Genetics and Biology of Neuromuscular Disorders: Unmet scientific need

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Neuromuscular Disorders

- Affects peripheral nerve and muscle
- Ability to perform voluntary movement
- Disability, quite often leading to complete paralysis
- Progressive
- Genetic and non-genetic origin

Causes of Neuromuscular Diseases

- Genetic mutation
- Viral infection
- Autoimmune disorder
- Hormonal disorder
- Metabolic disorder
- Dietary deficiency
- Certain drugs and poisons
- Unknown factors.
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Neuromuscular Genetic Disorders

- More than 1000 diseases
- Some form of therapy is available for about 1% of diseases but not cure
- Available therapy is extremely expensive and have to be taken lifelong
- Finding cure is therefore the only choice

Categories of Neuromuscular Disorders.

Disease category	Number of genes/loci
Muscular dystrophies	31
Congenital muscular dystrophies	25
Congenital myopathies	30
Distal myopathies	15
Other myopathies	22
Myotonic syndromes	9
Ion channel muscle diseases	19
Malignant hyperthermias	6
Metabolic myopathies	23
Hereditary cardiomyopathies	102
Congenital myasthenic syndromes	21
Spinal muscular atrophies (including motor neuron diseases)	34
Hereditary ataxias	57
Hereditary motor and sensory neuropathies	60
Hereditary paraplegias	41
Other neuromuscular disorders	24
Total	519

Complexity of Neuromuscular Disorders

There is no clear link between genotype and phenotype for many diseases unlike many other genetic disorders such as those related to hematopoietic diseases.

Multiple genes can cause similar disease: Spastic Paraplegia

Hereditary spastic paraplegia (HSP), also called familial spastic paraparesis (FSP), refers to a group of inherited disorders that are characterized by progressive weakness and spasticity (stiffness) of the legs.

Number of genes have been found that are associated with HSP. Some of these are:

PLP1, SPAST, SPG7, CYP7B1, KIAA0196, ALDH18A1, KIF5A, SPG11, RTN2, HSPD1, ZFYVE26, BSCL2, ERLIN2, SPG20, SPG21, DDHD1, B4GALANT1……

These genes are involved in a variety of functions.

Multiple genes can cause the same disease: Limb Girdle Muscular Dystrophy

Table 2. Autosomal recessive limb girdle muscular dystrophies.

Dystrophy	Chromosome location	Gene/protein	Gene symbol
LGMD2A	15q15-q21	Calpain3	*CAPN3*
LGMD2B	2p13	Dysferlin	*DYSF*
LGMD2C	13q12	γ-sarcoglycan	*SGCG*
LGMD2D	17q12-q21	α-sarcoglycan	*SGCA*
LGMD2E	4q12	β-sarcoglycan	*SGCB*
LGMD2F	5q33	δ-sarcoglycan	*SGCD*
LGMD2G	17q12	Telethonin/Titin-Cap	*TCAP*
LGMD2H	9q31-q34	Tripartite motif-containing protein 32	*TRIM32*
LGMD2I	19q13	Fukutin-related protein	*FKRP*
LGMD2J	2q31	Titin	*TTN*
LGMD2K	9q34	Protein o-mannosyltransferase 1	*POMT1*
LGMD2L	11p14	Anoctamin 5	*ANO5*
LGMD2M	9q31	Fukutin	*FKTN*
LGMD2N	14q24	Protein o-mannosyltransferase 2	*POMT2*
LGMD2O	1p34	protein o-mannose beta-1,2-n-acetylglucosaminyltransferase	*POMGNT1*

Laing 2012

Different mutations in the same gene can cause multiple diseases

Table 4. Range of Phenotypes Associated with Mutations in the Ryanodine Receptor Gene (RYR1).

Phenotype	Mode of inheritance	Reference
Central core disease	Autosomal dominant	(73)
Central core disease and malignant hyperthermia	Autosomal dominant and autosomal dominant susceptibility	(74)
Core-rod disease	Autosomal dominant	(75,76)
Uniform Type 1 fibres	Autosomal dominant	(77,78)
Central core disease presenting as minicore disease	Autosomal recessive	(79,80)
Exertional rhabdomyolysis	Autosomal dominant susceptibility	(81)
Centronuclear myopathy	Autosomal dominant	(82)
Minicore myopathy with ophthalmoplegia	Autosomal recessive	(83)
Congenital fibre type disproportion	Autosomal recessive	(84)

Laing 2012

Relating target genes with muscle function

Most of the time it is difficult to relate genes involved specifically with muscle/brain and the question arises why muscle is the target tissue.

Genes that have muscle specific function:

Muscle skeletal Receptor Tyrosine Kinase *MUSK*
Muscle related coiled coil protein *MURC*
Myosin heavy chain 8 *MYH8*
Myosin Heavy Chain 6 *MYH6*
Alpha Actinin2 *ACTN2*
Alpha Actin *ACTC1*
Actin Filament Binding Protein *FDG4*

Genes that have no known muscle specific function:

Acid alpha-glucosidase preproprotein *GAA*
Alanyl t-RNA synthetase *AARS*
Aldehyde dehydrogenase *ALDA13A2*
Beta-1,3-N-acetylgalactosamyltransferase 2 *B3GALNT2*
Calpain 3 *CAPN3*
Lamin A/C *LMNA*
Lysosome associated membrane protein 2 *LAMP2*
GNE

Some examples of mutational complexity with respect to phenotypic differences

1. A polyglutamine expansion in the androgen receptor as the cause of Kennedy's disease (X-linked spinal and bulbar muscular atrophy)

2. duplication or deletion of the same 1.5Mbp region on chromosome 17 containing PMP22 gene is the cause respectively of Charcot-Marie-Tooth disease (CMTIA)39 or hereditary neuropathy. Thus, two copies of the region equates to normal phenotype, deletion to one form of peripheral neuropathy and duplication to a different form of peripheral neuropathy.

3. A non-coding triplet (CTG) repeat expansion as the cause of myotonic dystrophy,

4. Deletion of one of two almost identical genes (SMNt as apposed to SMNc) on chromosome 5q causes all forms of differing severity of autosomal recessive proximal spinal muscular atrophy

5. de novo mutations are perhaps a more common cause of genetic diseases than previously thought

Examples of *de novo* mutations

Severe recessive early onset peripheral neuropathy Dejerine-Sottas syndrome

The majority of mutations in the skeletal muscle alpha-actin gene (ACTA1) have been shown to be de novo dominant mutations

Nemaline myopathy

Laing distal myopathy

Consequences of *de novo* mutations

The absence of family history may hinder diagnosis.

Somatic mosaicism

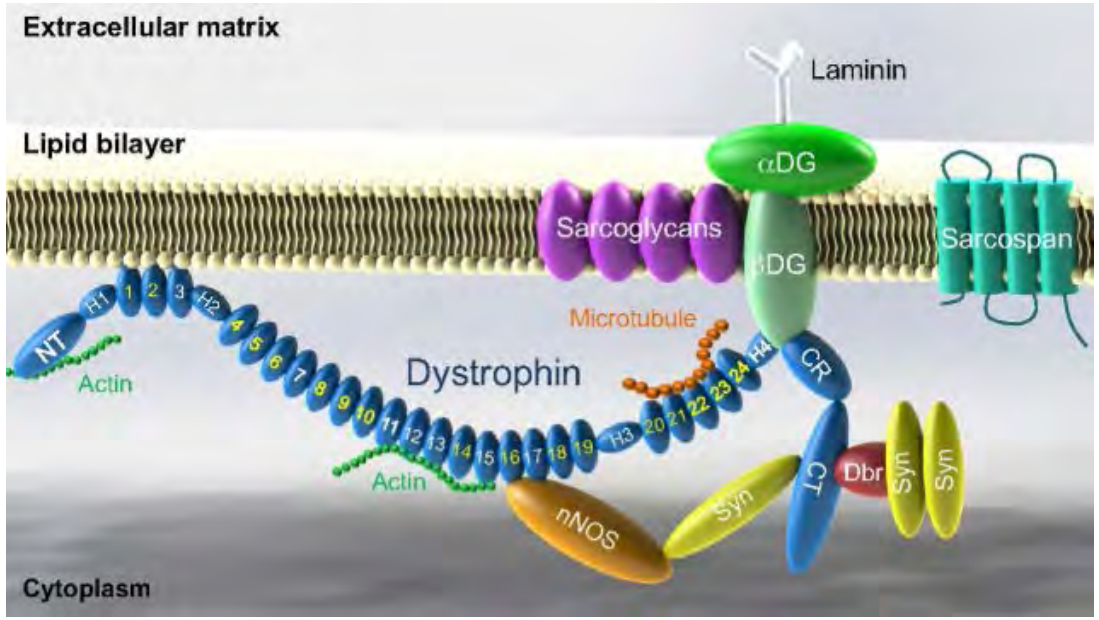
Reasons for Lack of Therapy for these diseases

- Number of patients are very few, therefore there is a lack of interest among researchers, drug makers and granting agencies
- Lack of understanding of the pathobiology of the diseases
- Lack of resources and research reagents.
- Absence of clinics that handle these patients
- Current regulatory processes are not compatible with ground realities though recent clinical trial guidelines do provide some concession to rare diseases

Duchene Muscular Dystrophy (DMD): An example of a well-studied system

- DMD affects mostly males at a rate of 1 in 3,500 births.
- There are over 200 types of mutations that can cause any one of the forms of muscular dystrophy. There are also mutations that occur within the same gene that cause other disease types.
- It is the most severe and common type of muscular dystrophy.
- DMD is characterized by the wasting away of muscles.
- Diagnosis in boys usually occurs between 16 months and 8 years.
- Death from DMD usually occurs by age of 30.

Biology of DMD



Dystrophin, the protein product of the mutated gene, normally sits at the muscle cell membrane but is lost or modified in DMD.

The amino-terminal of dystrophin interacts with F-actin.

The other end of dystrophin at the cell membrane binds a group of glycoproteins collectively known as the dystrophin-associated protein complex (DAPC). Among these are four sarcoglycans and dystoglycan, which binds via its extracellular portion to laminin-α2, a component of the extracellular matrix.

Dystrophin thus links the internal structure of the muscle cell with the external matrix and is an important structural component supporting the membrane.

Mutations in the sarcoglycans and laminin-α2 all cause different forms of MD.

Other diseases due to mutations in dystrophin gene

Other diseases are also caused by mutations in dystrophin gene

Becker muscular distrophy - **BMD** (1.1, 10.76)
Cardiomyopathy, dilated, X-linked - **XLCM**(1.1, 10.76)
Cardiomyopathy, Dilated, 3B - **CMD3B** (1.1, 10.76)

GNE Myopathy: Example of a less studied disease

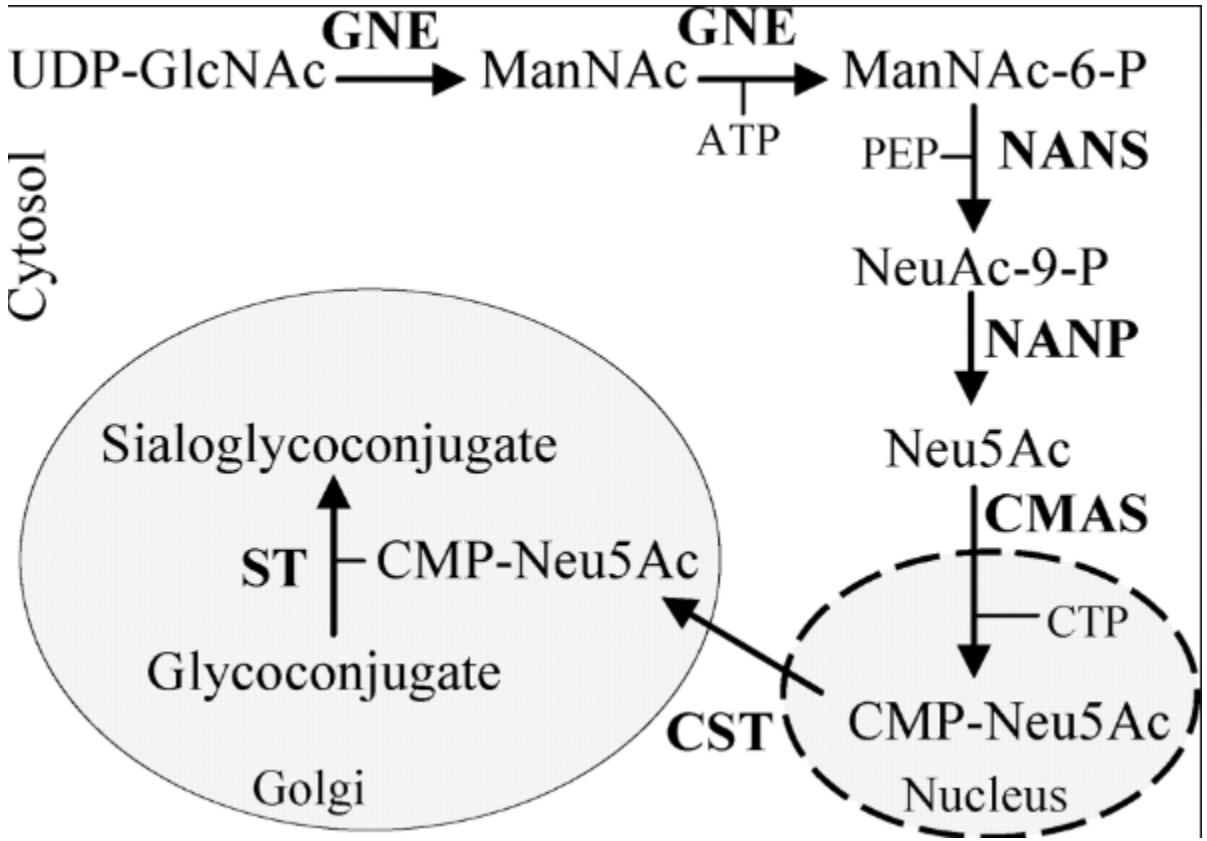
- Recessive genetic disorder
- Affects skeletal muscles sparing quadriceps
- Late onset about 20-55 years
- Progressive loss of skeletal muscle function starting from distal muscle eventually patients become wheelchair bound in about 10 years
- Diagnosis: clinical examination, gait analysis, pathology and DNA sequence
- About 200 diagnosed patients in India
- Currently no treatment available

GNE myopathy may not be that rare

- Recessive, mainly compound heterozygous, a few homozygous
- In India About 200 documented cases of GNE myopathy, an adult onset degenerative disease with no available treatment
- There are more than 150 mutations known worldwide, but 70% of Indian carry one pathogenic allele
- This allele is present in 15% of healthy Gujaratis and 1.5% of all Indians
- Estimated number of patients is between 30,000-50,000

Bhattacharya et al 2018 J Neuromuscular Dis

GNE Myopathy



GNE, a rate limiting enzyme in the sialic acid biosynthetic pathway

Mostly missence mutations

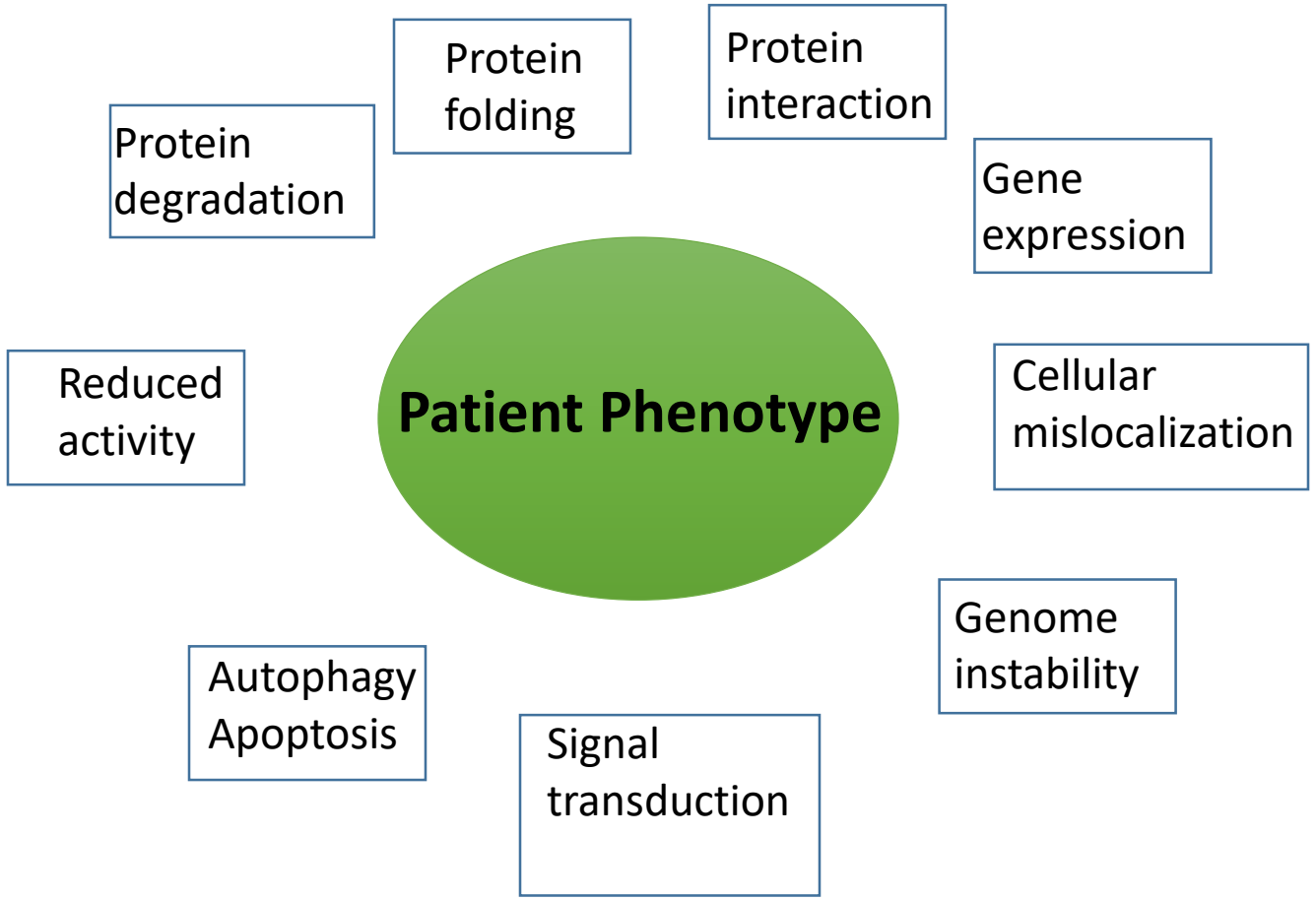
Nonsense mutations only in heterozygous situation

Null mutations in GNE is lethal

GNE Myopathy: Biology

- Hyposialylation of key molecules is a possible mechanism. Can lead to inclusion body formation
- Alternate mechanism, GNE participates in other functions through protein-protein interaction and mutant proteins have reduced ability to interact. Alpha-actinin is a possible interacting partner
- **NOT KNOWN**
- There is no relationship between genotype and phenotype
- Not clear why skeletal muscle is the diseased tissue

Possible Mechanisms of Pathogenesis in GNE myopathy



Genetic disorders provide natural systems for basic human biology research

- Unlike model systems one cannot generate mutations in genes of interest in human
- These diseases provide human mutants of a large range of genes
- Different types of mutations, such as nonsense, missense, deletion, insertion and copy number variations
- Generally house keeping genes are not involved

Need alternate path to drug discovery for rare diseases

- Patients or patient groups are not considered to be a stakeholder in drug discovery process
- Tripartite collaboration-Researchers, clinicians and patients groups
- Patients input in “outcome measures” and “endpoint” of trials
- Patient feedback on efficacy and quality of life
- Patient supported trials

Acknowledgement

Sudha Bhattacharya, JNU
A. Nalini, NIMHANS,
S. Khadilkar, JJ Hospital, Mumbai
Ashraf Mannan, Strand Life Sciences
Partha Majumder, NIBMG

Patients, volunteers, families of GNE Myopathy