17 deletion)	Helerozygous	Pathogenic

STRAN-000004491

Inborn errors of metabolism (>200 cases)



Case study



Clinical indications: 9 years- Female

Manifestations:

- Hypoglycemia, Increased lactate
- Seizures
- Altered sensorium
- Hepatomegaly

Suspected **Hereditary fructose intolerance**

No variants detected in the ALDOB gene

Differential diagnosis- FRUCTOSE-1,6-BISPHOSPHATASE DEFICIENCY

STRAN-000004491

Insertion of ALU sequence confirmed by Sanger

ALU insertion sequence: GenBank: KT305716.1
(https://www.ncbi.nlm.nih.gov/nucleotide/925171155?report=genbank&log$=nuclalign&blast_rank=2&RID=XTTFV7EE014)

Detail Sanger sequence with low coverage region (grey), repeat region (underlined) and insertion (blue)

```
97382778

TGTAGCTATGGCATTGCTGGTTCTACCAACGTGACAGGTGATCAAGTTAAGAAGCTGGACGTtggccgggcgcggtggctcacgcctgtaatcccagcactttgggaggccgagg
ACATCGATACCGTAACGACCAAGATGGTTGCACTGTCCACTAGTTCAATTCTTCGACCTGCAaccggcccgcgccaccgagtgcggacattagggtcgtgaaaccctccggctcc

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gtttttttttttttttttttttttttttttttttttttttttttttttttttttTTCTTCGACCTGCAGGAGAGGTTGCTGGACCAATACTTGTACAATTTCAGTAGGAAACGGTGCACACAAGAGCA 97382657
```

Case study : Final Report

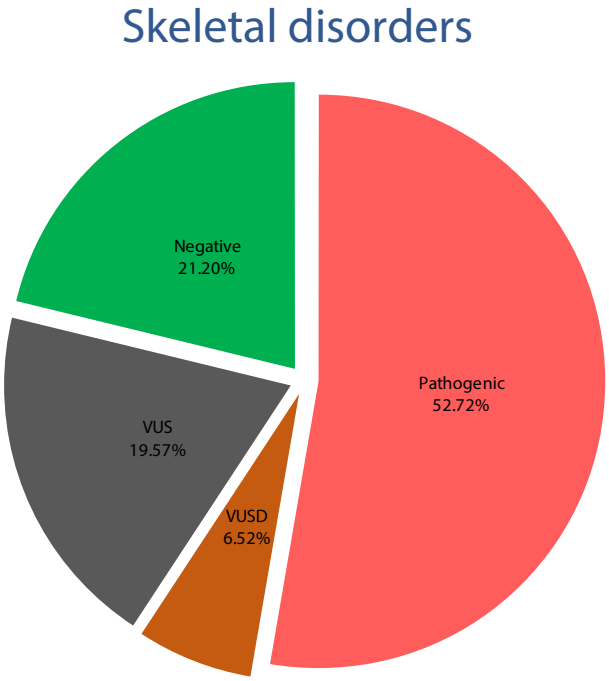
Results

A homozygous 'pathogenic' variant that results in a 331bp ALU sequence element insertion was detected in exon 2 of the FBPI gene

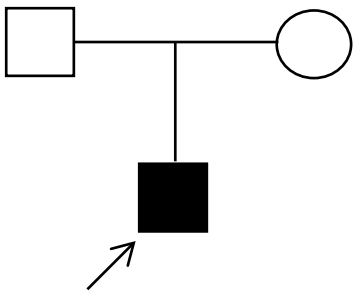
key findings

Gene	Variation	Zygosity	Clinical significance
FBPI	chr9:g.97382716_97382717insKT305716.1:g87_47 c.227 228insKT305716.1:g.87_417 p.Leu77GlyfeTer39	Helerozygous	Pathogenic

Skeletal disorders (>200 Cases)



Case study



Clinical indications: 4 months - Male

Radiological examination indicated
- lateral thoracic lumbar spine
- Proptosis
- thumb anomaly

Suspected **Rubinstein-Taybi syndrome**

Key genes : 2 (Rubinstein-Taybi syndrome)

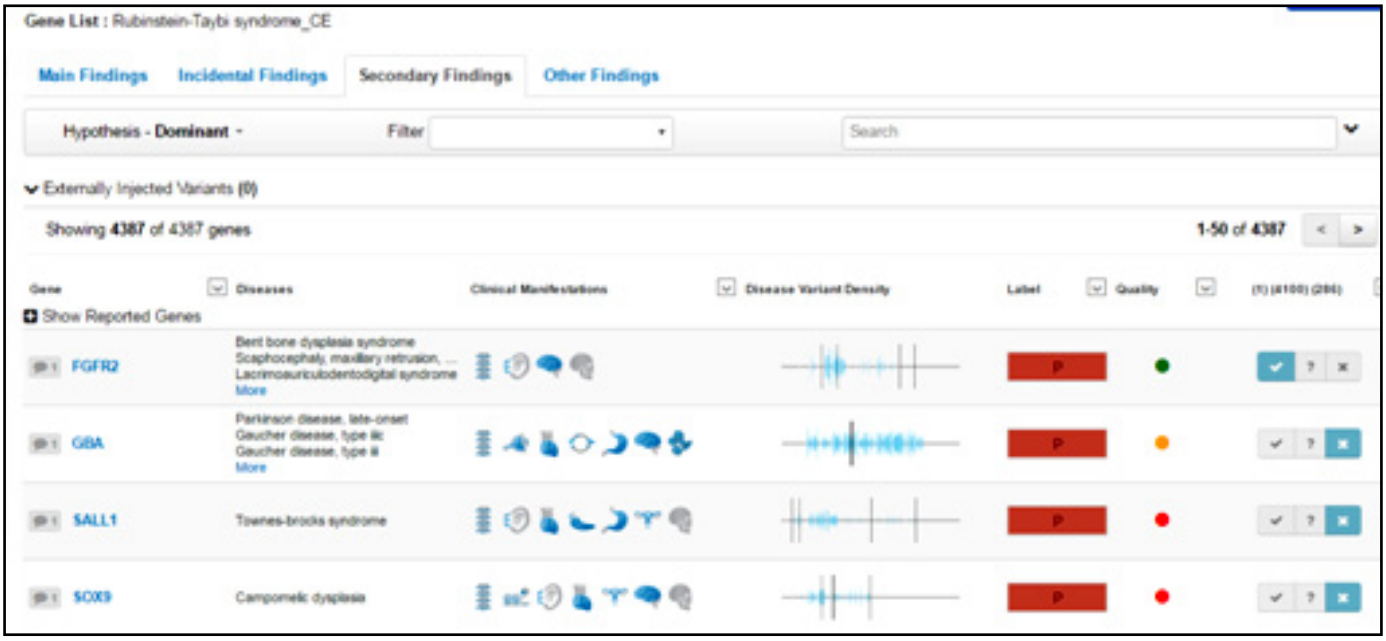
Total No. of variants in the panel: 13400

Total No. of variants in Key genes: 15

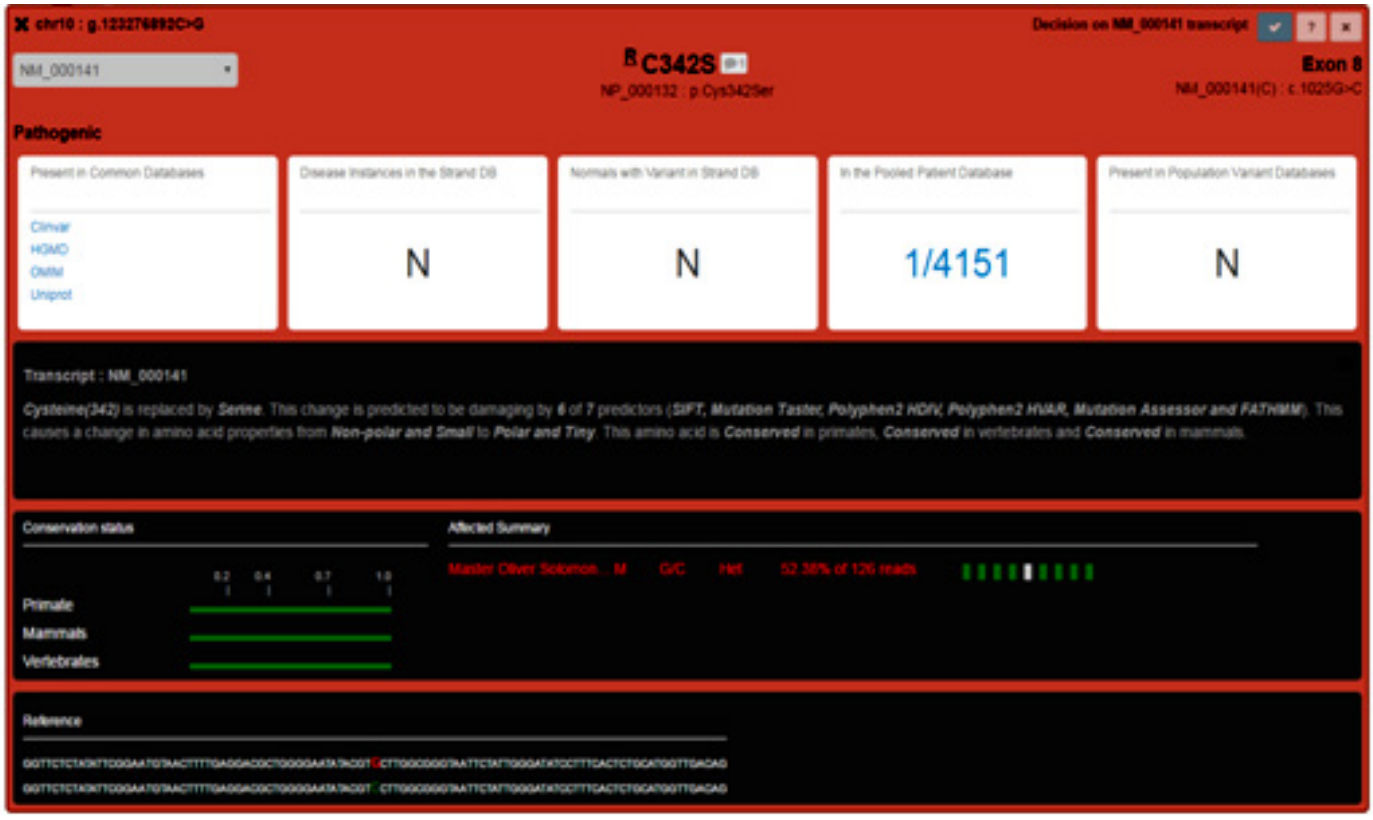
Total No. of variants in Secondary genes: 13385

STRAN-000004763

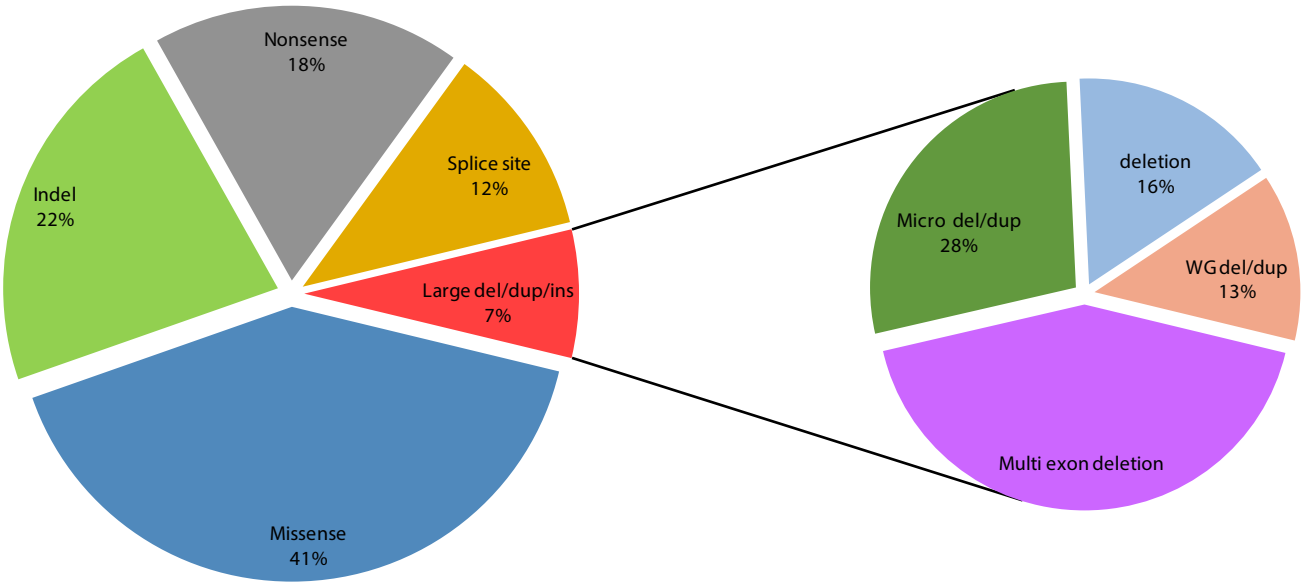
Diagnosis - CROUZON SYNDROME



STRAN-000004763



Mutational Spectrum



Among the large deletions, 1 in 4 cases were micro deletion/duplication, which are typically detected by aCGH (microarray) /Cytogenetic

Micro deletion/duplication cases

Sample	Initial suspicion	Identified microdeletion/ duplication	Possible diagnosis
STRAN-2857	Refractory epilepsy and dysmorphism	*1p36.23-1p36.33del (21 genes deleted)*	*1p36 deletion syndrome*
STRAN-5221	Macrocephaly and developmental delay	*4q21.21-q22.3del (20 genes deleted)*	*4q21-q22 deletion syndrome*
STRAN-5116	Epilepsy	*1q43-q44del (4 genes deleted)*	*1q43-q44 deletion syndrome*
STRAN-3209	Peripheral neuropathy	*17p12del (2 genes deleted)*	*Hereditary neuropathy with liability to pressure palsies (HNPP)*
STRAN-5204	Developmental delay and dysmorphism	*17q11.23dup (8 genes duplicated)*	*7q11.23 duplication syndrome*

Summary

- Neurological disorders, Inborn errors of metabolism and Skeletal disorders contribute to >60% of the ~1200 samples studies.
- Positive detection rate was seen in ~45%-50% of the cases tested for rare disease; higher than those detected by other whole exome panel (~25%-34%).
- In addition, ~7-10% 'potentially pathogenic' variants: 'novel' missense variants at conserved regions and are predicted to be damaging. With additional co-segregation studies and functional studies, these are likely to be re-classified. Increase in detection rate to upto ~55%-60%
- Large deletions have been identified in ~7% of cases by additional bioinformatic analysis.
- In ~8-10% of cases with inconclusive clinical diagnosis, multi-gene panel testing helped in arriving at a diagnosis.
- In ~2-3% of cases with a suggestive clinical diagnosis, multi-gene panel testing helped in arriving at a differential diagnosis thus significantly impacting the treatment and managements plans.

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Organization for rare diseases India (ORDI) – addressing the challenges and opportunities for the Indian rare diseases' community

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Summary

In order to address the unmet needs and create opportunities that benefit patients with rare disease in India, a group of volunteers created a not-for-profit organization named Organization for Rare Diseases India (ORDI; www.ordindia.org). ORDI plans to represent the collective voice and advocate the needs of patients with rare diseases and other stakeholders in India. The ORDI team members come from diverse backgrounds such as genetics, molecular diagnostics, drug development, bioinformatics, communications, information technology, patient advocacy and public service. ORDI builds on the lessons learned from numerous similar organizations in the USA, European Union and disease-specific rare disease foundations in India. In this review, we provide a background on the landscape of rare diseases and the organizations that are active in this area globally and in India. We discuss the unique challenges in tackling rare diseases in India, and highlight the unmet needs of the key stakeholders of rare diseases. Finally, we define the vision, mission, goals and objectives of ORDI, identify the key developments in the health care context in India and welcome community feedback and comments on our approach.

Introduction to rare diseases

By definition, a rare disease occurs infrequently in a population, but there is no universal definition. There are three elements to the definition as used in various countries – the total number of persons having the disease, its prevalence and non-availability of treatment for the disorder. This definition was introduced to identify disorders that are neglected by health professionals. A formal definition helps a nation to identify diseases that require financial incentives for discovery and development of drugs and biologics, so as to encourage product development as well as funding for basic and clinical research on those diseases. Many countries define 'rare' or 'orphan' diseases as those affecting less than a specific number of persons in the populations. For example, in the USA, it is defined strictly according to its prevalence, specifically

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'any disease or condition that affects less than 200,000 persons' (Shire Human Genetic Technologies, 2013). In Japan, the number is 50,000 persons, in Korea 20,000, in Taiwan 10,000, and in Australia 2000 (Lavandeira, 2002; Tang, 2013). The World Health Organization (WHO) has suggested that a rare disease should be defined as one with a frequency of less than 6·5–10 per 10,000 persons (Song, 2012), although, some experts feel this is rather high (Aronson, 2006). Stated as the prevalence per 10,000, the number used in the USA is 7·5, in Europe 5, in Japan 4, in South Korea 4, Australia 1·1 and Taiwan 1·0 (Song, 2012). In China, a rare disorder is defined as one that affects less than 1/500,000 persons, or one that has a neonatal morbidity of less than 1/10,000 (Song, 2012; Ma *et al.*, 2011). Thus, a country should define a rare disease in the context of its own population, health care system and resources. India, like many developing countries, currently has no standard definition. Considering the large population of India, we suggest the threshold for a disease to be defined as rare to be 1 in 5000. This would include diseases that have a higher prevalence, but do not have definitive therapy. Although this definition may suggest that the number of affected patients is small, it is important to note that when taken together, the number of patients living with a rare disease in India is over 70 million (Verma, 2000). By contrast, about 30 million Americans (Shire Human Genetic Technologies, 2013), and about 29 million persons in the EU are affected with rare disorders (Nogales, 2004).

The exact number of rare diseases is not known, but is estimated to be around 7000–8000 worldwide (Global Genes. RARE Facts and Statistics). With the rapid advances in genomic technologies in the last decade, the number is increasing steadily each year with new diseases and associated genes being discovered. About 80% of rare diseases are genetic in origin, many of which are thought to be monogenic (Global Genes. RARE Facts and Statistics). Rare diseases also include rare inherited cancers, autoimmune diseases, congenital malformations and infectious diseases amongst others. All rare diseases taken together affect about 6–8% of the world's population. About half of the rare diseases affect children causing significant social and economic burden, while the other half manifest in adulthood. Some examples of rare diseases include hemangiomas (Haggstrom, 2006), Hirschsprung disease (Butler Tjaden & Trainor, 2013), Gaucher disease (Rosenbloom & Weinreb, 2013), cystic fibrosis (Ehre, 2014), muscular dystrophies (Mercuri & Muntoni, 2013) and Pompe disease (Ausems *et al.*, 1999).

Treatments for rare diseases

According to a *Thomson Reuters* report (http://thomsonreuters.com/business-unit/science/subsector/pdf/the-economic-power-of-orphan-drugs.pdf), the global market for 'orphan drugs' (drugs that are meant to treat rare medical conditions) accounted for more than $50 billion in 2011. A majority of these diseases lack proper treatment options. A key challenge associated with rare diseases globally is the inability of the medical system to properly diagnose these diseases in a timely manner, leading to a delay in therapy. Early diagnosis is essential for proper disease management. The newborn screening program in the USA covers about 31 metabolic disorders, which, when detected in the neonatal period, can be treated to prevent disability. An example of this is phenylketonuria (PKU), which can be managed nutritionally to save the child from the devastating effects of PKU. Recently, a drug called 'Kuvan' was launched for the BH4 responsive version of PKU (BIOMARIN Pharmaceuticals; http://www.bmrn.com), which precludes the need for expensive dietary therapy. The average time to diagnose most rare diseases in the USA is about 7 years (Shire Human Genetic Technologies, 2013), causing significant anxiety and financial hardship to the families let alone increasing the morbidity in patients. In developing countries, the time to diagnosis is even longer. Even after proper diagnosis, there is little hope for cure. Only around 400 FDA approved 'orphan drugs' are available on the US market, and ~100 drugs approved by the European Medicines Agency (EMA) are available in the EU (Orphanet, 1997). Together, these approved drugs cover only about 11 million patients suffering from rare diseases leaving a majority of patients with no treatment options. Even where treatment is available, the cost is often prohibitive due to high development costs, fewer patients and lack of competition (Nogales, 2004). This is true for the enzyme replacement therapies (ERT) that have been approved for mucopolysaccharidosis types I, II, IV-A and VI, Gaucher disease, Fabry disease and others. The prohibitive costs limit their use to very few patients in India and other low resource countries. Charitable programs started for lysosomal storage disorders by companies like Genzyme, and on a smaller scale by Shire Human Genetic Technologies, are praiseworthy, and provide hope to some patients. These programs have also helped raise awareness among physicians, and stimulated them to make early and precise diagnosis. In India, enzyme therapies are provided either by the Pharma companies under their charitable programs, or by employers in India who are committed to giving 'free' health care to their employees and their dependents. A few associations of families of patients with rare disorders are also trying to persuade the government to cover the cost of therapies. Among the success stories in India is the case of providing Factor VIII to patients with hemophilia A and chelating agents to patients with thalassemia major. In an interesting development a plea was filed in the

Delhi High Court by the father of a 7 year old child suffering from Gaucher disease after being denied treatment by the All India Institute of Medical Sciences (AIIMS), New Delhi, for want of funds. He had lost four children with Gaucher disease. Justice Manmohan, remarking that 'health is not a luxury', and 'should not be the sole possession of a privileged few', asked the Delhi government to discharge its constitutional obligation and provide the child with ERT at AIIMS, free of cost, when required (Provide free treatment to Gaucher disease patient: High Court. News story at: http://www.newkerala.com/news/2014/fullnews-40628.html#.U22oX2xZrVI).

Global organizations devoted to rare diseases

Numerous organizations across the globe are tackling the challenge of rare diseases head on. The names of some such organizations, with URL addresses, are given below in alphabetical order:

- CORD: Canadian Organization for Rare Disorders (http://www.raredisorders.ca)
- EURORDIS: European Organization of Rare Diseases (http://EURORDIS.org)
- GARD: Genetic and Rare Diseases Information Center (https://rarediseases.info.nih.gov/GARD/)
- HMDSN: Hirschsprung's and Motility Disorders Support Network (http://www.hirschsprungs.info)
- INOD: In Need Of Diagnosis (http://www.inod.org)
- IRDiRC: International Rare Disease Research Consortium (www.irdirc.org)
- Jain Foundation (http://www.jain-foundation.org/)
- Madisons Foundation (http://www.madisonsfoundation.org/)
- NORD: National Organization for Rare Disorders (http://rarediseases.org)
- ORDR: Office of Rare Diseases Research (http://rarediseases.info.nih.gov)
- Orphanet (http://www.orpha.net)
- RARE – Rare disease, Advocacy, Research, Education (http://globalgenes.org/leadership)
- Rare Genomics Institute (RGI, USA) (http://raregenomics.org)
- Rare Health Exchange (http://rarehealthexchange.org)
- SWAN: Syndromes Without a Name (http://www.undiagnosed-usa.org)
- Vascular Birthmarks Foundation (http://birthmark.org)

ORDI will extend the work of these existing organizations in rare diseases, and collaborate at the international level, to advance the common objective of finding solutions to the problems of rare diseases and advocate for these patients. ORDI has partnered with RGI USA to institute a process for recruiting patients and their families with undiagnosed diseases (suspected to be familial) into exome-sequencing programs to identify potential causal mutations. ORDI is in discussion with other prominent international organizations to explore opportunities for collaboration and is already mutually cross-referenced with several of them.

Indian organizations devoted to rare diseases

Verma reviewed the burden of rare genetic diseases in India in 2000 and subsequently in 2002 and 2004 (Verma & Bijarnia, 2002; Verma, 2000, 2004). Although there has been improvement in the ability to reduce this burden over the years, still the services are inadequate and much remains to be done. Some of the organizations and resources for patients with rare diseases in India are listed below:

- ARDSI – Alzheimers and Related Disorders Society Of India (http://www.alzheimer.org.in)
- Birth Defects Registry of India (http://www.fcrf.org.in/bdri_abus.asp)
- Down Syndrome Federation India (http://downsyndrome.in/)
- Fragile X Society – India (www.fragilex.org)
- Genetic Alliance (http://www.geneticalliance.org)
- Hemophilia Federation (http://www.hemophilia.in/)
- Indian RETT Syndrome Foundation (www.rettsyndrome.in)
- Indian Association of Muscular Dystrophy (www.iamd.in)
- Indian Prader-Willi Syndrome Association (http://pwsindia.hpage.com)
- IPSPI – Indian Patients Society for Primary Immunodeficiency (www.ipspiindia.org)
- LSDSS – Lysosomal Storage Disorders Support Society (www.lsdss.org)
- MERD – Metabolic Errors and Rare Diseases (http://merdindia.com)
- Muscular Dystrophy Association India (http://mdindia.org/)
- Muscular Dystrophy Foundation India (http://www.mdfindia.org)
- Muskaan (intellectually disabled) (http://muskaan-delhi.com/)
- National Thalassemia Welfare Society (http://www.thalassemiaindia.org/)
- Pompe Foundation (http://pompeindia.org/)
- Rare Diseases India (http://www.rarediseasesindia.org)
- Retina India (http://www.retinaindia.org)
- Sjogren's India (http://www.sjogrensindia.org)
- Thalassemics India (www.thalassemicsindia.org)

These organizations render yeomen services to the patient community. Internationally, umbrella organizations such as NORD, EuroRDIS, Genetic Alliance and Global Genes RARE have played key roles in

engaging with stakeholders primarily in the USA and Europe. In India, an organization that can unite all rare disease stakeholders under a single umbrella, and speak in a single voice for them does not exist. The lack of such an umbrella organization has reduced the effectiveness of the above mentioned organizations as much of their resources are directed towards common causes such as raising general public awareness about rare diseases. As a result, progress in assisting patients with rare diseases is slow, leaving many patients hapless. The need for an umbrella organization that can provide a common framework for these disease-specific organizations to function effectively and to focus on their mission is clear. Such an organization could provide generic patient registries compliant with regulatory requirements in India, develop and maintain a comprehensive information portal about rare diseases, create and maintain a sample biorepository for use by Indian rare disease researchers in approved translational research studies, interface with international resources, broadcast best practices, raise public awareness about rare diseases, host national and international conferences and other events to engage key stakeholders, and create an ecosystem of incentives to accelerate research, development and delivery of affordable diagnostics and treatment options for patients with rare diseases. Bringing the various associations under one organization will give it greater leeway to lobby with the government, international agencies and philanthropists for help and support. It is ORDI's objective to fill these gaps. It must be stated that getting these different organizations in India to work under one umbrella organization would not be an easy task. In this context, the response from Indian organizations, since the launch of ORDI on 18 February 2014 in Delhi, has been most encouraging. The ORDI's nationwide rare disease telephone helpline receives on average, 3–4 enquiries every day. A total of 13 Indian organizations have already joined, as they are convinced that joint effort will be more rewarding than their individual efforts.

History of rare diseases in India

Extensive haplotyping studies have predicted that most present day Indian populations are descendants of ancestors who migrated out of Africa to the Indian continent about 65,000 years ago (Tamang *et al.*, 2012) Evidence suggests that today's Indian population is an admixture of two genetically divergent ancient populations, referred to as the Ancestral North Indians (ANI), who are genetically close to Middle East, Central Asian and European populations, and the Ancestral South Indians (ASI), who are less so (Reich *et al.*, 2009). This study estimated that ANI ancestry ranges from 39–71% among the various current Indian populations, with pure ASI groups represented by the indigenous Andaman Islanders (Reich *et al.*, 2009). Consanguineous marriages take place preferentially in many communities, while in other ethnic groups, endogamous marriages have occurred over long period of time. As a result, the frequencies of founder and common mutations are likely to be relatively higher in the Indian subpopulations. Rough estimates show that more than 56 million individuals in India are likely to be affected by single gene disorders (monogenic disorders) (Global Genes. RARE Facts and Statistics). With the lack of awareness in the general population about genetic disorders, and scarcity of specialized medical professionals and affordable genetic tests, the burden from these disorders is growing rapidly. This is, in part, due to the absence of a properly functioning social health care system in India, where the health professionals, including doctors and nurses, are not given enough exposure to medical genetics, molecular biology and rare disorders in their curriculum. There is also insufficient encouragement by the government for individual health insurance. Consequently, most of the population, especially in rural areas, does not opt for prenatal testing, predictive genetic diagnosis or timely genetic counseling with some families having more than one affected individual. The WHO has stressed the need for prevention, early diagnosis and management of genetic disorders in developing countries, and has issued detailed guidelines (http://www.doh.gov.za/docs/policy/humangenetics.pdf).

Over a decade since the completion of the Human Genome Project, awareness about genetic disorders among physicians as well as the general population of India is still lacking. As a result, early and affordable diagnostic tests, even where available, are not widely prescribed. To remedy this, a few accredited and reputed educational institutions, hospitals and laboratories across the country have initiated genetic diagnostic services covering selected disorders. A directory of accredited genetic testing service centers in India compiled in 2007 (Singh *et al.*, 2010) showed that there were 47 such centers offering genetic services, including cytogenetic (40 centers), biochemical (26 centers) and molecular diagnosis (26 centers), along with genetic counseling. A current directory of genetic centers and services is now available online http://www.geneticsindia.org. This is supported by the Indian Council of Medical Research (ICMR). It has listed 649 disorders, 66 genetic centers and 35 prenatal diagnostic centers. Another ten genetic counseling centers are known to the authors that are not yet listed on the website. It has also started listing the parent support groups for various diseases in India. The number and distribution of these centers are abysmally small to serve the massive Indian rare disease community. Many of the centers need to upgrade

capabilities to include recent advances such as next-generation sequencing (NGS)-based molecular diagnosis of rare diseases. Most of the genetic centers offer targeted tests that involve screening for common mutations, although in recent years, many centers provide sequencing of entire disease-associated genes. A number of private companies have set up NGS technologies, harnessing the excellent bioinformatics resources available in India. They are providing molecular diagnosis based on NGS of disease gene panels at a fraction of the cost of these tests abroad, making them affordable to the Indian population. However, some samples are still sent abroad for genetic testing. International laboratories (Quest, Centogene, BGI China and Core Diagnostics) have also set up offices in India. Some Indian national laboratories such as SRL Labs and Lal Path Labs also offer advanced medical and genetic tests. Therefore, an extensive network of collection centers has spread all over India. This enables every person, even in small remote towns, to provide samples for advanced genetic and biochemical tests. This has tremendously improved the diagnostic abilities for genetic tests in the country.

Education and genetic counseling (GC) are critical necessities to help patients and physicians deal with rare diseases. GC is needed at various levels such as prior to genetic testing, post-testing, prenatal diagnosis and family planning particularly in consanguineous marriages. GC needs to be made an integral part of all genetic testing centers in India. This has been realized by the private genetic laboratories and they have appointed genetic counselors on their staff. There are at least three institutions offering courses in GC for non-medical personnel. There is a move by one of the authors of this paper to initiate a GC course under the Indira Gandhi Open University. ORDI will provide expertise for this venture by enlisting experts from the USA to assist the faculty in India. This will, to some extent, fill the gap of qualified genetic counselors in India.

The burden of providing care for an individual affected by rare disease is not easy to meet in India due to lack of infrastructure. Therefore, determining carrier status for genetic diseases can help individuals make decisions about prenatal testing when both partners are carriers of a particular disease. In urban areas at least, most obstetricians screen for common infections and thalassemia. After that, the family history guides the screening strategy. Carrier testing for genetic diseases using NGS of a panel of genes is being offered by one company now, while others are in the process of validating such tests. A number of institutions, both in the public and private domains, provide GC to patients.

The National Board of Examinations, with support from the Department of Biotechnology, Government of India, is due to start a national postgraduate course in Medical Genetics and Genomics. The Medical Council of India (MCI) has expanded the curriculum for medical students to include genetics and molecular biology (Vision Document 2015: http://www.mciindia.org/tools/announcement/MCI_booklet.pdf). What is still woefully inadequate is the number of medical genetics departments in the country. To meet the shortage of medical doctors and to ensure that rural populations are better served, the Ministry of Health and Family Welfare, Government of India, pushed for a 3 year B. Sc. course in Community Medicine to be imparted to doctors in Ayurveda and other indigenous systems of medicine. The course has been approved by the MCI (Sinha, 2012). The number of seats in medical courses, at both the graduate as well as the postgraduate level has increased by 1·5 to 2 times in a short time span. Kapoor *et al.* (2013) reviewed the challenges and opportunities for newborn screening (NBS) in India and recommended widespread implementation of the NBS program across the country starting with the metro cities. At least six private laboratories offer newborn screening through tandem mass spectrometry and gas chromatography mass spectrometry at a nominal fee. Many private hospitals have instituted NBS programs. Some states like Gujarat, Chandigarh, Delhi, Maharashtra, Kerala, Goa and Tamil Nadu, have started pilot NBS programs, and it is expected that these will be further extended to cover larger populations in the near future.

India-specific challenges in the management of rare diseases

India faces numerous challenges in awareness, public perceptions, diagnosis, treatment and public policy on rare diseases. Some of the most significant ones are listed below.

Lack of awareness

There is significant lack of awareness about rare diseases among the lay public, and unfortunately even among physicians in India. The health care training and education system in India are more focused on training physicians and health care personnel to treat common diseases such as infectious diseases, diabetes mellitus, cardiovascular disease and common cancers. This has led to a pronounced dearth of trained physicians and health care personnel to care for patients with rare diseases.

Lack of infrastructure

Inadequate training and facilities to properly diagnose rare diseases in a timely manner is another disadvantage. If it takes an average of 7 years to diagnose a rare disease in developed nations like the USA (Shire Human Genetic Technologies, 2013) the average time

to diagnose is likely to be greater in developing countries (Christianson & Modell, 2004). It is unclear as to the average time that it may take to diagnose a rare disease in India. There is lack of adequate statistics and data on incidence as well as prevalence of rare diseases in India. A systematic catalogue of the different rare diseases in India does not exist. The availability of such basic information is essential for use by policy makers, physicians, scientists, drug or device manufacturers, patients and the community at large.

Prohibitive costs

A majority of patients with rare diseases in India cannot afford the high costs of treatments even when available. Most orphan drugs are not curative but palliative, and need to be administered regularly. These drugs are highly expensive and inaccessible to the majority of the Indian population affected by rare diseases.

Cultural influences

The incidence of rare diseases is believed to be higher in India compared to western countries due to practice of consanguineous marriages in many communities. Social stigmas for disabled individuals and patients with rare diseases continue to be a societal challenge that can only be addressed with education and awareness.

Government initiatives

There seems to be no official policy on rare diseases in India. There is no specific push for research and development in the field of rare diseases in India, although the programs by funding agencies like the ICMR, Department of Science and Technology (DST), Council of Scientific and Industrial Research (CSIR) as well as the Department of Biotechnology (DBT) are noteworthy. The Government should encourage and fund Indian academic research laboratories as well as pharmaceutical and biotechnology industries to take up scientific research work leading to the development of diagnostics and drugs for rare diseases. ORDI would persuade and assist the government to (i) enact an act similar to the Orphan Drugs Act (ODA) of USA, and (ii) create a fund to support work related to rare disease research, education and treatment by institutions that are prepared to take up this work. The Government has already started funding such work in a small way but we believe that a lot more needs to be done. A national plan for rare disease research needs to be developed.

Funding

The lack of significant public funding for rare disease research is another critical impediment in creating the much needed momentum for education, diagnosis and treatment of rare diseases in India.

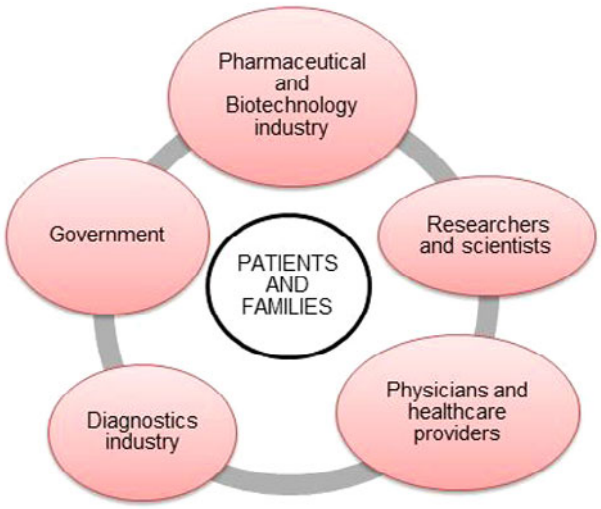

Fig. 1. Key stakeholders of rare diseases in India.

The rare disease stakeholders in India

Fig. 1 depicts the key stakeholders of rare diseases in India. The patients and their families are the most important stakeholders as they experience the disease first hand. The other important stakeholders such as physicians, health care providers, researchers, diagnostic laboratories, biotechnology and pharmaceutical companies, government, regulatory bodies, non-profit patient organizations and health insurance companies have a significant impact on the rare disease patients and their families.

Patients and families

Patients with rare diseases and their families need quick and easy access to information about affordable diagnosis and treatment options. A single portal for such information would serve the purpose and is urgently needed. The ORDI has already provided such a portal and receives many enquiries every day. The existing parent support groups also play an important role in providing such information, and in providing a forum for parents of children with specific diseases to exchange experiences.

Physicians and health care providers

Indian doctors need access to more clinical training, research and education on rare diseases. In addition, they need access to state-of-the-art diagnostic tests, information on clinical trials and currently available drugs for rare diseases in India. ORDI could help organize workshops to meet this need. The ratio of doctors to patients in India continues to be

significantly lower compared to those in developed countries and compared to the ratio recommended by WHO. Training in medical genetics needs to be scaled up urgently across the country.

Scientists and researchers

Indian scientists need access to rare disease patient registries and biorepositories to advance science and understanding of these diseases in order to translate research findings into diagnostic tests and therapeutics. Biorepositories that house patient samples and associated clinical, genomic, transcriptomic, proteomic and metabolomic data will be the main catalysts of therapeutic and diagnostic solutions for rare diseases. Defective genetic pathways contribute to a great extent to the pathogenesis of rare diseases (Global Genes. RARE Facts and Statistics). Molecular diagnostic technologies such as NGS offer great opportunities to sequence the genome or targeted regions of a patient's DNA at an affordable cost to identify mutations that may be associated with a disease. There is a need and immediate opportunity to accelerate the identification of as yet unknown critical genes involved in rare diseases. To establish a strong association between a mutation and disease, a large number of patient samples are often required. Dalal *et al.* (2012) report on an Indian cohort study involving 35 progressive pseudorheumatoid dysplasia patients harboring mutations in the *WISP3* gene. Such studies will clearly benefit the populations in the Indian subcontinent. Inherited disease-causing mutations could be detected by comparing DNA sequencing data from the trio (patient and parents). Being able to access patient samples could help identify associated mutations that could lead to drug targets and thereby therapies. Moreover, identification of rare disease genes and mutations can advance our overall understanding of underlying biological mechanisms of more common human diseases. For example, understanding the involution process in vascular tumors such as hemangiomas (Haggstrom *et al.*, 2006), a rare disease, will benefit the understanding of human solid tumor regression biology.

Pharmaceutical and biotechnology industries

The lack of an Orphan Drug Act type of legislation in India is hampering the development of indigenous development of drugs for rare diseases. While the opportunity for growth in the rare disease market is high from patients/providers' perspectives, industry faces mixed benefits and barriers. Orphan drugs are relatively non-profitable as rare diseases affect a small patient population, and usually are unaffordable to Indian patients as the drug cost per patient far exceeds the per capita income. Hence, the Indian pharma/biotech industry needs incentives for investing in research and development of orphan drugs. A stable and reliable regulatory environment conducive to investments in long-term R&D is also desired. If the Indian pharmaceutical companies take up manufacturing of products for rare disorders this would bring the cost down not only for the Indian market, but also for the whole world. An example is the manufacture and supply of anti-retroviral drugs by CIPLA, a pharmaceutical company in India that helped tremendously in controlling AIDS across the world. Some global pharma companies, such as Genzyme, have developed charitable access programs for orphan drugs in developing countries including India. The Gaucher initiative to provide Genzyme's first approved orphan drug (1999), called Cerezyme, is one such example. Such initiatives work with humanitarian organizations but a commitment from the Government of India to support the cause of rare diseases in the form of an ODA will clearly improve accessibility to orphan drugs. The policy to import orphan drugs into India also needs a fresh re-evaluation, as companies should be permitted to import free of duty.

Government of India

The support of the Indian government is critical to the success of health initiatives such as the one we are proposing for rare diseases. The government should play an active role in addressing the enormous health care challenges posed by rare diseases through various mechanisms such as public funding for research in orphan diseases, creating business friendly policies for pharmaceutical and biotech companies and developing a balanced regulatory framework that catalyses innovation and protects the safety of patients.

ORDI vision

Our vision is to make rare diseases as easily diagnosed and treated as common diseases are in India. Collection of epidemiologic data, catalysing research and facilitating creation of registries and biorepositories would be high on our agenda.

ORDI mission

We at ORDI aim to be the umbrella organization, uniting and providing a common forum for all individual disease-specific organizations in India. It will have branches in all the state capitals. It will collaborate with other parent support groups and help to initiate new parent groups for disorders that currently do not have one. It will obtain funds from corporate houses, pharmaceutical companies, private genetic laboratories and foundations both in India and abroad. It will formulate plans of action on various

topics involving rare disorders, such as epidemiology, natural history, mechanisms of disease and treatments. It will seek support for these plans from the Government of India – DST, ICMR, DBT, CSIR, other Government agencies, and philanthropists. Memberships will be available for parent support organizations, individual patients, various categories of health professionals, hospitals (both public and private), corporate houses and pharmaceutical companies for the following mission:

- Create awareness for rare diseases all over India using mass media, newspapers, television, social media, pamphlets and posters.
- Set up patient registries for the more prevalent 'rare disorders'.
- Represent the collective voice and concerns of over 70 million patients with rare diseases in India.
- Obtain concessions from the local governments (as health is a state subject in India) for travel, treatments and jobs.
- Work with the government of India to create an optimal business and regulatory environment for the diagnostics and drug development industry (pharmaceutical and biotechnology).
- Catalyse rapid development and delivery of affordable diagnostics and treatments for rare diseases in India through innovative collaborations and partnerships among stakeholders.
- Advocate investments in rare disease research, diagnostics and drug development.
- Work with the insurance regulatory agency to ensure non-discrimination in health insurance based on the genetic constitution of an individual.
- Organize national and international conferences in India on rare disorders to create awareness and promote research and development of therapies.
- Provide assistance for rare disease patients to the largest possible extent.
- Service a 24/7 rare disease helpline that has been launched to provide assistance to patients. A proposal by ORDI to develop this helpline into a comprehensive rare disease care coordination center is currently being reviewed by pharmaceutical companies in India.

Future perspectives

Although the challenges for rare diseases appear to be of Himalayan (pun intended) proportions, the advent of precision medicine, otherwise referred to as personalized medicine, offers hope in the diagnosis and treatment of rare diseases. Precision medicine is being applied in relatively common disorders such as cancer, immune diseases and infectious diseases. It is based on the premise that by stratifying patients with similar genomic, transcriptomic, proteomic and metabolic biomarker profiles, it is possible to direct specific therapies to achieve higher levels of efficacy and reduce drug toxicities. The 'blockbuster model' of pharmaceutical companies is no longer tenable in this scenario and hence they are already developing therapies for smaller patient populations with relatively common disorders such as cancer, immune diseases and infectious diseases. All of the recent epochal advances in drug development, diagnostics and regulatory paradigms are likely to have a beneficial impact in driving precision medicine into rare disease clinics across the world.

India has a high potential to cost-effectively develop and manufacture small molecule drugs, biologics and vaccines for rare diseases due to its inherent capabilities in drug and vaccine development and manufacturing (Smita, 2006; Chakma *et al.*, 2011). India is already considered as a global hub for vaccines as it supplies close to half of the world's childhood vaccines (Virk, 2010). In addition, the small molecule drug development and manufacturing capabilities of Indian pharmaceutical companies are well recognized, especially in the generic market segment (Genetic Engineering and Biotechnology News, 2006; Kale, 2012). The biosimilars (generic biologic drugs that are produced using recombinant DNA technology such as monoclonal antibodies, hormones and cytokine therapeutics) segment is likely to experience significant growth in India in the coming years with numerous biologics likely to go off patent adding to India's capabilities in the biologic manufacturing arena (Mukherjee, 2010).

The rapid growth and innovations in molecular diagnostics and bioinformatics globally are also likely to have a beneficial impact on precision diagnostics for rare diseases. The advent of NGS in the clinic has led to the application of multi-gene, exome and whole genome-based diagnostic tests. India's strength in information technology and bioinformatics will be highly beneficial in the development and democratization of precision diagnostics for rare diseases.

The government of India has a crucial leadership role to play in advancing progress in this area. We persuade the government agencies to sponsor various activities beginning with a national level assessment of the needs of various stakeholders in the rare diseases community. India can draw upon the model that the US government developed to support rare diseases. The US government funded rare disease community needs assessments on three occasions: first, in the 1970s, approximately 5 years prior to the enactment of ODA; second, in the 1980s about 5 years after the ODA and then as part of a Special Emphasis Panel on the Coordination of Rare Diseases Research in 1998. The surveys completed examined the needs and priorities of the patients/families, physicians and medical specialists, research investigators,

voluntary health organizations (patient advocacy groups), the pharmaceutical industry, and philanthropic foundations, regulatory agency (FDA), biomedical research agency (National Institutes of Health (NIH)) and other government agencies such as Health Resources and Services Administration (HRSA) and the Centers For Disease Control And Prevention (CDC). According to the office of rare disease research (ORDR) at the NIH, these surveys helped identify the needs and opportunities to implement specific recommendations provided by the stakeholders. In many cases, these recommendations even became part of the legislative initiatives such as the ODA in 1983 and then the Rare Disease Act of 2002. Innovative government funded programs such as the Rare Disease Clinical Research Network (RDCRN; http://rarediseasesnetwork.epi.usf.edu), have made significant positive impact to the overall cause of rare disease patients in the USA. We urge the government of India to similarly take a lead in initiating and funding such needs assessments formally, to pave the way towards developing a roadmap for tackling rare diseases in India.

Developments in India

In recent years, some significant developments have taken place that will change the future of health care in India. First, the launch of the Rasthtriya Bal Swasthya Karyakram (National Health Program for Children), on 6th February 2013 (Rashtriya Bal Swasthya Karyakram, 2013). It covers 270 million children starting from birth to 18 years of age, in a phased manner and moving towards the goal of Universal Health Coverage. This program screens for 30 health conditions among children including defects at birth, deficiencies and diseases, development disabilities and also helps manage these conditions. The following conditions have a significant genetic component and will be screened and managed: impairment of vision, hearing, neuro-motor system, delay of motor functions, cognition and language, autism and attention deficit hyperactivity disorder. Birth defects such as neural tube defects, Down syndrome, cleft lip and palate, club foot, developmental dysplasia of hip, congenital heart disease, congenital deafness and congenital cataracts will also be included. Second, is the significant lowering of the infant mortality rate (IMR) across India due to government initiatives in health care. The IMR is currently 42 per 1000 at the national level, 46 in the rural areas and 28 in the urban areas (Mukherjee, 2010). Many states have an IMR of less than 20. The WHO has recommended that genetic services should be established without fail in countries with an IMR of less than 50 (Sample Registration System Bulletin, 2013). Third, is the changing pattern of diseases in India from communicable and nutritional disorders to predominance of non-communicable disorders. Finally, an enlarging and expanding private health sector and genetic laboratory services are driving health care and public awareness for genetic disorders. These events are likely to effect a remarkable change in the care and cure of rare disorders in India. ORDI has its work cut out to play a major role in this transformation.

Contact us

Visit us online at www.ordindia.org. Email your comments and suggestions to contactus@ordindia.org. We have launched the first rare disease telephone helpline in India to guide patients with rare diseases through their journey by connecting them with experts and parent support groups: +91 8892 555 000.

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On 16 May 2013, the first broader brainstorming session was organized to gather feedback from various stakeholder groups in India. We received valuable suggestions from the diverse participants at this event hosted by Strand Life Sciences, Bengaluru, India, as well as offline from thought leaders in India and abroad. Individual team members have gathered feedback from a large number of relevant people via informal discussions, e-mails, the Facebook page and LinkedIn group discussions. Much of this feedback has defined the core principles of ORDI as a neutral, non-profit organization. The authors wish to thank all organizations and individuals for volunteering their time and sharing their perspectives during informal meetings, discussions, brainstorming sessions, and teleconference calls. Many of these organizations are listed in the body of this manuscript. In particular, we thank Dr Stephen C. Groft and Dr Rashmi Gopal-Srivastava at the NIH office of rare disease research for their guidance, support and contribution, based on 30+ years of experience in rare diseases in the USA and worldwide. Dr Rajat Agrawal from Retina India and Dr Linda Shannon-Rozell from vascular birthmarks foundation are among other organizations that offered detailed discussion and suggestions. We thank everyone who has contributed directly or indirectly to the formation of this important initiative.

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Annexure 2

Next-generation sequencing-based method shows increased mutation detection sensitivity in an Indian retinoblastoma cohort

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Purpose: Retinoblastoma (Rb) is the most common primary intraocular cancer of childhood and one of the major causes of blindness in children. India has the highest number of patients with Rb in the world. Mutations in the *RB1* gene are the primary cause of Rb, and heterogeneous mutations are distributed throughout the entire length of the gene. Therefore, genetic testing requires screening of the entire gene, which by conventional sequencing is time consuming and expensive.
Methods: In this study, we screened the *RB1* gene in the DNA isolated from blood or saliva samples of 50 unrelated patients with Rb using the TruSight Cancer panel. Next-generation sequencing (NGS) was done on the Illumina MiSeq platform. Genetic variations were identified using the Strand NGS software and interpreted using the StrandOmics platform.
Results: We were able to detect germline pathogenic mutations in 66% (33/50) of the cases, 12 of which were novel. We were able to detect all types of mutations, including missense, nonsense, splice site, indel, and structural variants. When we considered bilateral Rb cases only, the mutation detection rate increased to 100% (22/22). In unilateral Rb cases, the mutation detection rate was 30% (6/20).
Conclusions: Our study suggests that NGS-based approaches increase the sensitivity of mutation detection in the *RB1* gene, making it fast and cost-effective compared to the conventional tests performed in a reflex-testing mode.

Retinoblastoma (Rb) is a malignant tumor of the developing retina that occurs in children, usually before the age of five years, and it causes childhood blindness [1]. According to the World Health Organization (WHO), the average age-adjusted incidence rate of Rb in the United States and Europe is 2–5 cases per million children (approximately 1 in 14,000–18,000 live births) [2]. As per the latest National Cancer Registry Program (NCRP) report, in India, the age-adjusted rates of Rb incidence are estimated to be 1.9–12.3 and 1.3–6.7 per million in boys and girls, respectively [3]. Due to its early age of occurrence and the risk of second cancers (soft tissue sarcomas, osteosarcomas, and melanomas) at later stages of life, early molecular diagnosis and treatment options must be considered for better management of the disease [4,5].

India has the highest number of Rb cases, where almost 20% of the world's Rb patients reside in India [4]. In developed countries, children with Rb have a disease-free survival rate greater than 90%, compared to developing nations, where it is substantially lower, at 10–30% [6,7]. As with other developing countries, late diagnosis, lack of awareness, and the inaccessibility of specialized care are the major reasons for tumor metastasis in India [4]. The burden of Rb on the Indian health care system has been steadily increasing, thus stressing the need for cost-effective methods for early detection, surveillance, and disease management.

Rb is a tumor that occurs in both heritable (25–30%) and non-heritable (70–75%) forms. A heritable disease is defined by the presence of a germline mutation in the *RB1* gene (Gene ID: 5925, OMIM 614041), which is followed by a somatic mutation in the developing retina. It can result in tumors affecting either one (unilateral) or both (bilateral) eyes. In the non-heritable form of Rb, both mutations occur in the somatic cells, leading only to unilateral tumors [8]. Usually, a familial, bilateral, or multifocal disease is suggestive of a heritable disease, whereas older children with a unilateral tumor are more likely to have the non-heritable form of the disease [9].

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In India, the few studies that have been conducted to determine the prevalence of *RB1* mutations in various Indian cohorts reported mutation detection rates ranging from 33% to 85% for both unilateral and bilateral cases [10-14]. In one of the first studies in India, Ata-ur-Rasheed et al. screened 21 patients with Rb using the Sanger sequencing method and identified *RB1* mutations in seven patients, and the mutation detection rate was 33.3% [11]. In another study, Kiran et al. screened 47 patients by single-strand conformation polymorphism (SSCP) followed by sequencing and reported a mutation detection rate of 46% [13]. The screening of a relatively large cohort of 74 patients using a combinatorial approach including fluorescent quantitative multiplex PCR, fluorescent genotyping, restriction fragment length polymorphism (RFLP), and sequencing, Ali et al. reported a detection rate of 66% [10]. In a recent study, Deverajan et al. screened 33 patients from Southern India by targeted next-generation sequencing (NGS) and reported a mutation detection rate of 85% [12]. Collectively, from these studies, it is evident that there is a high variability in the reported detection rates of *RB1* mutations in various Indian cohorts.

The *RB1* gene shows a wide spectrum of mutations, including single nucleotide variations (SNVs), small insertions/deletions (indels), and large deletions/duplications. These mutations are distributed throughout the entire length of the gene, spanning 27 exons, and no hotspots have been reported. Conventional genetic testing of the *RB1* gene involves screening of all 27 exons and the flanking intronic regions by Sanger sequencing, followed by a deletion/duplication analysis by multiplex ligation-dependent probe amplification (MLPA). This sequential testing strategy performed in a reflex-testing mode is time consuming and expensive. New advances in genomic technologies, such as NGS, allow us to detect all types of variants, such as SNVs, indels, and structural variants, including large deletions/duplications, at a significantly lower cost than traditional methods. In the current study, we used an improved NGS-based method to screen the *RB1* gene in the DNA isolated from blood or saliva samples from an Indian Rb cohort (50 cases) and detected all types of germline mutations, including large deletions ranging from a single exon to a whole gene (>178 kb) deletion. Moreover, we report a mutation detection rate of 100% in bilateral Rb (22) cases.

METHODS

Clinical diagnosis and patients: Saliva or peripheral blood samples were obtained from 50 unrelated patients with an indication of Rb referred to our laboratory between March 2014 and January 2016. Informed consent was obtained from all subjects and sequencing of the patients' samples for this study was approved by the Institutional Ethics Committee of Strand Life Sciences. A clinical diagnosis of Rb was confirmed through a clinical examination conducted by the referring ophthalmologist. There were 20 patients with unilateral Rb, 22 patients with bilateral Rb, and 8 patients with unavailable information on laterality.

DNA was extracted from saliva samples using the PrepIT-L2P kit (DNA Genotek, Canada), as per the manufacturer's instructions. For blood samples, either the QIAamp DNA Mini Kit (Qiagen, Germany) or the Nucleospin kit (Macherey-Nagel, Germany) was used for DNA isolation, as per the manufacturer's instructions. The concentration of DNA was determined using the Qubit fluorimeter (Life Technologies).

Library preparation and targeted NGS: Targeted NGS was performed on patient genomic DNA using the Trusight Cancer sequencing panel (Illumina) that contains 1,736 genomic regions from 94 genes suspected of having a role in cancer predisposition, including the *RB1* gene. An analytical validation of our panel has shown a sensitivity of 98.2%, specificity of 100%, and reproducibility of 99.5%. The gene coverage analysis on this panel revealed that exonic and flanking intronic regions of the *RB1* gene (NM_000321) showed coverage of >99% (≥ 20 reads) with a mean read depth of 405X. The Nextera DNA library preparation protocol (Illumina) to convert input genomic DNA (gDNA) into adaptor-tagged indexed libraries was essentially performed as previously described [15]. The tagged and amplified sample libraries were checked for quality and they were quantified using the BioAnalyzer (Agilent). Up to 6–10 pM of the pooled library was loaded and sequenced on the MiSeq platform (Illumina), according to the manufacturer's instructions.

NGS – data analysis and interpretation: The trimmed FASTQ files were generated using MiSeq Reporter (Illumina). The reads were aligned against the whole genome build: hg19 using Strand NGS v2.5. Data analysis and interpretation were performed using Strand NGS v2.5 and StrandOmics v3.0 (a proprietary clinical genomics interpretation and reporting platform from Strand Life Sciences), as previously described [15]. In brief, StrandOmics is a clinical interpretation and reporting platform that combines knowledge from internal curated literature content (approximately 40,000 extra curated variant records), along with various publically available data sources such as Uniprot, OMIM, HGMD, ClinVar, ARUP, dbSNP, 1000 Genomes, Exome Variant Server, and Exome Aggregation Consortium (ExAC). In addition to databases, bioinformatics prediction tools, such as SIFT, PolyPhen HVAR/HDIV, Mutation Taster, Mutation Assessor,

FATHMM, LRT for missense variants, and NNSPLICE; and ASSP tools for variants in essential splice sites and exon–intron boundaries, have also been integrated to assess the pathogenicity of the variants. This integrated knowledge is then used to prioritize automatically a list of variants based on American College of Medical Genetics and Genomics (ACMG) guidelines [16], the inheritance model, disease phenotype, sequence conservation across various species, and allelic frequency in our laboratory's internal patient pooled database (PPDB). A variant was labeled 'novel' when it had not been previously reported in the literature or in any public database (as mentioned above).

Variant calling and classification: Reads with average base quality <Q20 were excluded from the variant calling process, and the Bayesian approach was used to identify the consensus genotype at the variant locus. Each called variant was assigned a Phred equivalent score that represents base-calling error probabilities. The identified variants in this study were called with a read quality >Q30 and a confidence score >50.

The identified variants were labeled according to the ACMG recommended standards for the interpretation and reporting of sequence variations [16]. The variants were classified into five categories: 1) pathogenic, 2) likely pathogenic, 3) variant of uncertain significance (VUS), 4) likely benign, and 5) benign.

Copy number variation analysis for large deletion/duplication: In addition to SNVs and small indels, a copy number analysis was performed to identify large deletions or insertions ranging from a single exon to whole gene deletion. This was done by taking each non-overlapping target region in turn, of which there are 1,736, and comparing normalized read coverage across 8–11 other samples from the same run. Normalized coverage-based copy number values (CNVs) and Z-scores [17] for each panel region were computed using StrandNGS v2.5. For each sample, potential copy number changes in the *RB1* gene were identified by manual interpretation based on the following cut-offs: CNV >3, Z-score >2 for duplications and CNV <1.2, Z-score <-2 for deletions.

Split read analysis for the identification of break points: Reads that did not align with an alignment score >95% were subjected to split read alignment [18]. Here, the input reads were split into two segments and each segment was mapped independently to the reference genome. The minimum size of the major segment was 35 bp and that of the minor segment was 15 bp. The split segments were required to align uniquely, with an alignment score of at least 97%. Based on these split read alignment scores, a structural variant (SV) caller was used to call out large deletions, insertions, inversions, and translocation events. These split read alignment and SV calling algorithms are integrated into StrandNGS v2.5, which was used to perform this analysis. A threshold of five split reads supporting the SV event was used for calling them out. Further confirmation of the SV event was performed by looking at the event in the StrandNGS elastic genome browser and verifying that the break points across all split reads are unique and that the other partially aligned reads support the same event. For deletion events spanning one or more exons, the CNV analysis would also show significantly lower normalized coverages at these locations, thus providing further evidence of the event.

Confirmation of the detected variants by Sanger sequencing or MLPA: All the pathogenic variants detected in the patient samples were confirmed by Sanger or MLPA. In case of SNVs and indels, primers flanking each variant were designed, and the genomic region encompassing the variant was amplified by PCR. Details of primer sequences and PCR conditions are provided in Appendix 1 (Appendix 1, Appendix 2, Appendix 3, Appendix 4, and Appendix 5 are available as online supplementary information). The PCR products were purified using the Gene Jet PCR Purification Kit (Thermo Fisher), according to the manufacturer's instructions. The purified PCR products were sequenced using both forward and reverse primers (which were used for the PCR amplification) using the BigDye® Terminator v3.1 kit (Life Technologies). The sequencing PCR products were purified and subsequently analyzed by the 3500DX Genetic Analyzer (Life Technologies), as described previously [15]. MLPA was performed with 50 ng of gDNA, according to manufacturer's instructions, using the SALSA MLPA P047-*RB1* kit (MRC-Holland, The Netherlands). Probe amplification products were run on the Genetic Analyzer 3500DX (Life Technologies). MLPA peak plots were visualized and normalized, and the dosage ratios were calculated using the Coffalyser.Net software (MRC-Holland, The Netherlands). A threshold ratio of >1.3 denotes duplication and a ratio of <0.7 denotes deletion.

RESULTS

The mutation spectrum in the patients with Rb: In total, we screened 50 DNA samples of unrelated patients with Rb for mutations in the *RB1* gene using NGS. The demographic profile and clinical characteristics of all the subjects are provided in Appendix 2. In 33 patients, pathogenic or likely pathogenic variants (hereby referred to as mutations) were identified (Table 1 and Figure 1), accounting for 66% (33/50) of all cases (Figure 2A). The spectrum of identified mutations includes 19 SNVs (11 nonsense, three missense, and five splice site variants), eight indels (six deletions, one indel, and one duplication), and six large deletions (single

exon deletion to whole gene deletions; Figure 3). All the SNVs and indels identified by NGS were confirmed by Sanger sequencing, and large deletions were confirmed by MLPA analysis, which implies 100% concordance between the NGS findings and Sanger/MLPA data. We detected 29 unique mutations, of which 12 were novel (Table 1). None of the 12 identified novel mutations in our study were found in the 1,200 control chromosomes. Interestingly, among the 11 nonsense mutations identified in our study, the majority (91%) were substitutions of arginine residue to stop codon due to a C to T transition (Table 1). We also detected two recurrent nonsense mutations: p.Arg455Ter (3X) and p.Arg579Ter (2X; Table 1). We detected three missense mutations (p.Gln702Lys, p.Cys712Arg and p.Trp563Cys), all of which lie in the A/B "pocket" domain of the protein [19,20].

Correlation between laterality and mutation detection rate: To determine whether the mutation detection rate in our screen was correlated with the laterality of the Rb patients, we stratified the patients into three categories, namely bilateral, unilateral, and unknown laterality. Of the 50 patients, 22 were diagnosed with bilateral Rb (BRb), while 20 patients showed a unilateral form of Rb (URb). For eight patients, laterality information was unavailable. In BRb patients, the mutation detection rate was 100% (22/22; Figure 2B). In URb cases, the mutation detection rate was 30% (6/20; Figure 2C) and in unknown cases, mutations were detected in 62.5% (5/8) of patients (Figure 2D). Overall, the mutation detection frequency was 66% (33/50 cases; Figure 2A).

Detection of large deletions in the RB1 gene: Using the CNV analysis, we detected six large deletions in our cohort. The spectrum of deletions ranged from a single exon deletion (one case) to multi-exon (three cases) to whole gene deletions (two cases; Table 1). The deletions identified by NGS in the patient samples (RB6, RB30, RB31, RB32, and RB33) were confirmed by MLPA (Appendix 3). In two of these samples (RB6 and RB31), we were able to detect the exact break point of the identified deletion in the genomic sequence by a split-read alignment analysis (Appendix 4). In patient RB6 with URb, the deletion of exons 8–11 was detected by CNV analysis. Using the split-read alignment of the sequence reads, the 5′ break point could be identified at 2,574 bp upstream (chr13:4893377) of exon 8 and the 3′ break point was mapped 678 bp downstream (chr13:48943418) of exon 11 of the *RB1* gene (c.719–2574_1127+678delinsC; Appendix 4). In patient RB31 with BRb, a partial deletion of 21 bases (chr13:49050959) at the 3′ end of exon 25 and a complete deletion of exons 26 and 27 were detected by CNV analysis. Using a split-read alignment of the sequence reads, the 3′ break point could be identified at 3,849 bp (chr13:49059971) downstream of 3′ UTR in the *RB1* gene [c.2643_(*1915+3849) del] (Appendix 4). The exact break points of the identified deletions were confirmed by Sanger sequencing (Appendix 4).

Identification of genetic mosaicism in URb cases: Individuals who have URb without an identified heterozygous germline *RB1* mutation are at risk for low-level mosaicism [1]. In our screen, two patients (RB12 and RB15) were found to carry nonsense variants: p.Arg445Ter (c.1333C>T) and p.Arg455Ter (c.1363C>T), respectively. In the RB12 case, the c.1333C>T variant had 21.7% supporting reads (out of 461 reads; Appendix 5) and in RB15, the c.1363C>T variant had 17% supporting reads (out of 909 reads; Appendix 5). When Sanger sequencing was performed, in the electropherogram, the relative peak intensity of the 'T' allele was much weaker than the reference 'C' allele in the specimen DNA samples (Appendix 5). Thus, in these individuals, there could be a possibility of genetic mosaicism in relation to the identified *RB1* mutation.

DISCUSSION

Germline mutations have been reported throughout the *RB1* gene in Rb patients, and only a few of these reported mutations are recurrent. Previously, several Indian studies conducted screening of the *RB1* gene in Rb patients and reported mutation detection rates in the range of 33% to 85% [10-14]. These studies highlight the limitations of the techniques used in these studies because, in principle, 100% of bilateral Rb patients carry germline mutations in the *RB1* gene. To confirm the molecular diagnosis of Rb, several different genetic testing methods have been used traditionally, such as Sanger sequencing, quantitative multiplex PCR, cytogenetic testing, MLPA, and array-Comparative Genomic Hybridization (aCGH) [14,21-23]. Sanger sequencing is used to detect point mutations and indels; when negative, another method (as mentioned above) is used to detect large deletions/duplications/insertions. This sequential mode (reflex) of testing is time consuming and expensive.

Compared to the reflex-testing mode, our current study shows that a NGS-based method is able to screen the complete *RB1* gene and can detect all types of mutations, including large deletions. In our study, among patients affected with BRb (22 cases), the mutation detection rate was 100%. Recently, a NGS-based test was used by Li et al. to screen the entire *RB1* gene to detect all types of *RB1* mutations, such as point mutations, small indels, and large deletions or duplications on a single test platform [24]. Our strategy had notable similarities with that reported by Li et al., including 100% concordance between the NGS output and

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Table1. List of mutations in the *RB1* gene, identified in patients with Retinoblastoma (Rb).

Patient No.	Exon/ Intron	Type of mutation	c.DNA	Amino acid change	Rb presentation	Reference/ Novel
RB1	Ex 1	Indel	c.46_68delGCCGCCGCGGAACCCCCGGCACC	-	Unilateral	Novel
RB2	Ex 2	Indel	c.178_182delTTATG	p.Leu60SerfsTer48	Bilateral	8651278[b]
RB3	Ex 4	Indel	c.400_407delTTACTAAA	p.Leu134ArgfsTer3	Bilateral	Novel
RB4	Int 5	SS	c.539+1G>A	-	Unilateral	10991691 [b]
RB5	Ex 6	Indel	c.540-6_545delTAATAGGATATC	-	Bilateral	Novel
RB6	Ex 8-11	LD	c.719-2574_1127+678delinsC	p.Lys240ArgfsTer3	Unilateral	Novel
RB7	Ex 8	NS	c.751C>T	p.Arg251Ter	Bilateral	20447117 [b]
RB8	Int 9	SS	c.940-1G>A	-	Bilateral	Novel
RB9	Ex 11	NS	c.1072C>T	p.Arg358Ter	Bilateral	rs121913301[a]
RB10	Int 12	SS	c.1215+1G>A	-	Bilateral	rs587776783 [a]
RB11	Ex 13	Indel	c.1281delA	p.Glu428ArgfsTer29	Unknown	Novel
RB12	Ex 14	NS	c.1333C>T	p.Arg445Ter	Unilateral	rs121913302 [a]
RB13	Ex 14	NS	c.1363C>T	p.Arg455Ter	Unilateral	rs121913302 [a]
RB14	Ex 14	NS	c.1363C>T	p.Arg455Ter	Unknown	rs121913302 [a]
RB15	Ex 14	NS	c.1363C>T	p.Arg455Ter	Bilateral	rs121913302[a]
RB16	Int 15	Indel	c.1421+5_1421+17delTTTTTTACTTTT	-	Bilateral	Novel
RB17	Ex 17	NS	c.1654C>T	p.Arg552Ter	Bilateral	25742471 [b]
RB18	Ex 17	MS	c.1689G>C	p.Trp563Cys	Unknown	15605413 [b]
RB19	Int 17	SS	c.1696-3T>G	p.Asp566ArgfsTer45	Bilateral	Novel
RB20	Ex 18	NS	c.1723C>T	p.Gln575Ter	Bilateral	rs587778864 [a]
RB21	Ex 18	NS	c.1735C>T	p.Arg579Ter	Bilateral	rs121913305 [a]
RB22	Ex 18	NS	c.1735C>T	p.Arg579Ter	Bilateral	rs121913305 [a]
RB23	Ex 19	Indel	c.1953T > TA	p.K653fs	Bilateral	Novel
RB24	Ex 19	Indel	c.1959_1960dupA	p.Val654SerFsTer14	Bilateral	15605413 [b]
RB25	Ex 20	MS	c.2104C>A	p.Gln702Lys	Bilateral	12541220 [b]
RB26	Ex 21	MS	c.2134T>C	p.Cys712Arg	Unilateral	9671401 [b], 10486322 [b]
RB27	Int 22	SS	c.2325+1G>A	-	Unknown	15884040 [b]
RB28	Ex 23	NS	c.2359C>T	p.Arg787Ter	Bilateral	rs137853293 [a]
RB29[#]	Ex 24-27	LD	c.(2479+1_2490-1)_(2787+1_2788-1)del	-	Bilateral	Novel
RB30	Ex 25	LD	c.(2520+1_2521-1)_(2663+1_2664_1)del	-	Bilateral	Novel
RB31	Ex 25_27*	LD	c.2643_(*1915+3849)del	-	Bilateral	Novel

Patient No.	Exon/ Intron	Type of mutation	c.DNA	Amino acid change	Rb presentation	Reference/ Novel
RB32	-	WGD	c.(?_-166)_(*1819_?)del	-	Unknown	cUnknown
RB33	-	WGD	c.(?_-166)_(*1819_?)del	-	Bilateral	cUnknwon

Abbreviations: Indel: small insertion or deletion, LD: Large deletion, MS: Missense, NS: Nonsense, SS: Splice site, WGD: Whole gene deletion, adbSNP database, bPMID, *Partial deletion of exon 25 with complete deletion of exons 26 and 27, c Breakpoints not known, #All LD and WGD except in the RB29 sample were confirmed by MLPA (Multiplex Ligation-dependent Probe Amplification).

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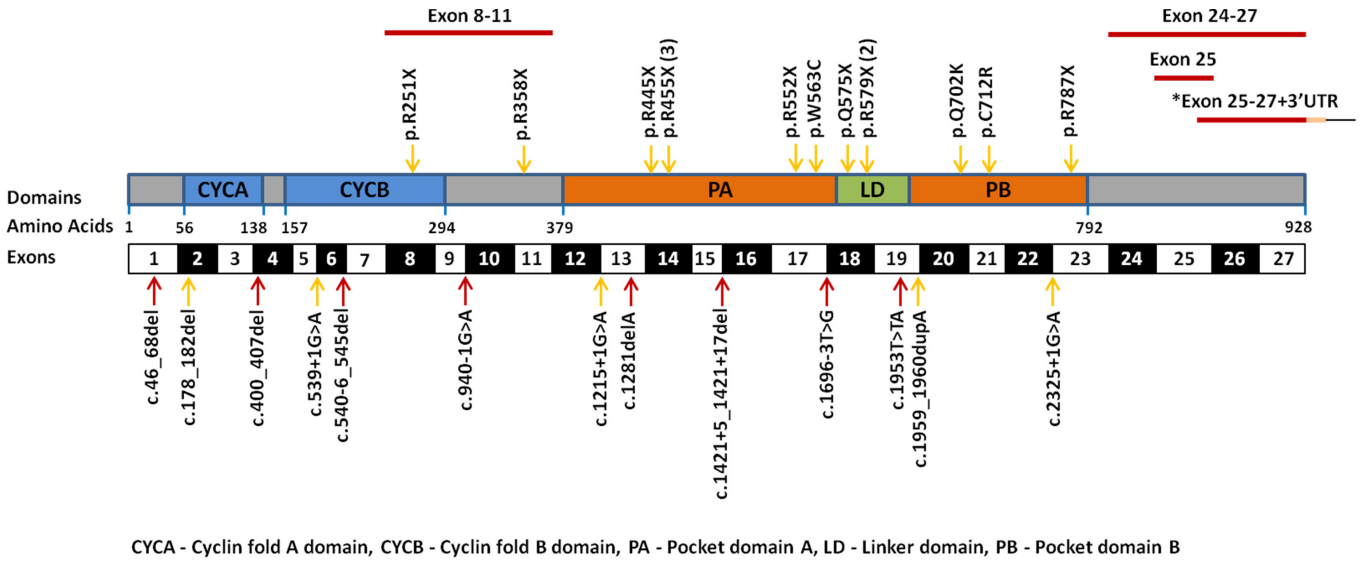

Figure 1. Schematic diagram representing the structural domains of the RB1 protein, along with 27 exons, and representing the localization of the identified mutations in the retinoblastoma (Rb) cohort. The novel mutations identified in our study are indicated by red arrows and known mutations are marked by yellow arrows. Among the 29 unique mutations identified in 33 patients in our Rb cohort, 16 mutations are located in the region encoding for the A/B pocket domain and five mutations are located in the cyclin domain of the RB1 protein. We identified six large deletions in our cohort, but two whole gene deletions are not shown in the representation; the other four deletions are indicated by red bars. Note: *partial deletion of exon 25 with complete deletion of exons 26–27 and 3′UTR (untranslated region).

Sanger confirmation and the detection of low-level mosaic *RB1* mutations using the NGS test [24]. In Indian Rb cohorts, conventional testing was able to detect mutations in the range of 36% to 83% in BRb cases [4]. In a recent study, Devarajan et al. used a NGS-based approach to screen the *RB1* gene in an Indian cohort and reported a detection rate of 85.7% (18/21) in the BRb cases [12]. Interestingly, in another recent study, Grotta et al. used a combined approach of NGS and aCGH and still could detect mutations in only 96.5% (28/29) of the BRb cases through this reflex mode of testing [22]. Overall, it appears that our NGS-based testing has a higher sensitivity than previous studies using both conventional tests and other NGS-based tests [10-14].

In our study, among the URb cases, the mutation detection rate was 30% (6/20). In previous studies with Indian cohorts with a significant number of URb cases, the mutation detection rate was reported in the range of 18% to 23.8% [21,23].

Through a CNV analysis, embedded in our NGS-based approach, we could detect six large deletions in our cohort ranging from a single exon to whole gene deletion. Among the six deletions, four were detected in BRb cases, one in an URb case, and one in a case where laterality was unknown. The overall detection rate of large deletions in our study was 12% (6/50) and in BRb cases, it was 18.2% (4/22), which is similar to the findings previously reported (9.5% to 20.5%) in other Indian cohorts [14,21,23,25]. Moreover, using the split read alignment of the sequence reads [18,26], we could identify the precise break points in the *RB1* gene in two of six deletions. We could confirm the break points of these two deletions using PCR amplification of the break point regions and Sanger sequencing. The identification of break points in cases with a large deletion by split read alignment allows us to establish a precise Sanger sequencing-based assay that is fast and economical for screening other at-risk family members.

In our study, we identified 11 nonsense mutations. Interestingly, ten of these 11 variants involved a substitution of arginine residue with a stop codon. At the nucleotide level, all mutations were C to T transitions. Previously, it has been reported that in the *RB1* gene, the majority of nonsense mutations occur due to C to T transitions at CpG dinucleotides (CpGs) as a result of the deamination of 5-methylcytosine to thymidine within these CpGs [27]. The occurrence of nonsense mutations at CpGs in the *RB1* gene appears to be determined by several factors, such as the constitutive presence of methylation at cytosines within CpGs, the specific codon within which the cytosine is methylated, and the region of the gene within which that codon resides [27]. In four of the mutated CGA codons (p.Arg251 in exon 8, p.Arg445 and p.Arg455 in exon 14, and p.Arg579 in exon18) of the *RB1* gene, a high frequency of constitutive methylation has been reported [27]. We detected the p.Arg455Ter mutation 3X

and the p.Arg579Ter mutation 2X. These two variants have been previously reported as recurrent mutations in patients affected with Rb [28].

We detected three missense mutations in our cohort and all of these mutations were located in the A/B pocket domain (379–792 residues) of the protein. The A/B pocket domain is essential for the interaction of the RB1 protein with the E2F transcription factor [29]. Previously, Richter et al. reported that 13 of 15 missense mutations identified in their study were located in the A/B pocket domain, thus suggesting that missense mutations occur frequently in this domain of the RB1 protein and highlighting the functional importance of this domain in the protein function [28].

In two cases (RB12 and RB15), the supporting read fractions for the identified variants were much lower (approximately 20%) than the expected ratio of 50%, suggesting the possibility of mosaicism. The incidence of mosaicism was estimated to be 30% and 6% in sporadic BRb and URb cases, respectively [30]. The use of deep sequencing technology, such as NGS, which has an increased sensitivity, enables us to detect low-level mosaicism in the *RB1* gene. The identification of a mosaic mutation in Rb cases has important clinical implications, as it confirms a genetic diagnosis and alters genetic counseling, surveillance, and disease management measures.

India has the highest number of patients with Rb, accounting for approximately 20% of the global Rb population [4]. The number of new cases is increasing each year, as the population of India is on the rise. As a result, treatment and disease management measures for patients with Rb are causing an increased financial burden on the Indian health care system. In the *RB1* gene, heterogeneous mutations

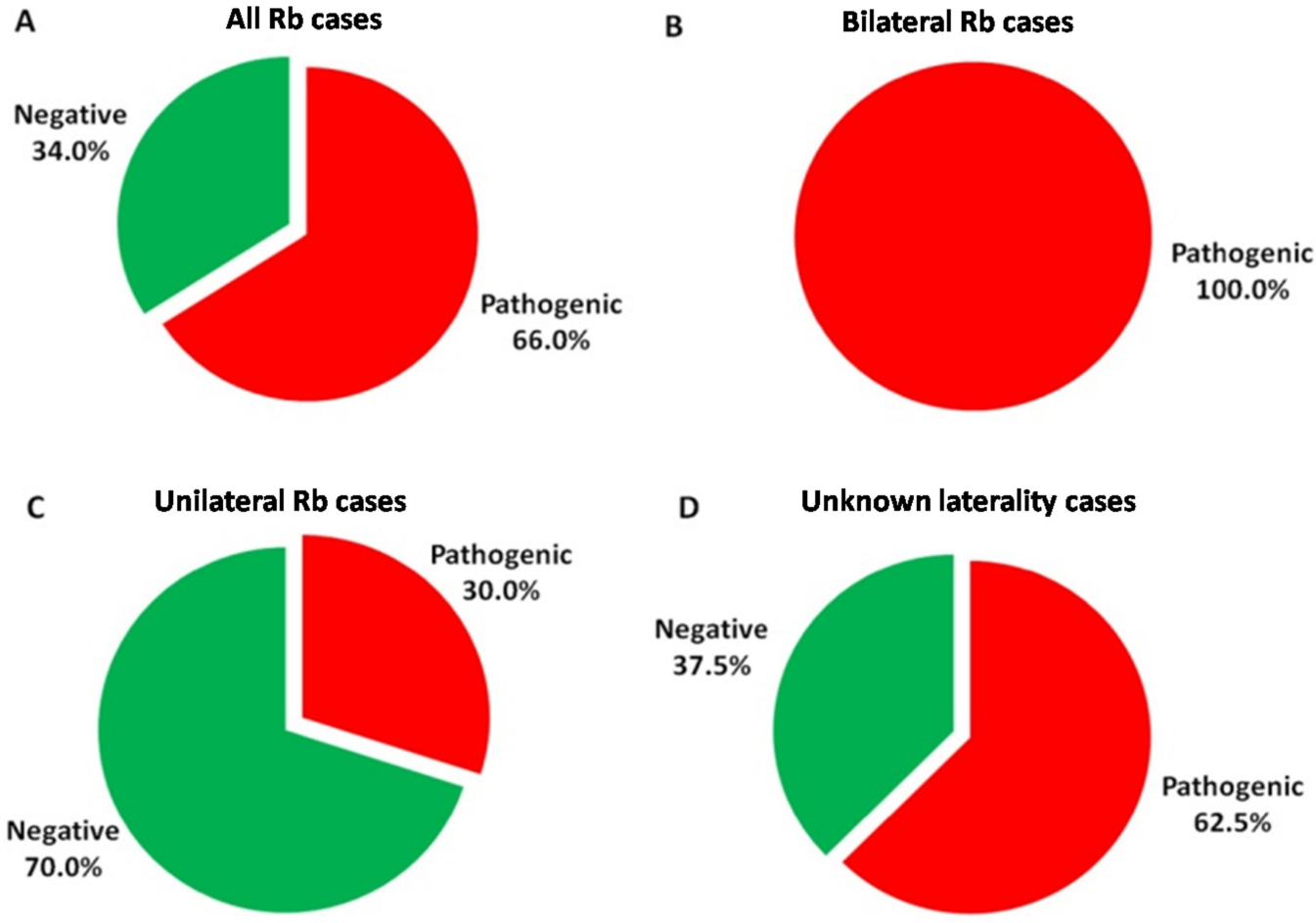

Figure 2. Mutation detection rate in the retinoblastoma (Rb) cohort. We stratified our cohort based on the Rb presentation into three groups, namely bilateral Rb (BRb), unilateral Rb (URb), and unknown literality, to determine whether the mutation detection rate was correlated with Rb presentation. **A**: A pie chart depicting an overall mutation detection rate of 66% (33/50 cases) in all Rb cases screened in our cohort. **B**: In BRb cases, the mutation detection rate was 100% (22/22). **C**: The mutation detection rate was 30% (6/20) in URb cases. **D**: In unknown laterality cases, the mutation detection rate was 62.5% (5/8).

are distributed throughout the entire length of the gene, suggesting that in terms of conventional tests, no single technology will be fully sensitive and efficient; a combination of tests will be necessary for confirmation of a genetic diagnosis, which is time consuming and costly. Our study indicates that NGS-based comprehensive testing of Rb patients will be at least six times more economical than reflex mode testing by Sanger, followed by MLPA for negative cases. In India, there is a pressing need for a cost-effective and comprehensive genetic testing method for the diagnosis and early detection of Rb. In the current study, we report a 100% mutation detection rate in patients with BRb. Our study suggests that a NGS-based approach increases the sensitivity of mutation detection in the *RB1* gene and helps in the confirmation of a genetic diagnosis in patients and at-risk family members compared to conventional tests performed in reflex testing mode. Our finding strongly supports the incorporation of a NGS-based approach for the routine genetic testing of Rb in India, as it is highly sensitive, accurate, fast, and economically feasible.

APPENDIX 1. PCR CONDITIONS AND PRIMER SEQUENCES FOR MUTATIONS IDENTIFIED IN THE RB1 GENE

To access the data, click or select the words "Appendix 1."

APPENDIX 2. THE DEMOGRAPHIC PROFILE AND CLINICAL CHARACTERISTICS OF PATIENTS WITH RETINOBLASTOMA (RB).

To access the data, click or select the words "Appendix 2."

APPENDIX 3. DETECTION OF LARGE DELETION IN THE *RB1* GENE IN THE PATIENT SAMPLES.

To access the data, click or select the words "Appendix 3." Detection of copy number variation (CNV) and confirmation by MLPA (multiplex ligation-dependent probe amplification). Figures on the left panel (A, C, E, G, I and K) represent CNV analysis and on the right panel (B, D, F, H and J) represent MLPA analysis. In the MLPA plot, x-axis represents genomic regions and y-axis represents dosage quotient (DQ). DQ distribution of 0.8–1.2 represent normal copy, 0.4–0.65 represents heterozygous deletion and 1.3–1.65 represents heterozygous duplication. In the sample RB6, the CNV analysis showed a heterozygous deletion of exon 8–11 (**A**), which was confirmed by MLPA analysis (**B**). Similarly, the CNV analysis in the sample RB30, revealed a heterozygous deletion of exon 25 (**C**), which was confirmed by MLPA analysis (**D**). The CNV analysis in the sample RB31, revealed a heterozygous deletion of exon 26–27 and a partial deletion of exon 25 (depicted in dotted circle; **E**), which was confirmed by MLPA analysis (**F**). The CNV analysis

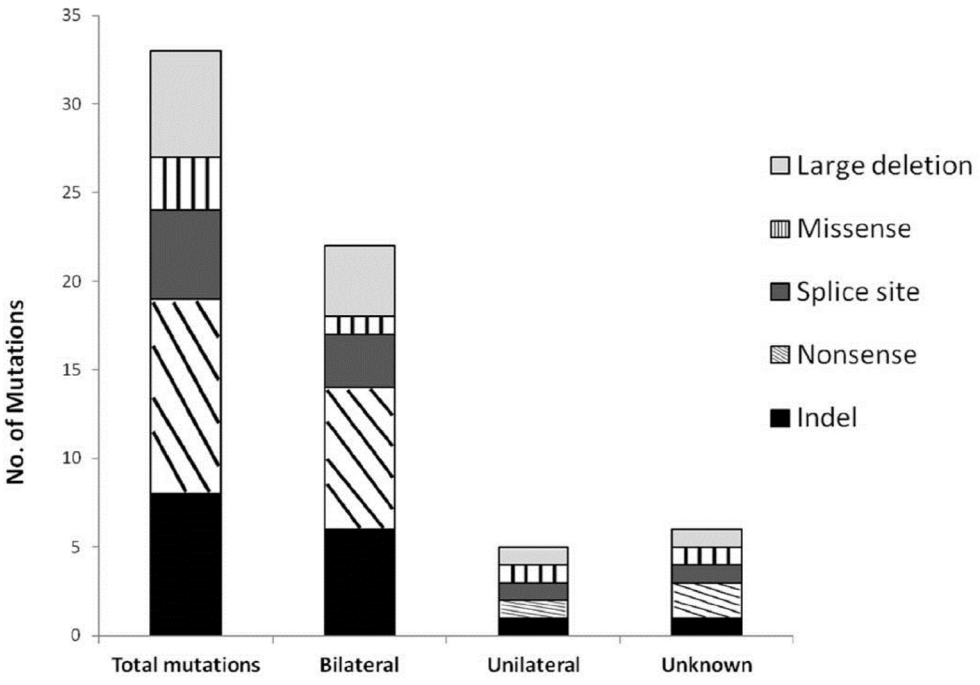

Figure 3. Types of mutations and spectrum in the retinoblastoma (Rb) cohort. The spectrum of mutation types detected in our cohort was missense, nonsense, splices site, indel, and large deletion types. In overall cases, we detected 11 nonsense, three missense, five splice site variants; eight indels; and six large deletions. In bilateral Rb (BRb) cases, eight nonsense, one missense, three splice site variants; six indels; and four large deletions were detected. In unilateral Rb (URb) cases, one nonsense, one missense, and one splice site variants; one indel; and one large deletion were detected, and in unknown literality cases, two nonsense, one missense, and one splice site variant; one indel, and one large deletion were detected.

in the sample RB32 showed heterozygous whole *RB1* gene deletion (**G**), which was confirmed by MLPA analysis (**H**). The CNV analysis in the sample RB33 showed heterozygous whole *RB1* gene deletion (**I**); although the CNV data showed higher heterogeneity, however, this deletion was confirmed by MLPA analysis (**J**). In both cases of whole gene deletion (RB32 and RB33), MLPA analysis revealed that in addition to the whole *RB1* gene deletion, the upstream and downstream genomics regions flanking the *RB1* gene were also deleted. The CNV analysis in the sample RB29, revealed a heterozygous deletion of exon 24–27, this sample also showed higher heterogeneity in the CNV data compared to other samples; however, considering deletion of multiple continuous exons, it is unlikely that it is false positive (**K**), as, additional DNA was unavailable for the sample RB29 therefore MLPA confirmation could not be performed.

APPENDIX 4. SPLIT-READ ALIGNMENT ANALYSIS.

To access the data, click or select the words "Appendix 4." By split read alignment of the sequence reads, we could detect the precise breakpoints in the *RB1* gene in 2 out of 6 deletions. In the sample RB6, for the identified deletion (c.719–2574_1127+678delinsC), the break points were: 2574 bp upstream (chr13:4893377) of exon 8 (5′ break-point) and the 3′ break-point was mapped to 678 bp downstream (chr13:48943418) of exon11 of the *RB1* gene (**A**). The identified deletion in the sample RB6, was confirmed by Sanger sequencing (**B**). In the sample RB31, for the identified deletion [c.2643_(*1915+3849)del], the break points were: 21 bases (chr13:49050959) at 3′ end of exon 25 and complete deletion of exon 26 and exon 27 and the 3′ break-point was mapped to 3849 bp (chr13:49059971) downstream of 3′UTR of the *RB1* gene (**C**). The identified deletion in the sample RB31, was confirmed by Sanger sequencing (**D**).

APPENDIX 5. INDICATION OF GENETIC MOSAICISM IN THE UNILATERAL RB (URB) CASES.

To access the data, click or select the words "Appendix 5." In the sample RB12, NGS data showed that the variant, c.1333C>T, had 21.7% supporting reads (out of 461 reads; **A**). In the Sanger electropherogram, the identified variant, c.1333C>T, was detectable and the relative peak intensity of the variant nucleotide 'T' was weaker as compared to reference nucleotide 'C' (**B**). In sample RB15, NGS data showed that the variant, c.1363C>T, had 17% supporting reads (out of 909 reads; **C**). In the Sanger electropherogram, the identified variant, c.1363C>T, was detectable and the relative peak intensity of the variant nucleotide 'T' was weaker as compared to reference nucleotide 'C' (**D**).

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Draft Rare Disease and Orphan Drug Policy

BANGALORE

Government of Karnataka

Draft Rare Diseases and Orphan Drugs Policy

Submitted by Vision Group for Biotechnology to

Secretariat of Health and Family Welfare

VGBT, Karnataka

03/05/2017

Vision

To create a robust, equitable and accessible health system and enact a rare disease policy for best practices in diagnosis, care, treatment and management of orphan/rare disease patients in Karnataka

Mission

- To look into the health requirements of the rare disease community in Karnataka
- To evolve best health care practices for rare disease patients
- To leverage Karnataka's multidisciplinary strength in health care, pharma, biotech and research to deliver optimal treatment and care
- To conceive and implement effective schemes and programs to achieve the goal of accessible and equitable health care for rare disease patients

Recommendations

1. Constitute a steering committee towards implantation of rare diseases policy and orphan drug act in the state of Karnataka.
2. Develop state-of-art diagnostic centres for statewide adoption of early detection (including new born screening, carrier screening and prenatal testing) for appropriate intervention.
3. Develop rare disease centres of excellence with high-quality expert care using the existing infrastructure of hospitals and research institutes like Indira Gandhi Institute of Child Health, NIMHANS, CHG and Kasturba Medical College, Mangalore.
4. Streamline patient care in collaboration with primary and tertiary care centres.
5. Promote research and develop a greater understanding of rare diseases.
6. Provide incentives in the form of tax subsidies and fast tracking in regulatory pathways for the indigenous development of orphan drugs, and therapeutic strategies such as gene editing and regenerative therapies for rare disease patients.

7. Promote collaboration between clinicians, researchers, biomedical specialists, pharma and biotech sector.
8. Support education and training of health care professionals and genetic counsellors to work with patients and their families.
9. Work out insurance and CSR endowment schemes for public health funding for these patients and their families to financially withstand the challenge of caring for rare diseases.
10. Enable social support for patients and families.

Table of Contents

1. Rationale

1.0 India has approximately 70 million children and adults affected by some form of a rare disease including rare cancers and auto-immune conditions. Diagnosis of a rare disease takes an average of 7-8 years due to lack of robust diagnostics and access. 50% of children with a rare disease do not live beyond the age of 5. Families with rare diseases usually bear out-of-pocket expenses which spirals them into a vicious circle of poverty. So far, rare diseases have been outside the public health discourse and this issue needs to be addressed with a specific rare disease policy.

The absence of any policy relating to rare diseases implies lack of multidisciplinary, coordinated, accessible and affordable diagnostics, treatment and care. There is little or no access to clinical research and innovations in rare disease drugs and treatment. People with rare diseases do not have access to health insurance either from the government or private insurers. Being out of the realm of the public health discourse, government hospitals and institutions are not equipped to deal with the medical complexities of rare diseases. In the light of these problems, a specific policy is imperative at the state level to create the best possible health system for people with rare diseases. Even the High Courts of Delhi and Kerala have recognized that Article 21 of the Constitution of India imposes an obligation upon the State to ensure that an effective framework to ensure the health of its citizens is developed. This includes ensuring that patients suffering from rare diseases have access to adequate and affordable healthcare (Mohd. Ahmed v Union of India and Ors., Delhi High Court, 2014; Manoj M. v State of Kerala and Ors., Kerala High Court, 2016).

In the context of the above issues, the Government of Karnataka has decided to bring out a rare disease and orphan drug policy.

2. Background & Objectives

2.0 The aim of formulating a policy is to create a comprehensive document in the coordinated multi-disciplinary care for children, adults and their families who are affected with rare diseases. It also aims to ensure that people with rare diseases have the best quality evidence-based care and

treatment, which is accessible and affordable. The diagnosis, management and treatment of rare diseases require the highest level of partnership between various stakeholders and this can be achieved by establishing robust links. There is also a need to strengthen the best research, diagnosis and service provisions that already exist in Karnataka and elsewhere. To sustain the highest quality of care, collaboration at all levels needs to be established.

2.1 At least 80% of rare diseases have an identified genetic origin and 50% of all new cases manifest in childhood. Other causes of rare disease are infections, allergic disorders and teratogens in pregnancy. These rare disorders affect multiple organs and systems and are often complicated with other associated morbidities that further complicate management.

2.2 The aim of this policy is to identify and adopt a systemic approach to rare disorders to achieve the following:

- Early identification and prevention of rare diseases where possible
- Early diagnosis and timely intervention
- Optimal co-ordination of care
- Facilitation of audit and research within the system
- Empowering those affected by rare diseases

2.3 The rare disease policy intends to achieve the following objectives:

- **Promote equity of access** – allowing families with affected members with a rare disease to follow clear, well defined care pathway, through a systematic 'pyramid of care' approach which is accessible and uniform over all centers. The health systems must improve access to safe and quality diagnostics, drugs, treatment and care for patients and families. This objective includes a strategy to create a fair health insurance scheme without excluding pre-existing genetic disorders which is currently a main clause of exclusion
- **Prioritize care optimization** – prioritizing rare diseases has public health importance for our population towards optimal utilization of the available resources. The strategy of including rare diseases within public health discourse will have a multiplier effect on the public health system in improving quality through upgraded technologies and state-of-art care

- **Family centered approach to care** – to establish linkages to converge all components of treatment services, specialist healthcare and social support around the needs of patients, their families and other care-givers
- **Deliver evidence-based cost-effective diagnosis and therapy of rare diseases** which should evolve through the best use of available regional and national resources
- **Capacity building** – to develop a structure for easy accessibility to the best evidence-based care and treatment. Support for education and training programs that train health and social care professionals to better identify rare diseases, optimize diagnosis and access to treatment for affected. The strategy of capacity building needs to include the medical education sector to educate and upgrade the skills of health and allied professionals to handle rare diseases
- **Specialized clinical centers to provide expert, high-quality clinical care and expertise to patients, families and caregivers** - financial and technical support to develop infrastructure such as laboratories and treatment units for the delivery of accessible care, treatment and research
- **Surveillance** – to develop future strategies to expand and scale up the program within a resource constrained setting. Surveillance also strategically implies a robust prevention and control program for rare diseases
- **Promote excellence in research** which will enable a better understanding of the magnitude, profile and therapy of rare disorders. This will strategize the promotion of clinical and biomedical research and innovations towards new drugs and therapeutics. Multidisciplinary partnerships will be facilitated across academia, hospitals, public health agencies, pharma and biotech and NGOs to support rare disease research
- **To develop an orphan drug policy** to scale up and fast track development of therapies for these disorders
- **Deliver rapid and effective translation of advances in management of rare diseases** into clinical care by creating appropriate infrastructure, care pathways and clinical competences
- **Raise public awareness of rare diseases** among common public as well as health experts and researchers. This will create a network of understanding and the need to put in place prevention and control measures

3. Definition and Classification of Rare Diseases

3.0 The definition and classification of rare diseases is arbitrary in different countries. There is no universally accepted definition of rare diseases though it is generally accepted that a “rare disease occurs infrequently in a population”. A rare disease is defined through three key elements:

a) the total afflicted population
b) prevalence
c) frequency of occurrence and

Rare diseases often pose challenges of availability and accessibility to treatment as do several other neglected or orphan diseases.

3.1 Nearly 7 crore of the India population is afflicted with some form of rare/genetic disorders. Of the total, 30-40 lakh people are affected by one of the globally known approximately 7000 rare/genetic diseases in Karnataka. However, an accurate estimate is lacking because we do not have a quantitative database with classifications on the number afflicted by these 7000 rare diseases. It is important to first have a definition in place so that a country may develop a public health policy and encourage the development of relevant treatment options and protocols. In USA, a rare disease is defined by its prevalence as “a disease that affects less than 200,000 persons” (Rajasimha, et al., 2014). Currently, India does not have a standard definition for rare diseases. Considering India’s total population, Rajasimha, et al., (2014) suggest that a rare disease be defined as 1 in 5000. Given Karnataka’s approximate figures of 30-40 lakh affected persons, it would be realistic to define a rare disease in Karnataka as occurring 1 in 6000.

3.2 It is believed that approximately 80% of rare disease is due to genetic causes given the high rates of consanguineous marriages (community education to reduce this phenomenon is necessary) within various Indian communities (Rajasimha, et al., 2014). Estimation of prevalence, population count and genetic surveillance is important towards formulating a robust state/national policy.

4. Identification, Prevention and Diagnostics

4.0 Many rare diseases are present at birth and are either caused by:

- A genetic problem (for example sickle cell disease and Thalassemia Major) or

- Deficiencies or exposures to substances around the time of conception or during pregnancy (for instance, spina bifida is associated with a folic acid deficiency around conception and early pregnancy or intrauterine infections)

4.1 Some of these diseases may manifest later in the childhood or even in adulthood (Thalassemia Minor, Duchenne muscular dystrophy, Huntington chorea, Disorders of Sexual Development).

4.2 Newborn Screening

The Govt. of Karnataka has announced a new born screening program on pilot basis which is now expected to be scale up to cover the entire state. Testing will be made available to all new born children for a range of rare disorders including phenylketonuria, congenital hypothyroidism, Glucose-6-phosphatase deficiency, congenital adrenal hyperplasia and galactossemia. With all of these diseases, early intervention results in better long-term outcome for the affected individual.

Screening programs raise complex ethical, legal and social issues for the people who are offered screening – either as an adult for their own information or as parents on behalf of their child. There is a need to establish a state screening committee, which should advise the Govt. on all aspects of screening in newborn as well as beyond newborn period. Using research evidence, pilot programs and economic evaluation, the committee should assess the evidence for screening programs against a set of internationally recognized criteria covering:

- The epidemiology of the disorder
- The test methodology
- The treatment options
- The effectiveness and acceptability of the screening program

Early, effective screening means that parents/patients can be immediately referred to specialist centres for diagnosis and onward management. The committee should regularly assess current screening programs against new evidence for screening of other conditions and to ensure that these programs are both useful and cost effective.

4.3 Preventive Testing

Preventive steps towards reducing the risk of rare/genetic disease births are a significant component of a public policy. Pre-family counseling with education and information on the risk of genetic transmission of diseases is desirable. While adhering to ethical considerations, it is also necessary to take such preventive steps. Government-sponsored genetic counseling centres at district levels could be setup to advice and counsel couples who are about to start families. Voluntary testing for disease traits among couples can reduce the incidence of genetic disease births. Preconception and prenatal screening should be voluntary and confidential.

4.4.1 Carrier testing

Carrier testing involves testing people who are at increased risk of being carriers of a specific inherited disorder. This may be because a relative is known to be a carrier or has the condition or because certain genetic conditions might be more prevalent in their community.

Cascade testing can also be used to identify 'at risk' relatives of an affected person in pre-symptomatic stage. Used effectively, it can reduce morbidity and mortality. For example, when a child with Wilson's disease is diagnosed in hepatic failure, cascade testing can identify other younger siblings who may benefit from early treatments, preventing cirrhosis and death.

Carrier testing for autosomal recessive disorders assumes importance in the context of high rate of consanguineous marriages in our state, for example in Thalassemia,carrier testing of at-risk relatives is not usually offered until the diagnosis of index case in the family. However, with the availability of surveillance data, high-risk populations can be mapped and carrier testing can be offered to such groups. This will allow more informed choices about having a family, pre-conception or fetal screening or testing a child in early life. All this will need to be addressed taking into consideration socio-ethical issues, patient information confidentiality and organized management of the return of results of such testing.

4.4.2 Preconception and antenatal care

The community-based strategies developed to reduce the number of babies born with congenital disorders (disorders that are there from birth) are most effective and give parents reproductive choice through continuing programs that:

- Raise awareness on adequate nutrition and periconceptional folic acid supplement for all women likely to become pregnant
- Awareness about avoidance of exposure to harmful substances or organisms before and during pregnancy (for example by having the rubella immunization, creating awareness about common teratogens and medications especially over the counter (OTC) drugs
- Serum screening and ultrasound scans in 1st and 2nd trimester to screen women during pregnancy for genetic structural birth defects in the fetus
- Safe and institutional delivery facilities for all pregnant women

4.4.3 Diagnosis and early intervention

The initial presentation of most of these rare diseases mimics common childhood illnesses as a result of which diagnosis is delayed and often missed. This delay in diagnosis leads to missed opportunities for timely interventions. Often the diagnosis of rare diseases requires specialized expertise and laboratory tests.

Although rare diseases are covered in the curriculum of undergraduate and postgraduate medical training, it is unrealistic to expect primary care staff such as general pediatricians and other non-pediatric health care staff (who are very often the first point of contact) to recognize all rare diseases. Many diseases are so rare that it is unlikely for a primary health care staff member to see a single case in their whole career.

Timely and accurate referral to specialized centres can therefore be achieved by teaching primary health care staff to recognize a handful of key warning signs, highlighted through care pathways. In 2008, a large study identified five aspects of diagnosis that are particularly difficult for general physicians (Kostopoulou, Delaney & Munro, 2008). These include:

- Atypical presentations
- Non-specific presentations

- Very rare conditions
- Co-morbidity (more than one disease present)
- Perceptual features that could be missed

Therefore, there is a need to evolve simplified algorithms to help health care professionals with limited resources for early identification these disorders and appropriate referral.

Strategy: It is necessary to create a robust screening and testing program from the perspective of public health towards the prevention and control of rare diseases. A network of health centres, hospitals and labs must be facilitated for quick and accurate diagnostics, prenatal testing and newborn screening. Given the high costs of genetic testing and sequencing, it is necessary for the government to step in to subsidize these tests to be made available at a network of centres across Karnataka. It is necessary to facilitate tie-ups with private genomic labs for this purpose. Newborn screening must be made available at all government hospitals and high-risk couples wanting to start a family must be allowed to avail low-cost carrier screening.

Outcome: Identification, prevention and early diagnosis of rare diseases can put in checks and balances to control the escalating population of rare disease cases. Early diagnosis has a clear rationale as early intervention can prevent complications and even lifelong disablement. For example, a child with Pompe if diagnosed within the first year of birth can be given the required enzyme immediately thereby stopping the progression of muscle atrophy and degeneration. The quality of life and life expectancy can be radically improved with early diagnosis and therapeutics.

5. Treatment and Care

5.0 Clearly defined care pathways

It is essential to have clearly defined; easily accessible and effective care pathways. To achieve this, a pyramid model of care is proposed which includes primary care at the base, regional centres and specialist clinical centres at the apex. There should be common protocols for identifying patients at risk of rare diseases at every level of care. Affected individuals should be referred to a coordinated diagnostic service so that they can get a rapid and accurate diagnosis of the suspected disorder. This is an important component of the overall policy, which should define the health care

deliverables and delivery system for these rare diseases. Operational guidelines need to be developed to help in implementation of the policy.

5.1 Prioritization

As these rare diseases are a very heterogeneous group, it is difficult to evolve common guidelines for the identification and intervention in all situations. Many of these disorders are so varied and so rare that it may not be possible to formulate a single policy for each of them. As resources available are scarce, allocation of resources for optimal value for the money is important, as is the prioritization of these rare diseases for the inclusion in the rare diseases management program. Prioritization should depend on disease burden in the state, availability of cost effective diagnostic methods and therapy that can modify the course of the disease substantially. The specialist committee should decide on the prioritized list of rare diseases to be included in the program and regular re-evaluation of such a programme.

5.2 Genetic testing

As more than 80% of the rare disorders have a clearly defined genetic basis, it is important to have access to laboratory services, which can ensure high quality genetic testing for inherited disorders. The care pathway for the rare disorder should include the guidance to clinicians as to when they should request a test. There is a need to formulate policy to order a test, transport the samples, conduct a high quality tests at an affordable cost and to disseminate the test result and future options to the family including link to care, treatment and prevention. There is also need to main the highest degree of quality while performing these tests.

5.3 Coordination of Care

Interdisciplinary and intra-disciplinary coordinated care is essential when several specialists and hospital departments are involved in a patient's care for optimal utilization of resources, time and cost. It is essential to coordinate care across the 'boundaries' between different services, so that care is effective, accessible and convenient to patients (for example, it should not disrupt their work or education).

Telemedicine especially means geographical distance does not have to be a barrier to coordinated care. It can improve access to specialist medical services that might not be available in some areas.

Some of the scope of telemedicine includes tele consultation with specialists, tele review, tele tracking the progress of the children and tele learning to improve the capacity of the health care professional involved in the care. This improves the accessibility to the highest quality of care at an affordable cost.

Primary care services often manage a patient's day-to-day care including immunization, nutritional support and monitoring of overall child's health. It is therefore important that general practitioners involved in the routine care of these children feel supported and that they can manage care efficiently. Following diagnosis, a patient should have an evidence based care plan that identifies the anticipated course of the condition and sets out the responsibilities of specialist, general and primary care services in care management. Good communication between patients, their families and professionals is essential to ensure that the primary care plan is agreed and the care team has information and appropriate specialist support. The ultimate aim will be to ensure that the agreed care plan is delivered effectively.

As therapies for many of these rare diseases are long term if not lifelong, maintaining optimal adherence to the therapy is a challenge as well as paramount to the success of such therapy. One of the strategies to improve adherence is easily accessible well-coordinated multidisciplinary care with family as the centre of care and pretreatment counseling to prepare them for the long term therapy. For successful coordination of care and optimal adherence to the life-long therapy, it is extremely important to identify the primary care givers who are willing to take the responsibility. The primary care giver should be adequately prepared for the long-term care and therapy needs and should be made aware of the expected outcome of such therapy before initiation of therapy. It is also important to assess the social and economic support available to the family, which plays a vital role in the success of such therapy. Support groups can offer such social support system and can help in bringing back the non-compliant back to loop of care pathway.

Responsibility for coordination will depend on the case and the circumstances. For those receiving complex treatment where only one discipline is involved, a highly specialized professional might have responsibility for coordinating their care. Where there are many disciplines, the clinical geneticist may have that responsibility. In any case, the aim should be to ensure that care is always coordinated in a hub and spoke fashion.

5.4 Specialist clinical centres

Specialist clinical centres or centres of excellence can provide an opportunity to acquire and maintain knowledge through research and interaction with patients. They bring together multidisciplinary teams of health and social care professionals to manage patient care and local resources effectively and efficiently. The centres need not necessarily be in specific locations but may be 'virtual', using appropriate technologies to bring experts together. Timely referrals to appropriate centres can be important in reducing the time it takes to receive a diagnosis. The use of new technologies such as telemedicine will increasingly mean that patients can access expert services remotely. This reduces the need for patients to travel and allows the creation of networks of experts who work together across hospitals. These centres should also be uniquely placed to provide a focal point for undertaking research and for implementing evidence based practices across all aspects of the patient pathway.

In providing specialist health and care services, specialist medical professionals assist with the coordination of professional care and provide information & advice to patients and professionals, identifying where the care pathway can be improved. Of vital importance in scientific communication is genetic counseling, the art of communication of a scientifically complex topic in nonprofessional terms as applicable to the family. Expert training and experience is required in this form of communication. Successful counseling is the mainstay in families understanding of complex diseases and when and where to seek appropriate care.

Although specialist clinical centres may provide all the essential expertise, in almost all cases most of the care is provided locally – by local hospitals, primary care teams, social care and education teams, and in the patient's home. Therefore, centres must have protocols in place to share their expertise with local services. This will require the development of shared protocols for effective communication and information sharing between the centre, local teams and the patient.

Strategy: It is imperative to create specialist services like clinical genetics for rare disease treatment and management. Rare disease treatment and care cannot be handled by simple generalist approaches. A concerted, coordinated, multidisciplinary approach is the best strategy towards optimal care and management. The state will come up with a prioritized list of rare diseases with

relevant care pathways. Crisis intervention centres for rare diseases and counseling for patients and families will be coordinated.

Outcome: Setting up and coordinating specialist care centres for rare disease treatment and care will prevent unnecessary delays and waiting for the relevant treatment. This can be lifesaving as delays often lead to death and disablement. Access to genetic testing and analysis will allow for personalized and precision treatment according to disease genotypes and phenotypes. Personalized care is valuable to patients in improving their life chances and survival rates.

6. Paramedical and Palliative Care Support

Often people with rare/genetic diseases require simple procedures like injections, tracheostomy draining etc. Paramedical care centres can be setup to provide basic nursing and care services to patients and families. Such a centre also provides employment opportunities as people from lower-income groups with limited education access can be provided training and placement.

Strategy: Creation of paramedical and palliative care support will take off the burden on the core healthcare system managing rare diseases and needs to be setup and facilitated to take care of simple procedures in nursing and care.

Outcome: A robust paramedical and palliative care support will expedite delays in accessing simple procedures without the patient being taken around to hospitals and secondary care centres.

7. Orphan Drugs and Devices

7.0 Orphan drugs policy

Support and funding for affordable therapeutics in orphan disease application is a significant component of a public policy. Companies developing therapeutics for orphan diseases need to be given government support and adequate funding. Karnataka needs to put into place an “orphan drug act” (ODA) which would be the first-of-its-kind in India. A good starting point in putting together such an act is the US ODA which was passed in 1983 which facilitates the development

and commercialization of drugs and biologics to treat rare diseases. Under the current US ODA, orphan drug developers have three incentives:

- federal funding of grants and contracts for clinical trials of orphan disease products
- tax credit of 50% of clinical testing costs
- exclusive right to market the orphan product for 7 years from the date of marketing approval

There is also a need to facilitate clinical research in rare diseases by waving off clinical trial application fees, priority review & approval of study protocols and accelerated development programs.

An orphan drug act should also incentivize indigenous development and production of drugs for rare diseases in the form of tax holidays and subsidies.

7.1 Orphan Diseases Devices, Dietary Supplements

Along with incentives for developing therapeutics for this sector, there are several additional products like medical devices (infusion pumps), aids and diet foods (often used for phenylketonurics, PKU) which are often required in the care and treatment of rare/genetic diseases. These are mostly imported at exorbitant prices and place a heavy burden on patient families. An Orphan Drugs Policy should be extended to incentivize companies to develop domestic alternatives for imported substitutions.

7.2 Compassionate use policies

As delay in treatment is an important criterion in rare disease intervention, many countries have put in place a Compassionate Use Program. This permits the doctors treating a rare disease patient to request a manufacturer for access to a drug that is in the process of getting approval for public use. Compassionate use policies specify conditions under which this access can be granted and are usually subject to a drug having cleared clinical phase II studies and the patient having a favorable benefit/risk ratio.

Strategy: Enactment of legislation for an orphan drug act in Karnataka is necessary to encourage and push pharma and biotech sector take up drug development for ignored rare diseases.

Outcome: An orphan drug act will enable faster drug development as regulatory processes are expedited for rare diseases. This will positively impact the rare disease patients who will get faster access to lifesaving drugs.

8. Disease Surveillance Programs

As the burden of these rare diseases is largely unknown, it is important to assess the prevalence of these diseases individually to formulate, prioritize and scale up the program. The evidence generated through this surveillance program should guide the future course of the program. This can be an integral to the existing screening program or can be through a formulation of registry.

8.0 Monitoring

Monitoring is an integral part of any program for its ongoing success. Monitoring and evaluation should guide future changes in the policy. There is a need to develop monitoring indicators both technical as well operational, which help in assessing the impact of the program.

8.1 Documentation

High quality healthcare, diagnosis and intervention rely upon accurate methods of recording health information to detail the incidence and prevalence of disease, and to enable service planning and international collaboration. To enable updated documentation requires development of infrastructure and training of appropriate human resources.

8.2 Assessing treatments

It is important to have appropriate procedures for evaluating the benefits and costs of diagnosis and treatment so that patients with rare diseases get the most effective care. These procedures should be transparent and robust enough to be able to take account of the particular challenges that occur when evaluating treatments for rare diseases.

Strategy: The state will facilitate the setting up of rare disease registries for robust surveillance, data collection and analysis for a set of prioritized rare diseases in Karnataka. Through such registries, monitoring, documentation and evaluation of care pathways and policies will be made possible.

Outcome: Surveillance programs and linkages to registries are crucial not only for population studies of rare diseases in the state but also towards strategies of prevention and control.

9. Research

Research is an integral part of a programme that is developed at the cutting edge of scientific and medical knowledge. Research can be at three main levels:

- 9.0 Epidemiological research - into prevalence, causation, prevention and socio-cultural aspects of rare disorders. Information gleaned from this type of research will be important to feedback into the programme for improving prioritization and programme efficiency
- 9.1 Translational research - is of vital importance when laboratory and scientific discoveries can be translated into improvement of patient care services. Gene manipulation techniques and gene editing research are being carried out extensively in the West and if we are to reap the benefits of gene therapy research, we need to establish research labs, which can do the same. This is critical if therapeutic interventions have to reach the Indian population. The Vision Group for Biotechnology (VGBT) in Karnataka has submitted a proposal for an institute of integrated and synthetic biology. Entrepreneurial efforts for example, (Aten Biotherapeutics) in this domain have also started in the Bengaluru.

- 9.2 Operational research - Ongoing audit of all systems and processes are important in assessing positive and negative trends in achieving health care goals. This will also help identify lacunae, strengthen systems that are robustly functioning and create avenues for better operational models at all levels of the programme.

Strategy: The state will encourage and facilitate networking between academia, research institutes and biotech to enable “bench to bedside” translational research for rare diseases.

Apart from core research, operational research will also be encouraged to improve care pathways and delivery of services.

Outcome: Research can contribute significantly towards improving the life quality and life expectancy for people with rare diseases as newer drugs and therapeutics are developed.

10. Health Education

Integrating rare disease curriculum in medical education is important to create awareness and competence in the medical community. Rare disease intervention has been prevalent in the United States for more than three decades, and its value has been widely acknowledged. We need to integrate a rare disease curriculum into medical education. This can provide a significant boost to the critical problem of public awareness, as well as promoting research into rare diseases. CHG has a well-developed CME programme for physician exposure to recent developments in human genetics. Such curricula need to be disseminated on a wide scale through the use of digital media. Massive Online Open Courses (MOOCs) developed in English and Kannada can be created to prepare medical personnel from physicians to health workers in remote locations of the state in recognizing genetic diseases in their community health interventions. There should be special training programs for genetic counsellors to work with patients and families.

Strategy: Medical education curriculum will be strategized to include modules and components on rare diseases and clinical genetics. A special course on genetic counselling will be introduced through an MSc program open to life science undergraduates.

Outcome: As it frequently happens, medical personnel are themselves unaware of several rare diseases leading to misdiagnosis or total missing out on diagnosis. Developing upgraded curriculum to involve rare disease and genetics in medical education will significantly improve doctors' diagnosis and subsequently streamline the right treatment for people with rare diseases.

11. Facilities and Support for Rare Disease Patients & Families

11.0 Health Insurance

Insurance exclusions are a major issue for people with rare diseases who are forced to pay out of their pockets for every procedure/hospitalization. Private insurance companies exclude people with pre-existing conditions. Genetic pre-dispositions are identified by the IRDA (Insurance Regulatory and Development Authority) Act to be pre-existing and hence insurance companies are permitted to exclude coverage. There are no government health schemes covering people with rare diseases. This discrimination in insurance exclusions increases the financial burden on families. At this stage, IRDA intervention is necessary to ensure that private insurance companies at least provide hospitalization coverage at reasonable premiums. Health insurance under government schemes also need to include people with rare/genetic diseases. If people with rare diseases have to access quality healthcare, insurance exclusions must be dealt with to ensure maximum coverage during health crises.

The government can also work out insurance and support through a public health fund for rare diseases supported by CSR activities in the state.

11.1 Access to Education

Access to education is another serious issue faced by children/adults with rare diseases. Families often spend huge amounts of out-of-pocket money for medical treatment and may not be able to support educational facilities for children. Scholarships/financial aid for children/adults with rare diseases must be instituted to support access to education. The state must also be able to identify a network of institutions that can support students with rare diseases by making their spaces accessible and inclusive.

11.2 Caretaker aid

In countries like Australia, state health services appoint trained caretakers/nurses to provide care to people affected with rare/genetic/chronic conditions. These caretakers could be paid student interns from medical colleges. This provides relief to the family who is burdened with chronic care for a child or adult 24/7 and a way of motivating medical college students to understand more about rare/genetic conditions.

Strategy: The state will facilitate the provision of basic health insurance facilities for people with rare diseases through schemes similar to *"Yeshaswini."* The state will also provide education to patients and families dealing with different rare diseases. This could be in the hospitals and secondary care centres providing treatment for rare diseases. Public health workers can be trained to provide basic health counseling and education to families. The state will also facilitate provision of caretaker services through mobilizing government medical college students and interns.

Outcome: Health insurance is vital to cover emergency hospitalization treatment. So far, people with rare diseases are outside the health insurance schemes due to pre-existing clause exclusions. By setting up basic insurance scheme for people with rare diseases, Karnataka will be the first state to take a non-discriminatory stand on healthcare insurance services. Education of patients and families will enable better understanding of the disease and preparedness for medical emergencies. Caretaker aid can take off some of the burden on families who serve as primary care givers thereby improving the family's mental health and well-being.

12. Resource Mobilization

Funding for a programme of this magnitude will have to be sustained and defined. Some of these could be provided by the government as a corpus grant.

- Existing pediatric programmes such as *Bala Sanjeevani, Yeshaswini* and other insurance schemes for the economically backward groups can support some of the patients requiring investigations and therapy for rare disorders
- *Rashtriya Bal Seva Karyakram* and *National Rural Health Mission* can contribute to specific programmes in detection and health education
- Identifying and tapping CSR funding can contribute towards additional funding and developing a larger corpus resource
- Support organizations working for rare diseases and disease specific parent support groups can help raise funding for research and education
- Philanthropic organizations can be approached by regional health services for programme support

13. Review

For the successful operation of a programme of this magnitude, it is vital to have systematic review and objective assessment of all processes. It is proposed that the policy is reviewed at 2-3 yearly intervals to evaluate short term achievements and revise focus of emerging health priorities. With the development and enforcement of this policy document Karnataka state can hope to develop as a leader in the country on rare diseases and their management.

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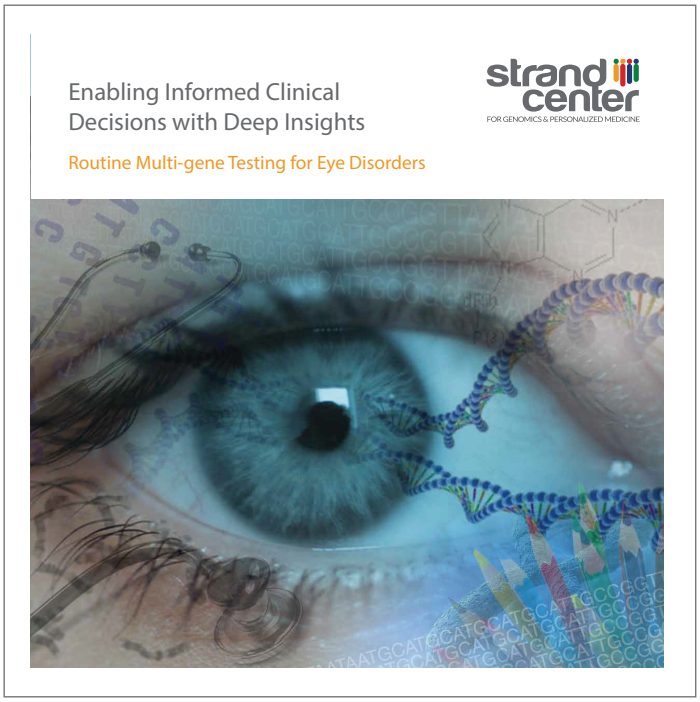

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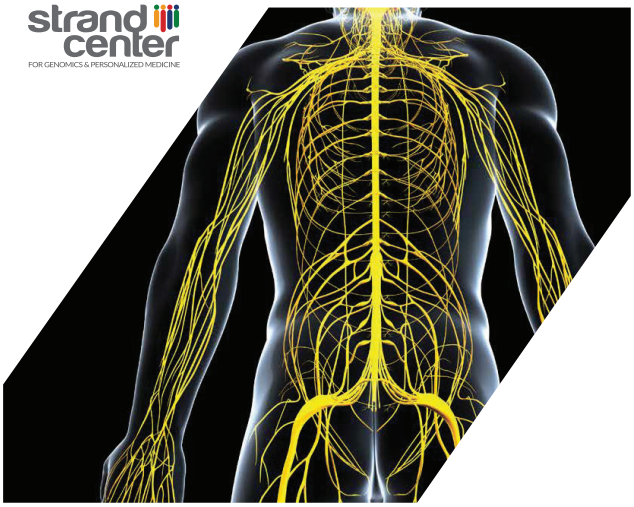

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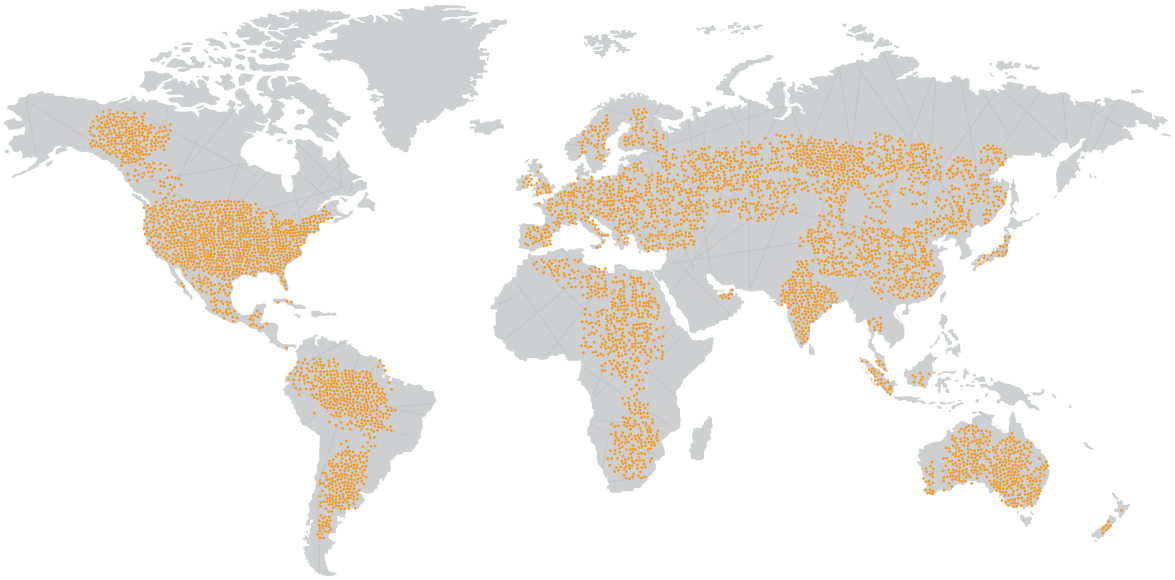

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