2013 GAETANO CONTE PRIZE LECTURE

GNE myopathy: a personal trip from bedside observation to therapeutic trials

Zohar Argov

Kanrich Professor of Neuromuscular Disease, Hadassah-Hebrew University Medical Center, Jerusalem, Israel
Based on the Gaetano Conte prize lecture delivered at the 11ᵗʰ meeting of the Mediterranean Society meeting (Athens 2013).

The trip is not over yet as definite therapy for GNE myopathy is not yet available. Also the exact mechanisms by which GNE defects lead to isolated muscle disease in humans are not fully recognized. But in the Gaetano Conte lecture of 2013 I have tried to describe how much a progress was made in several research laboratories and clinical institutes in the investigation of this unique myopathy.

Introduction

This is a personal review of the history and current status of a rare myopathy that spans my 35 years' career in the neuromuscular field, starting from a bedside observation to ongoing therapy trials. As a neurologist in training I returned from Newcastle UK after a WHO sponsored fellowship period at the Muscular Dystrophy Laboratories under Professors John Walton and Frank Mastaglia. Soon after my return in late 1979 I was called for a consultation to a hospitalized man who was at his 60's. I found him sitting in his wheelchair at the hospital corridor playing with his little granddaughter: he extended his legs straight forward and she was riding on them at the ankles. This was a very unusual feature for a person with progressive myopathy that required major strength in the quadriceps (usually the muscle first involved in most muscular dystrophies and acquired myopathies). By 1982 I identified 9 patients with this unique syndrome of quadriceps sparing myopathy who all were Jews of Iranian (Persian) origin. They all had adult onset myopathy, with modest elevation of serum CPK and biopsy that showed various numbers of muscle fibers containing 'rimmed' vacuoles. I termed this disorder quadriceps sparing myopathy (QSM) and sent it

to be published in a special issue (foreschrift) honoring Sir John Walton's departure from Newcastle to Oxford in 1982. It took until 1984 to be published in the *Journal of Neurological Sciences* (1), by then I already accumulated 27 such patients (added as an addendum in that publication). Few reports of a similar disorder were published in the following two decades from other countries. Because of the similarities in the histological feature of the QSM with the sporadic IBM, the disease carried the term hereditary inclusion body myopathy (HIBM) or IBM2 (2). The understanding that this is not only a unique clinical entity but a well-defined genetic disease came from the work of Stella Mitrani-Rosenbaum, with whom I continue to collaborate for the past 20 years, who by 1995 linked the Persian Jewish QSM to chromosome 9 (3). This led to a special meeting in Napoli in 1996 where the diagnostic criteria for this recessive myopathy were set by a group of experts from around the world and published in a special issue of Acta Myologica (4). In 2001 Iris Eisenberg, a PhD student in Prof Mitrani-Rosenbaum's laboratory, identified mutations in the gene encoding N-acetylglucosamine epimerase/N-acetylmannosamine kinase (termed *GNE*) as the disease causing defect (5). The linkage data and later the identified gene defects helped in determining that what was thought to be a different disorder previously described in Japan as distal myopathy with rimmed vacuoles (DMRV or Nonaka's myopathy (6)) is in fact the same condition (7). A consortium of researchers working on various aspects of this disease has decided recently to unify the name for this myopathy and call it GNE myopathy (8).

I will review some issues related to GNE myopathy with a personal perspective.

Address for correspondence: Zohar Argov, Department of Neurology Hadassah-Hebrew University Medical Center, Jerusalem, Israel. E-mail: zohara@ekmd.huji.ac.il

Clinical features

These have not changed much since the original descriptions. It usually starts at the third decade of life but few cases were reported with onset in the early teens and some had late onset in the 5th decade. The typical onset in the vast majority of patients is distal weakness in the legs (drop foot). The disease is usually slowly progressive spreading to the proximal musculature and the upper limbs but patients may maintain independent walking for many years relying on the hip strength. A major determinant of early loss of ambulation is quadriceps weakness. Although most patients retain the quadriceps sparing feature through several decades, about 5% have various degrees of weakness in this muscle early on. However, the unique clinical pattern of strong quadriceps in spite of major involvement in other leg muscles is still the best clinical hint for the diagnosis of GNE myopathy and is unequalled in any other neuromuscular disorder. Unusual patterns of onset in proximal lower limb musculature and even in the upper limbs is still associated with quadriceps relative or total sparing. The cause of the quadriceps sparing remains one of the enigmas of this condition (9). The understanding of what preserves this muscle could be of major help to future therapy.

Genetic features

To date, several dozens of mutations in the *GNE* were associated with this myopathy. A clear phenotypic-genotypic correlation has not emerged although recently in the cohort of patients from Japan heterozygous patients seem to have a more severe course (10). This observation remains to be confirmed in other countries. There are, however, several important genetic data that emerge from the various reports of patients with this recessive myopathy. While the mutations are spread along the various exons of the *GNE* gene, most of them are missense mutations. Apart from few ethnic clusters (see Epidemiology) most patients are compound heterozygotes, carrying usually two missense mutations or a combined missense and nonsense mutations. No patient with two nonsense mutations was described. Since *GNE* knockout is lethal in mice it is presumed that such condition is also nonviable in humans. It is my personal belief that *GNE* double nonsense genotype results in a completely different disorder, possibly not only limited to muscle but this remains to be proven. Another enigma in GNE myopathy is the finding of homozygous person for the common mutation in Persian Jews (with other affected members in the family) who has no myopathy at age 78 years. We are currently working to understand this unusual situation in recessive disorders, because it can provide yet another clue for the disease pathogenesis and its therapy.

Epidemiology

The largest ethnic cluster of GNE myopathy is in the community of Jews originating from Iran and neighboring Middle Eastern countries (11). They all share homozygosity to a specific *GNE* mutation (M743T using the new nomenclature- see (8)). With about 150 patients identified in Israel and about 50-70 in other countries (mainly the USA) this large cohort represent a prevalence of 1:1500 in this community with an estimated carrier rate of 1:20-30 (11). A similar number of patients but in a much larger and mixed population was recorded in Japan (under the term DMRV) but in this country the overall prevalence is smaller. Although two mutations are more common (considered to be founder mutations) numerous patients are either compound heterozygotes to those or to a combination with other mutations. This is a unique situation where a rare myopathy is more frequently reported in two countries but the epidemiology of the mutations is so different in them. Is it more awareness in the two countries from which the original descriptions emerged? The disease has been reported from numerous other countries (Asian and Europeans) and is certainly a world-wide disorder, thus recognition may not be the sole explanation to this variable epidemiology. It should be pointed out that no patient was reported from South America but again this maybe a result of lack of awareness and diagnostic methods. Estimates of the total number of patients are difficult as only few patients are currently identified in China and India, but it should still be regarded as a rare disease with several hundred to possible few thousands of patients.

Pathophysiology

GNE is encoding a bi-functional enzyme in the sialic acid synthetic pathway. As a result, one could consider it to be a 'metabolic myopathy' with reduced product (hyposialylation). However, when summarizing the biochemical investigations in this myopathy one is faced with few more enigmas. GNE activity is only partially reduced in patients ranging from 30-60% reduction. Such range of reduction is not expected to cause a phenotype in the 'classical' metabolic myopathies. Testing overall sialylation of muscle showed that it is only marginally reduced in some patients and more severe in others (12, 13). Searching for specific muscle glycoconjugate (protein or lipid) did not reveal a crucial hyposialylated metabolite that could determine the disease pathophysiology.

The HIBM research laboratory at Hadassah has spent a lot of time and effort in determining other 'unknown' functions of GNE that could be part of the pathophysiology of this myopathy (14). While some other functions

may have been identified, the process by which a *GNE* defect leads to muscle disease remains to be fully elucidated. I believe that the lack of normal GNE in muscle is contributing to the degenerative disease process in GNE myopathy, not only through the metabolic pathway.

In trying to develop a mouse model for GNE myopathy it became clear, yet again, that mice are not human. An attempt to develop a knock-in model with the common Middle Eastern mutation resulted in a different lethal disorder in early life (few days) due to a kidney disease (15). Adding yet another enigma to the story, our laboratory has managed to produce a line of mice homozygous for this mutation who do not show any kidney or muscle disease after more than 12 months of life (16). What was rescued here and whether it is similar to the protective effect in our elder subject homozygous for this mutation is currently unknown. Clearly an answer to this puzzling issue carries a promise for therapy.

One cannot ignore though the role of hyposialylation in *GNE* defects. Both the knock in model and the transgenic model for the D176V (currently termed D207V) common Japanese mutation produced in the Tokyo laboratory could be treated with sialic acid or its metabolic precursors with major improvement (15, 17). These observations set the stage for the current and future therapy trials.

Therapy

GNE myopathy has currently no proven effective therapy. However, an important human trial based on the hypothesis of reduced sialic acid as a major contributor to the myopathy pathogenesis was initiated by Ultragenyx Pharmaceutical (18). 46 patients have completed 24 weeks of treatment with oral slow release sialic acid preparation (SA-ER). Preliminary results show that the serum levels of free sialic acid doubled. Modest positive effect was seen in changing the progressive weakness course in the upper limbs with statistically significant slowing over this short period. Results of 48 weeks SA-ER treatment are pending. A phase 1 trial with a metabolic derivative (ManNAc) was also completed and a phase 2 trial is pending at the NIH- USA. Will such metabolic correction, 'bypassing' the defect, suffice to arrest the progression or restore the muscle function? At this stage there is no clear answer to this question but efforts are continued.

If indeed the lack of normal GNE in muscle is of major importance to the disease process, then gene therapy will have to be applied. Under Prof Mitrani-Rosenbaum, the laboratory at our Institute is devoting its major effort toward such therapy, using viral mediated gene delivery to muscle. A construct based on AAV8-human wild type *GNE* driven by MCK promoter has been designed.

Such a construct has been injected into normal mice intravenously and yielded a persistent intramuscular human GNE mRNA. An experiment to determine whether this construct will be able to prevent or reverse the myopathy in the Japanese transgenic mouse model is underway.

Acknowledgment

References

1. Argov Z, Yarom R. Rimmed vacuole myopathy sparing the quadriceps. A unique disorder in Iranian Jews. J Neurol Sci 1984;64:33-43.

2. Askanas V, Engel WK. New advances in inclusion-body myositis. Curr Opin Rheumatol 1993;5:732-41.

3. Mitrani-Rosenbaum S, Argov Z, Blumenfeld A, et al. Recessively-inherited inclusion body myopathy in Iranian Jews maps to chromosome 9p11-p21. Hum Mol Genet 1996;5:159-63.

4. Argov Z, Sadeh M. Recessively-inherited inclusion body myopathies: Clinical and laboratory features. ACTA Myologica 1998;1:41-4.

5. Eisenberg I, Avidan N, Potikha T, et al. The UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase gene is mutated in recessive hereditary inclusion body myopathy. Nat Genet 2001;29:83-7.

6. Nonaka I, Sunohara N, Ishiura S, et al. Familial distal myopathy with rimmed vacuole and lamellar (myeloid) body formation. J Neurol Sci 1981;51:141-55.

7. Nishino I, Noguchi S, Murayama K, et al. Distal myopathy with rimmed vacuoles is allelic to hereditary inclusion body myopathy. Neurology 2002;59:1689-93.

8. Huizing M, Carrillo-Carrasco N, Malicdan MC, et al. GNE myopathy: new name and new mutation nomenclature. Neuromuscul Disord. 2014 Mar 13. pii: S0960-8966(14)00079-0. doi: 10.1016/j.nmd.2014.03.004. [Epub ahead of print].

9. Argov Z, Mitrani-Rosenbaum S. The hereditary inclusion body myopathy enigma and its future therapy. Neurotherapeutics 2008;5:633-7.

10. Mori-Yoshimura M, Monma K, Suzuki N, et al. Heterozygous UDP-GlcNAc 2-epimerase and N-acetylmannosamine kinase domain mutations in the GNE gene result in a less severe GNE myopathy phenotype compared to homozygous N-acetylmannosamine kinase domain mutations. J Neurol Sci 2012;318:100-5.

11. Argov Z, Eisenberg I, Grabov-Nardini G, et al. Hereditary inclusion body myopathy: the middle eastern genetic cluster. Neurology 2003;60:1519-23.

12. Hinderlich S, Salama I, Eisenberg I, et al. The homozygous M712T mutation of UDP-N-acetylglucosamine 2-epimerase/N-acetyl-mannosamine kinase results in reduced enzyme activities but not in altered overall cellular sialylation in hereditary inclusion body myopathy. FEBS Lett 2004;566:105-9.

13. Salama I, Hinderlich S, Shlomai Z, et al. No overall hyposialylation in Hereditary Inclusion Body Myopathy myoblasts carrying the homozygous M712T GNE mutation. Biochem Biophys Res Comm 2005;328:221-6.

14. Sela I, Milman Krentsis I, Shlomai Z, et al. The proteomic profile of hereditary inclusion body myopathy. PLoS One 2011;6:e16334.

15. Galeano B, Klootwijk R, Manoli I, et al. Mutation in the key enzyme of sialic acid biosynthesis causes severe glomerular proteinuria and is rescued by N-acetylmannosamine. J Clin Invest 2007;117:1585-94.

16. Sela I, Yakovlev L, Becker Cohen M, et al. Variable Phenotypes of Knockin Mice Carrying the M712T Gne Mutation. Neuromolecular Med 2012;15:180-91.

17. Malicdan MC, Noguchi S, Hayashi YK, et al. Prophylactic treatment with sialic acid metabolites precludes the development of the myopathic phenotype in the DMRV-hIBM mouse model. Nat Med 2009;15:690-5.

18. ClinicalTrials.gov: NCT01517880.

19. Mitrani-Rosenbaum S, Yakovlev L, Becker Cohen M, et al. Sustained expression and safety of human GNE in normal mice after gene transfer based on AAV8 systemic delivery. Neuromuscul Disord 2012;22:1015-24.