Perspectives in GNE Myopathy: Research, Clinical Management & Patient Care

Held on February 16-17, 2018, at Indian National Science Academy, New Delhi

Summary for patients

The Science-

It is important to understand how the disease is caused as that will guide us about the best approach for therapy. It emerged from this meeting that we have two schools of thought. The Japanese group (Dr. Nishino) holds the view that GNE myopathy is caused primarily by the reduction of sialic acid (SA) in the body. They find that level of SA on the surface of muscle cells is very low in GNEM patients compared with control. Therefore, increasing the levels of sialic acid (or its precursors like ManNAc) in the body should relieve the symptoms.

In favour of this view are the results with mouse model where some of the symptoms were reversed upon feeding with SA. In patient muscle cells (myotubes) grown in the lab, SA deficiency on the cell surface was reversed by addition of SA or ManNAc.

Against this view is the data from Israel group (Dr. Argov, Dr. Stella) who believe that SA deficiency does not explain all the problems of GNEM. Rather they feel that the GNE protein may have other important roles, like binding to other molecules in the cell. In patients some of these binding interactions may be weak- leading to problems in muscle cells.
In support of their view is the observation that SA levels are only slightly reduced in many patients, but the disease is there. However, they or other groups working on GNEM do not yet have the data to clearly show the involvement of GNE protein in other cellular processes. We need more research in this aspect.

The scientific data presented in this meeting by the above two groups and by the Indian groups (Dr. Ranjana Arya working in GNE myopathy; and Dr. Kaushik Chakraborty working on protein folding) give us the following picture about the GNE protein, its possible functions, and what possibly goes wrong in GNEM, as summarized below-

The GNE protein is directly involved in SA synthesis in the cell (already well established fact). However, the mutations in GNEM patients are distributed throughout the GNE protein and not only in those parts needed for its activity to make SA. The protein from patients retains the activity to make SA but at a reduced level.

There does not seem to be a general defect of sialylation in GNEM (Remarks: Sialylation is the attachment of SA to other molecules in the cell. SA functions by attaching to a large number of molecules on the outer surface of the cell). General sialylation takes place in GNE patients. Looks like the defect in GNEM could be that SA does not attach to some very specific molecules which are important. These need to be investigated.

Some of the defects in GNEM could arise due to the GNE mutations affecting cell survival pathways.

The GNE protein is needed for muscle development. It directly binds to a protein called actinin, which is an important structural protein of the cell needed for muscle function. The

association of GNE and actinin is weakened in GNEM.

The proteins in our cells need to be folded correctly for proper function. In GNEM there is likely to be protein misfolding which gives rise to inclusion bodies seen in muscle biopsy. We are not clear why this misfolding occurs in GNEM.

Important unanswered Questions-
Is GNEM a disease of low sialic acid concentration?
What is the origin of protein aggregates in GNEM muscle, and are these toxic?
Why does the disease appear at a later age, and only in some muscle tissues?

Therapies-

Since mechanism of disease is not entirely clear in GNEM, it is not advisable to design a single therapeutic strategy. Rather, we could try different approaches simultaneously. Gene therapy could be considered a very promising approach as it does not depend on knowledge of the disease causing mechanisms. It simply provides a correct copy of the gene to the affected tissues.
Below is a description of some of the possible therapies.

Gene therapy-
Dr. Stella described their results with gene therapy using Adeno Associated Virus (AAV) vectors in the GNE mouse model (of Japan). They used the AAV capsid serotype Cap AAVrh74 of Jerry Mendell, and the MCK promoter specific for expressing the gene in muscle.
Their data showed that there was no toxicity in mice due to gene therapy treatment. The female mice showed some improvement in weight after gene therapy. However, they could not show whether gene therapy had any therapeutic effect in terms of muscle improvement as the GNE mice were not showing muscle defect any more. That means the mouse model is not working very well and we perhaps need to make another mouse model (or any other animal model). However, it was felt that lack of consistent animal model that provides proof of concept should not be a barrier for gene therapy trials in humans. Unfortunately her experiments also show that MCK may not be that tissue specific.

What is the status of gene therapy preclinical studies being done by Dr. Jerry Mendell?
Jerry Mendell has the complete set up for gene therapy. They have Regulatory team , Viral Vector Manufacture team, Clinical team: Physicians, nurses, physiotherapists, pathologists; and Laboratory team.
In June 2017 FDA was given the study data from mouse model for preIND approval. FDA has asked for better toxicology design at higher doses. However, it is not clear questions raised by FDA which it appears that Jerry Mendell has answered. This work is in progress though many people believe that Dr. Mendell may not be very interested in this trial. Currently there is money for Phase1/2 gene therapy trial with 6 patients. Further funds need to be raised. What is the time frame? It is not clear.

SA/ManNAc supplementation (Clinical Trials)-
As we know the SA trial by Ultragenyx has been discontinued due to insufficient improvement in patients. Dr Nishino feels the Ultragenyx trial failed for some technical reasons- like, they changed the criteria in phase 3 for determining efficacy of SA. The ‘active declining period’ used was at an earlier time point when the muscle decline had not yet set in. Therefore not much difference was seen with respect to placebo.
Dr. Nishino informed that NIH will be recruiting 50 patients for multicentre trial (10 clinical

sites) of ManNAc phase 3 trial to determine clinical efficacy. Primary end point- Quantitative muscle strength. Primary analysis- slowing in rate of progression.
For non-US patients- ENMC workshop is planned for 2019. It will meet to brainstorm for future therapies/clinical trials.
Dietary sources of SA were listed by Dr. Nishino-
Milk and egg are rich sources. However one has to consume very large quantities to get sufficient SA. For example, 4 l goat milk, and 16 l cow milk are needed to get 1 gm SA.

Other therapies-
In GNE myopathy the defective protein is a cytosolic enzyme and not a structural protein. Thus it is expected that even small quantities of normal functioning protein will suffice to overcome the disease mechanism. Small molecules that interact with proteins have the potential to modulate protein function.
Potential applications of small molecules

- Can increase expression of the gene
- Can modify the properties of the mutant protein to increase its activity
- Can help mutant protein to fold properly, not allowing inclusion bodies to form
- Can modulate autophagy

It may be possible to test already approved molecules for other diseases to see if they can benefit us. We need a suitable cell-based or animal model of GNE myopathy to test this.

Other suggestions from Dr. Nishino for possible alternative drugs- Arimoclomol- a drug to slow muscle degeneration.
N-Acetyl Cysteine (NAC) for muscle atrophy; other SA compounds like sialyllactose which might have better uptake than SA.

Gene therapy resources in India

Dr. Arkasubhra Ghosh (GROW Labs, Narayan Nethralaya, Bengaluru) has extensive experience in gene therapy using AAV vectors. In his postdoctoral work in USA he had developed gene therapy systems for Duchene muscular dystrophy. After joining GROW labs as Director he has set up clinical grade vector production facility for AAV vectors. Recently they succeeded in gene delivery into cornea using rabbit model.
Dr. Ghosh stressed the urgent need to check which types of AAV vectors would be efficient and safe to use in the Indian population depending on whether they generated an immune reaction. This needs to be tested in our population. We cannot blindly use the vectors developed in other countries.
We should formulate our national guidelines for use of gene therapy in India.

Stem Cell possibilities in India

Dr. Sujata Mohanty (AIIMS, New Delhi) has set up a state-of-the-art stem cell facility at AIIMS. They have taken the skin cells from patients, including one DMD patient and made induced pluripotent stem cells (iPSCs). Have been kept frozen.
They could differentiate iPSCs into myoblasts. Efforts to be made to get 3D muscle tissue from stem cells after differentiation.

Clinical experience of GNEM in India

The maximum number of confirmed GNEM patients in India have been correctly diagnosed by Dr. Nalini at NIMHANS, Bengaluru and by Dr. Khadilkar's group at Bombay Hospital, Mumbai. Dr. Nalini has seen patients from different states of the country, except from north

western parts of India. They were so far getting genetic testing from Newcastle (Dr. Lochmuller) and Japan (Dr. Nishino) but now they will do it themselves at NIMHANS.
Age of onset in Indian patients is generally 25-29 yrs. It is later for patients with both mutations in kinase domain compared with those in epimerase domain, or one mutation each in epimerase and kinase domains. Mutations are spread throughout the GNE gene. Most patients are compound heterozygous (that is, with two different mutations in each chromosome). The most common mutation in patients is Val727Met. It is found in 70% patients. Another mutation Ile618Thr is also common, especially in Rajasthan and Gujarat. The Val727Met mutation is very common in the entire Indian population, and more so in Gujarat. It is not found in other parts of the world, except in Thailand.
Dr. Nalini spoke about the Computerized 'Gaitrite' pressure sensitive mat walkway system which they are using to objectively evaluate the gait of patients. This will be useful to monitor progression in gait anomalies.
Strand genomics ((Bengaluru) also do DNA testing for GNEM. Dr. Mannan from Strand informed about the extensive sequencing done by them for a large variety of rare genetic disorders.
Misdiagnosis of GNEM is very high in India. Most neurologists are not familiar with it. We need more experts in GNEM to correctly diagnose the disease from clinical symptoms (need to learn from Japan where patients are correctly diagnosed in the first attempt because doctors are taught about this disease).

Registry

One of our priorities is to generate a GNEM patient registry in India. This is needed to approach Govt for policy decisions, for fund-raising for our disease, for drug-development initiatives and clinical trials.
Dr. Madhulika Kabra (AIIMS, New Delhi) has been spearheading the Rare disease policy formulated by Govt. She informed that ICMR has launched the rare disease registry initiative last year. Funding for this work is expected this year. A multicentric national registry is to be prepared. Amongst the diseases included in phase I are some neuromuscular disorders (DMP, SMA, LGMD) but not GNE.
We can approach ICMR and include GNEM also (Contact Dr. Reeta Rasaily at ICMR).
We will need harmonized consent form, and patient information sheets. Details can be obtained from ICMR. NIMHANS, Bengaluru can be suggested as the nodal centre for data monitoring for GNEM.
It was suggested that patients take the initiative to collate the data on known, genetically verified GNEM patients in India. Dr. Nalini (NIMHANS, Bengaluru) has data for 82 GNEM patients while Dr. Khadilkar's group (Bombay Hospital, Mumbai) have data for 58 GNEM patients.
We can learn from the formats of GNEM patient registries elsewhere. Registry of Japanese patients (REMUDY).

Genome sequencing-

So far GNEM patients only know about the mutations in their GNE gene. It is puzzling that different patients, and even siblings show different pattern of disease onset and progression. Could other genes in each individual contribute to this variation? Dr. Madhuri Hegde (Perkin Elmer) spoke about whole genome sequencing (WGS) efforts to look at the contribution of variants in other genes to the disease outcome in GNEM. She has been funded by NDF to do WGS on 100 GNEM patients. She will combine it with metabolomic study to get comprehensive data that could reveal the reasons for different disease outcomes in different

patients.
Dr. Ravi Gupta (MedGenome) is running a project (Genome Asia) on large scale genome analysis of 100,000 Asian individuals. This will help to understand population history and ethnicity of Asians. Needed for precision medicine.

Patient Organization and advocacy

We need to come together and assert ourselves. We need greater visibility. We should work to spread awareness amongst the government and philanthropic segments to raise funds for research and trials. We could try to rope in some celebrity as our brand ambassador. Could also approach all sections of media. Can use social media to help in correct diagnosis by posting the important symptoms.
Dr. Nishino suggested we could approach the Japanese patient group- Patient Association of Distal Myopathies (PADM). Very active group. Have made News and TV programs to popularize myopathies in Japan.
As a patient group we need to lobby with Govt. to make it easier for pharma companies to take initiative- for example by removing bureaucratic hurdles which pharma companies have to face. We should try to interest new start-up pharma companies.
Need to approach Govt. for support, with a concrete proposal (See below in Govt policy).
Dr. Umapathi (NNI, Singapore) exhorted all members of society to change their perception of disability as it can happen to anyone at any time and is a common concern. We have to fight against the widespread exclusion of disabled people from the general public space. They need to be proportionately represented and influence policy for their benefit.

Exercise and alternative therapies

Dr. Argov advised that general exercise is good for GNEM. Aerobic exercise good. Prevents muscle from getting into 'detraining'. Walking, static pedalling good. No need to lift weights. Too much is bad. Should not have muscle pain.
Dr. Harpreet Singh (Physiotherapy Dept., AIIMS) said that most of the time patients come to physiotherapist when it is quite late. Early referral is necessary to help in saving muscle. Generally light to moderate exercise is recommended. Aquatic exercises (in swimming pool) are beneficial.
Dr. Vishnupriya (Yoga instructor, New Delhi) emphasized on focused exercise with full involvement of the mind. Doing exercise mechanically is not so beneficial. One must feel the muscle being exercised through the mind.
Dr. Bhavana Prasher, Dr. Ramniwas Prasher (Ayurveda) explained the tenets of ayurveda. The pathologic presentation of GNE myopathy is described in ayurveda. The therapeutic approaches include dosha cleaning (shodhana), dosha balancing (shamana) and bulk promoting/regeneration (brimhana, rasayana). Dr. Ramniwas Prasher can be contacted for treatment.

Government Policy

Dr. V.K. Paul- NITI Aayog and Dr. V.M. Katoch (former Dirctor General, ICMR) gave very positive suggestions.
Rare diseases have been ignored as it is a game of numbers. The disease burden of morbidity and mortality is huge in other diseases. But public health concept has to go beyond numbers. The problems of each individual must be addressed in a fair way- not taking away the right of

others.

In response to a PIL from a rare disease patient, the Honble Delhi High Court charged GOI to come out with policies on rare diseases. The document came out last year. A parallel initiative has also been taken by Delhi Govt. So the Govt. is now taking note of rare genetic diseases.

Rare disease patients must make noise, write about their diseases, hold meetings and increase visibility. Need to build resources for diagnostics and connect patients. The intent is there in India and the framework is there in the form of rare disease policy. Need to build on it. Patient groups should guide the Govt how to move forward through concrete plan of action. Need effective policy for drug development. Patient groups should give suggestions about drug development and drug adaptation. Suggestions should be concrete, balanced, with timelines and milestones to be achieved. Govt could give incentives like tax exemption to boost drug development for rare diseases. Govt should be approached to build infrastructure, and to dedicate funding for research in these diseases. Dr. Jagannathan (Vice-president, INSA) said that the Indian National Science Academy will be happy to participate in promoting research in rare genetic disorders.