

COMMENTARY

ADVANCES IN THE UNDERSTANDING AND MANAGEMENT OF NEUROMUSCULAR DISEASES

Alberto J.L. Macario^{1,2} Everly Conway de Macario¹¹ Department of Microbiology and Immunology, School of Medicine, University of Maryland at Baltimore-Institute of Marine and Environmental Technology (IMET), Columbus Center, Baltimore, MD, USA.² Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo, Italy.

CORRESPONDENCE:

Alberto J. L. Macario, M.D.

e-mail: albertomacario@iemest.eu

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Abstract

Skeletal muscles are essential for virtually all human activities but they would not function without the nerves that serve them. Life safety in our moving world indoors and outdoors depends on an optimal neuromuscular system. Molecular chaperones are indispensable for development and maintenance of muscles and nerves throughout the life span of an individual. It follows that chaperone failure may affect seriously the neuromuscular system. Indeed, chaperone deficiency does occur and can cause disease, a chaperonopathy. Many chaperonopathies affect primarily muscles and nerves, causing neuro-myopathies. These pathologic conditions are discussed here and illustrative examples are presented, focusing on the information provided in an open access eBook recently published and available to all. The notion that chaperones can be pathogenic must be borne in mind by physicians and health professionals in practice and research; it will enable them to improve differential diagnosis and patient management. Chaperonopathies may be involved in the mechanism of many diseases, not just those affecting preferentially muscles and nerves, but they go undiagnosed because currently they are not in the medical curricula. This article is an attempt to correct, at least partially, this deficiency.

Key words: neuropathies; myopathies; chaperonopathies; chaperonotherapy; archaea; Bardet-Biedl Syndrome; Spinal Muscular Atrophy; Pelizaeus-Merzbacher Disease; Distal Hereditary Motor Neuropathies; Multisystem

Proteinopathies; Limb Girdle Muscular Dystrophy; Amyotrophic Lateral Sclerosis; Melusin; p97; Leukodystrophies.

Abbreviations: HSP, or Hsp, heat shock protein; BBS, Bardet-Biedl Syndrome; CCT, Chaperonin containing T-CP1; MKKS, McKusick-Kaufman Syndrome; SMA, Spinal Muscular Atrophy; SMN, Survival Motor Neuron; snRNA, small Nuclear RNA; snRNP, Small Nuclear Ribonucleoprotein; ALS, Amyotrophic Lateral Sclerosis; PMD, Pelizaeus-Merzbacher Disease; ER, Endoplasmic Reticulum; UPR, Unfolded Protein Response; dHMN, Distal Hereditary Motor Neuropathy; MSP, Multisystem Proteinopathy; FALS, Familial Amyotrophic Lateral Sclerosis; CMT2Y, Charcot-Marie-Tooth Disease, Type 2Y; LGMD1D, Limb Girdle Muscular Dystrophy type 1D; SOD1, Superoxide Dismutase 1.

Foreword

The central and peripheral nervous systems were heroes in my (AJLM) life of medical student, marvelous entities floating in the imagination of young men and women learning the complex vital processes in health and disease. The two heroes, together with the muscles, seemed to impulse life, action, moving forward as the only accepted alternative of youth. Old textbooks already showed how important the two systems were for the physician, no matter his/her specialty. The semiology of these systems was one of the fundamental areas of training for medical students in the old days. The front cover of an old text book on this matter I recently unearthed is shown in Figure 1. Pertinent to this article are two figures in that book, here Figure 2 and 3, still valid in their

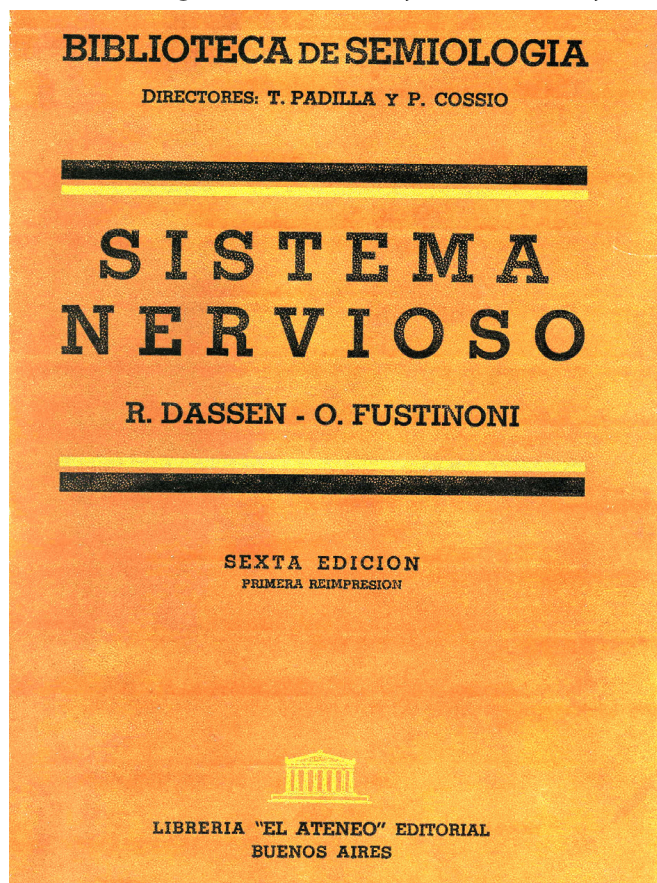
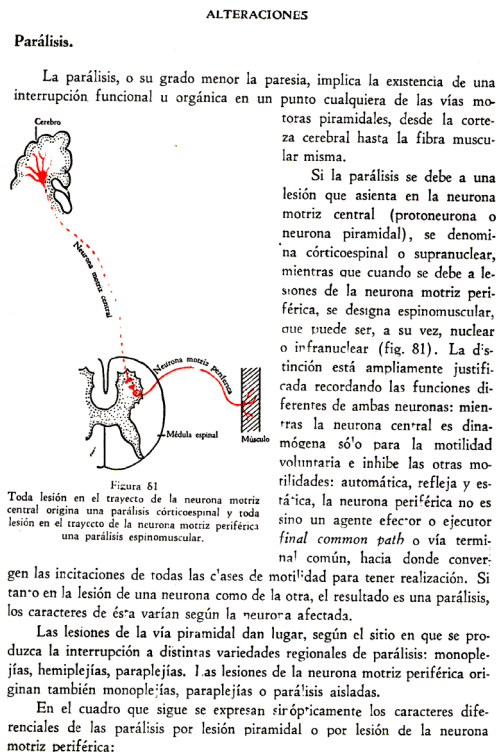
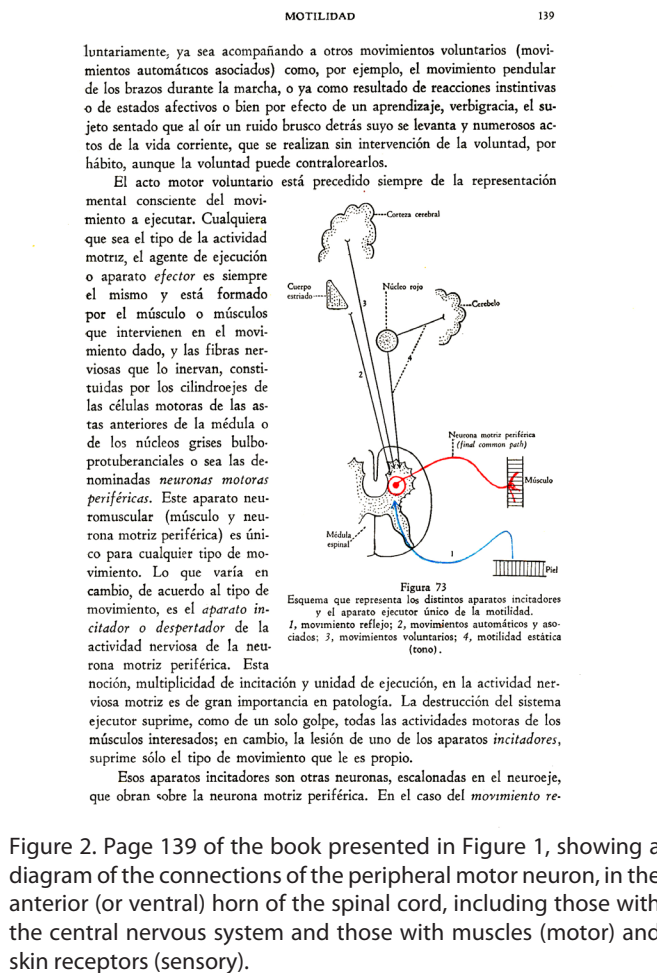


Figure 1. Front cover of a classic book in Spanish on the semiology (semiotics) of the nervous system, published in 1955.



motor neuron and its connections both ways, up and down so to speak. Figure 3, shows how a lesion of the central or peripheral motor neurons would cause a corticospinal or a spinomuscular syndrome, respectively (namely a central or a peripheral paresis or plegia).

Nowadays we know a great deal more than in those days, particularly at the molecular level. For instance, molecular chaperones have been recognized as essential factors in the development and functioning of the nervous system, both central and peripheral. The immediate inference is that abnormalities in molecular chaperones will have a serious, deleterious impact on both systems. And many abnormalities of chaperones do occur, because of gene mutation or other reasons and cause diseases. Some of these diseases affect the neuromuscular system as it will be discussed below.

Introduction

The neuromuscular system is made of all the skeletal muscles in the body and the nerves that

connect them with the spinal cord and brain. The coordinated action of muscles moves the body and its parts as necessary for all mechanical activities. One might say that muscles and nerves are essential for life safety and security, the soul of this Journal. Yes, other tissues and organs are essential for life, as much or more than muscles and nerves, but in what concerns to safety and security these latter two are unrivaled in importance. Thus, it is of the essence to take good care of them, for instance with daily systematic well-programmed exercises. Unfortunately, there are many diseases that affect primarily muscles and nerves and they are, indeed, invalidating. These diseases can be genetic or acquired, and are usually characterized by pain, and by motor, gait, and balance abnormalities, often accompanied by disruptive accidents at home, at work, and elsewhere. Thus, these diseases are a major threat to life safety and security.

In the development and physiology of muscles and nerves intervene a range of factors, among which molecular chaperones (also named heat shock proteins or Hsp, also written HSP) are of exceptional importance. This is because chaperones are necessary for the correct folding and assembling, and thereby for the functioning of many other molecules and structures that integrate muscles and nerves. If one or more chaperones fail, the impact on muscles and nerves can be disastrous. And chaperones do fail if affected by pathogenic mutations or other abnormalities, such as aberrant post-translation modifications, or simply because they are quantitatively below or above the normal range of concentrations at the site they exercise their functions. The pathologic conditions caused by quantitative and/or qualitative defects of chaperones are the chaperonopathies (Macario and Conway de Macario, 2005; Macario et al.,

Table 1. Articles in the eBook on genetic chaperonopathies of the nervous and muscular systems

Art. #	Molecule / gene	Disease	Date	Country	Corresponding author / e-mail address	Refs.
1	BBS6, BBS10, BBS12	Bardet-Biedl Syndromes	VII-31-2017	Spain	Diana Valverde/ dianaval@uvigo.es	54
2	SMN	Spinal Muscular Atrophy	VI-08-2017	Malta	Ruben J. Cauchi/ ruben.cauchi@um.edu.mt	74
3	ER chaperones	Pelizaeus-Merzbacher	II-24-2017	Japan	Ken Inoue/ kinoue@ncnp.go.jp	48
4	Archaeal chaperones	Chaperonins Groups I and II	I-09-2017	USA	Everly Conway de Macario/ econwaydemacario@som.umaryland.edu	51
5	DNAJ; sHSP (DNAJB2, HSPB1, HSPB3, and HSPB8)	Hereditary Motor Neuropathies	XII-14-2016	Spain	Carmen Espinós/ cespinos@cipf.es	78
6	Human AAA+ chaperone p97	MSP; FALS; CMT2Y	XII-01-2016	USA	Di Xia/ xiad@mail.nih.gov	118
7	HSPE1 (HSP10, Cpn10)	Neuropathy	X-07-2016	Denmark	Anne S. Bie/ anne.sigaard.bie@regionh.dk Peter Bross/ peter.bross@clin.au.dk	59
8	DNAJB6	Myopathies; LGMD1D	IX-30-2016	Italy	Marina Mora/ marina.mora@istituto-besta.it	42
9	Melusin	Heart stress	IX-12-2016	Italy	Mara Brancaccio/ mara.brancaccio@unito.it	55
10	HSPD1 (HSP60, Cpn60)	SPG13; MitCHAP-60	VIII - 31 - 2016	Denmark	Peter Bross/ peter.bross@clin.au.dk	45
11	Cu/Zn-Superoxide Dismutase (SOD1)	Multiple Sclerosis	VIII - 09 - 2016	Japan	Yoshiaki Furukawa/ furukawa@chem.keio.ac.jp	50
Abbreviations: Art. #, Article number; Date, date of publication; Country, country where corresponding author works; Refs., number of references cited; BBS, Bardet-Biedl Syndrome; SMN, Survival Motor Neuron protein; ER, Endoplasmic Reticulum; HSP, Heat Shock Protein; MSP, Multisystem Proteinopathy; FALS, Familial Amyotrophic Lateral Sclerosis; LGMD1D, Limb Girdle Muscular Dystrophy type 1D; CMT2Y, Charcot-Marie-Tooth Disease, Type 2Y; SPG13, Spastic Paraplegia 13; MitCHAP-60, Mitochondrial Chaperone 60.						

2013; and updates at <http://www.chaperones-pathology.org>).

Advances in the understanding, diagnosis, and treatment of genetic chaperonopathies affecting preferentially muscles and nerves have been compiled in an open access eBook, recently published by FRONTIERS in Molecular Biosciences as a Research Topic (<http://journal.frontiersin.org/researchtopic/4348/pathologic-conditions-of-the-human-nervous-and-muscular-systems->

and_Degradation/researchtopics/Type_I_Chaperonin_Mechanism_and_Beyond/6050).

Diseases caused by abnormal chaperones or normal ones caught in pathogenic pathways can be classified in various ways like any other pathologic condition. For instance, considering the main abnormal feature of a sick chaperone, i.e., the one underpinning the pathogenic mechanism, chaperonopathies may be by defect, excess, or mistake (Table 2).

Table 2. Classification of chaperonopathies by pathogenic mechanism and specific chaperonotherapy

Chaperonopathy by:	Mechanism, features	Chaperonotherapy
Excess	Quantitative, e.g., due to gene dysregulation; upregulation; other	Negative: Chaperone-gene knockdown; chaperone-protein blocking (chemical compounds)
	Qualitative, e.g., gain of function	Negative: Chaperone-gene knockdown; chaperone-protein blocking (chemical compounds)
Defect	Quantitative, e.g., gene downregulation; absence or misplacement; sequestration; excessive demand (defect relative to excessive substrate availability); other	Positive: Chaperone-gene/protein replacement; chaperone-gene induction (e.g., mild stressors); combined
	Qualitative, e.g., due to structural defect genetic (e.g., mutation) or acquired (e.g., aberrant post-translational modifications)	Positive: Chaperone-gene/protein replacement; chaperone function boost (chemical compounds); combined
Mistake	"Normal" chaperones contribute to disease, e.g., tumors that need chaperones to grow; autoimmune conditions in which a chaperone is the autoantigen and/or induces production of pro-inflammatory cytokines	Negative: Chaperone-gene knockdown; chaperone-protein blocking (chemical compounds); combined

associated-with-mutant-chaperones-mo).

The topic is of great importance in Medicine and Public Health, since it affects entire families and presents them with extremely difficult choices on marriage and procreation. Therefore, the articles in the eBook, available online for free, should be of interest to a wide range of professionals working in the medical and biological sciences, including physicians and clinical pathologists in practice and in applied and basic research.

The eBook is an assembly of contributions from diverse authors from various countries on a variety of chaperonopathies, and features informative text, tables, and figures, as well as pertinent bibliography (Table 1). So, a lot of useful information is made available to health professionals that, hopefully, will reach a large audience, thereby benefitting many patients and their families. It should also be mentioned, that a related publication in FRONTIERS in Biomedical Sciences, can be found at (http://www.frontiersin.org/Protein_Folding,_Misfolding_

The notion that a chaperone can cause disease should be in the mind of every physician and researcher in biomedical sciences, so they may be able to think in the face of a patient that a pathogenic pathway, different from the others known and involving abnormal chaperones can occur, and which is amenable to specific diagnostic and treatment procedures. It follows, that chaperones can be central not only in the differential diagnosis exercise but also in the treatment strategy to implement, in which chaperones can be used either as targets or as agents. For example, patients with chaperonopathies by defect may benefit from positive chaperonotherapy, namely from means such as chaperone-gene replacement, aiming at correcting the deficiency. The various forms of chaperonotherapy pertaining to the different types of chaperonopathies are indicated in Table 2. Numerous projects are currently on their way testing in experimental models the various types of chaperonotherapy, and some are also

applied in clinical settings. These investigations should be encouraged because there are many patients with chaperonopathies, genetic and acquired, who along with their families need treatment and support. Patients and their close relatives and friends, all suffering, are a constant feature in the life of physicians. So, one may think of chaperonotherapy every time one has to deal with a chaperonopathy. Developments in chaperonotherapy must be based on laboratory and clinical research and, therefore, the information in the articles presented below should be instrumental for researchers to undertake investigations aimed at preventing and curing chaperonopathies.

Chaperone-gene mutations are likely to impair or abolish function, namely they cause chaperonopathies by defect, as illustrated by various examples discussed below. It is, however, important to bear in mind that some mutations can cause a gain of function, namely they cause chaperonopathies by excess that require negative chaperonotherapy, consisting in blocking or eliminating the abnormal chaperone (Table 2).

The articles

Note: The articles are numbered here in the order in which they are listed in Table 1. The tables and figures referred to below can be found in the article being discussed.

Article 1. Bardet-Biedl Syndrome (BBS) comprises a group of genetic diseases called ciliopathies since the cell cilia are defective, but many cases can also be considered as chaperonopathies, because out of the 21 or so genes known to be implicated in the causation of BBS, three belong to the family of CCT molecular chaperones (Alvarez-Satta et al., 2017).

The CCT chaperone family comprises nine typical, two as yet to be characterized, and three non-canonical members (see Table 1 in Article 4). The latter three members are called BBS6 (MKKS), BBS10, and BBS12.

Main points to remember are: 1) BBS is a pleiotropic, rare genetic disorder included in the group of ciliopathies that can also be considered a member of the growing class of chaperonopathies; 2) Three out of 21 genes currently involved in BBS encode chaperonin-like proteins: BBS6 (MKKS), BBS10 and BBS12, which can be mutated in up to 50 % of affected families and are usually related to more severe phenotypes; 3) Chaperonin-

like BBS proteins define a particular branch of highly diverged proteins from the CCT family of group II chaperonins, so they conserve the typical chaperonin domain architecture but have specific insertions, entailing different functions not related to an ATP-dependent folding activity unlike canonical CCT chaperonins; 4) On the other hand, BBS6 (MKKS), BBS10 and BBS12 proteins have very specific functions pertaining to the initiation of the assembly of the BBSome (a multiprotein complex essential for ciliary trafficking activity) by stabilizing the first protein to be incorporated and also mediating its interaction with canonical CCT chaperonins; and 5) This view, that recognizes the fact that a good portion of BBS cases are chaperonopathies, opens new avenues for research toward developing accurate differential diagnostic protocols and novel means for generating tools applicable in the treatment of these severe chaperonopathies by defect. Positive chaperonotherapy offers hopes to patients and their families.

Article 2. Spinal Muscular Atrophy (SMA) is directly associated with decreased levels of the Survival Motor Neuron (SMN) protein (Lanfranco et al., 2017). A multimolecular complex that includes the SMN protein functions as a molecular chaperone in the assembly of small nuclear ribonucleoproteins (snRNPs), the spliceosome machine. The loss of the chaperoning ability of the multimolecular complex during snRNP assembly leads to transcriptome abnormalities implicated in the causation of the neuromuscular SMA phenotype. In summary: 1) Motor neuron disorders, especially SMA, can result from defective chaperoning activity; 2) SMN, the causative factor for SMA, is part of a multiprotein complex that functions as a molecular chaperone, hence, interacts with and assists in the assembly of small nuclear ribonucleoproteins (snRNPs), which are the operating components of the spliceosome; and 3) Numerous *in vivo* studies support the possibility that loss of chaperoning in snRNP assembly and the consequential transcriptome abnormalities are the primary drivers of the selective neuromuscular phenotype in SMA.

It is concluded that SMA may be considered a chaperonopathy and, consequently, amenable to chaperonotherapy, namely to a correction of the defect in the chaperoning of snRNP assembly. Furthermore, since disturbances in snRNP

assembly are part of the pathogenic mechanism of other neuromuscular disorders such as adult-onset Amyotrophic Lateral Sclerosis (ALS), developing means to improve the chaperoning mechanism necessary for the correct assembly of snRNPs could be a therapeutic strategy applicable to various neurological disorders.

Article 3. Mutations in the *PLP1* gene, encoding an important myelin protein, are associated with the hypomyelinating leukodystrophy Pelizaeus-Merzbacher Disease (PMD) (Inoue, 2017). Mutations in genes for membrane proteins (depicted in Figure 1) may result in the production of polypeptides with a tendency to accumulate in the endoplasmic reticulum (ER) of the myelin-producing oligodendrocytes, eliciting the Unfolded Protein Response (UPR), which involves ER chaperones (listed in Figure 2). In this case, we have a secondary chaperonopathy; the mutated gene does not code for a molecular chaperone, but its product nonetheless has a negative impact on chaperones, which become pathogenic. The main points of the article are: 1) The UPR and associated ER chaperones can play a major role in the molecular pathology of inherited diseases caused by point mutations in genes encoding membrane proteins; 2) PMD is a severe hypomyelinating leukodystrophy caused by *PLP1* mutations, which appear to elicit cell toxicity by overwhelming ER stress triggered by misfolded mutant *PLP1*, leading to the activation of pro-apoptotic branch of UPR; 3) PMD-causing mutations in *PLP1* also causes binding to calnexin, delayed clearance from the ER, and impaired ER-Golgi trafficking; 4) Some therapeutic interventions targeting the UPR and ER chaperones are evaluated, using cellular and animal models of PMD; and 5) The possibility of developing chaperonotherapy for PMD and other leukodystrophies is emphasized and specific suggestions are made.

Article 4. In the last decade, considerable progress has occurred in the identification and clinical characterization of a range of chaperonopathies. However, progress has been slow in the understanding of the molecular mechanisms underpinning the tissue and organ lesions observed in patients. Even less has been learned about the impact of the pathogenic mutations on the intrinsic properties (e.g., resistance to denaturation caused by stressors

such as heat or acidity, molecular flexibility, and ability to oligomerize into functional oligomers and to associate with other oligomers to form functional networks) and on the functions of chaperone molecules, functions such as protection of other proteins from denaturation by stressors, chaperoning of nascent polypeptides, dissociation of protein aggregates, and so on. In part, this slow progress is due to a scarcity of reliable experimental models that reproduce closely the human situation. This article presents experimental models useful to fill this void, based on the use of archaeal organisms (Conway de Macario et al., 2017). Archaea are prokaryotes that possess groups of genes that encode proteins very similar to those encoded by the human ortholog genes, and among these similar genes are those coding for molecular chaperones (Figure 1). The genes encoding chaperonins in humans are all presented in Table 1, and the known genetic chaperonopathies associated with mutations in them are described in Table 2; thus both Tables encompass very useful information on all the human chaperonin genes and on the diseases caused by mutations in these genes. Lastly, an archaeal experimental model used to study pathogenic mutations in CCT subunits is described, and illustrative results are discussed.

Article 5. This article deals with pathogenic mutations in four chaperone genes associated with a good portion of cases of Distal Hereditary Motor Neuropathies (dHMN) that are chaperonopathies (Lupo et al., 2016). The genes implicated are *DNAJB*, a member of the HSP40(DNAJ) family, and *HSPB1*, *HSPB3*, and *HSPB8*, which belong in the small HSP (sHSP) family. Table 1 presents a useful summary of pertinent mutations, phenotypes, and bibliography. Most interesting is the clear demonstration of the connection between chaperones and chaperonopathies and the illuminating distinction between proteinopathies and chaperonopathies. The latter are proteinopathies but not all these are chaperonopathies, a distinction to be borne in mind by physicians and pathologists in the course of differential diagnosis in any given patient and to indicate the proper treatment, for instance to determine if chaperonotherapy would be indicated, and if so, which type, positive or negative.

Main points to keep in mind are: 1) dHMN are a

group of rare hereditary neuromuscular disorders characterized by an atrophy that affects mostly peroneal muscles in the absence of sensory symptoms; 2) To date, 23 genes are thought to be responsible for dHMN; 3) Four dHMN genes encode chaperones: DNAJB2, HSPB1, HSPB3, and HSPB8; 4) DNAJB2 codifies for a member of the HSP40/DNAJ co-chaperone family, whereas HSPB1, HSPB3, and HSPB8 codify for three members of the small heat shock protein family; and 5) Chaperones play relevant roles in a variety of processes, such as the correct folding of newly synthesized proteins or as a response to protein misfolding. Despite these disparate functions, mutations in some of these chaperones lead to diseases with a similar clinical picture, suggesting that they share common pathways.

Article 6. This article presents a comprehensive treatment of pathogenic mutations of the human protein p97, with a list of mutations and their accompanying phenotypes in Table 1 (Tang and Xia, 2016). Mutations of p97 (also called VCP for valosin-containing protein) are associated with some Multisystem Proteinopathies (e.g., MSP1 also called Inclusion Bodies Myopathy with Paget's disease of bone and Frontotemporal Dementia), Familial Amyotrophic Lateral Sclerosis (FALS), and Charcot-Marie-Tooth Disease, Type 2Y (CMT2Y). The main conclusions are: 1) Cytosolic p97, a member of the broad AAA family ATPases, is involved in an increasing number of cellular processes primarily by segregating protein substrates from large protein complexes and organelles; 2) Extensive studies of p97 have begun to unveil the molecular mechanism underlying its functional multiplicity. The N domains are capable of interacting with many adaptor and/or cofactor proteins in a regulated fashion, which modify protein substrates for processing and direct p97's action towards various cellular locations; 3) While the ability of the D2 domain to hydrolyze ATP is indispensable for substrate extraction, the D1 domain play a regulatory role, controlling the N domain conformation switch and coordinating D2 activity by cycling through different nucleotide states; 4) Pathogenic mutations, located mostly in the N and D1 domains of p97, have been linked to various muscular and neurological disorders (that may be included within the chaperonopathies by defect) as a result of defects in the control mechanism of the D1 domain due to altered

interactions between the N and D1 domains; and 5) Unfortunately, the understanding of the intimate molecular mechanisms of diseases caused by p97 mutations is still quite poor. Nevertheless, the clear explanations provided of the unsolved problems are stimulating and excite the imagination to think of experiments to advance this field toward rapid disease diagnosis, and eventual development of specific chaperonotherapy.

Article 7. Mutations in the Hsp10 gene had not been described before this article (Bie et al., 2016). The conclusions are: 1) This is the first report on a disease-associated mutation in the *HSPE1* gene encoding mitochondrial HSP10; 2) The HSP10-p. Leu73Phe *de novo* mutation causes decreased conformational stability and *in vivo* levels of the mutant Hsp10 protein; and 3) Decreased levels of HSP10 in patient cells coincide with decreased SOD2 protein levels, a known substrate of the HSP60/HSP10 chaperone complex, and in turn results in increased mitochondrial superoxide levels.

The possibility is acknowledged that in the patient reported other contributing pathogenic factors, in addition to the mutant HSP10, might participate in the generation of the whole clinico-pathological picture. This multifactorial characteristic is frequently observed in chaperonopathies and many other diseases, genetic and acquired. Last but not least, this article contains material that will stimulate research not only on HSP10, but also on its functional partner, HSP60.

Article 8. Mutations in many of the chaperone genes known to exist in the human genome have been implicated in chaperonopathies, and this article focuses on *DNAJB6* and its various mutations and phenotypes already described (Table 1) (Ruggieri et al., 2016). The main points are: 1) The *DNAJB6* protein is a member of the DNAJ/Hsp40 family, and serves as co-chaperone for the Hsp70 chaperones; 2) *DNAJB6* is a potent inhibitor of misfolded poly-Q protein aggregation; thus is an attractive therapeutic agent for chaperonotherapy to prevent aggregate toxicity to the cells; 3) Mutations in the *DNAJB6* gene have been associated to the Limb Girdle Muscular Dystrophy 1D (LGMD1D) form, characterized by myofibrillar disintegration and accumulation of *DNAJB6* and its ligands in

the patient muscle tissue as well as in animal models; 4) Lately, several novel mutations and new phenotypes of *DNAJB6* have been described that widen the spectrum of *DNAJB6*-related myopathies; and 5) Molecular studies and the localization of mutations restricted to the G/F domain suggest this domain is implicated in substrate recognition and modulation, hence on specification of Hsp70 function.

Article 9. It is becoming increasingly clear that molecular chaperones are involved in heart development, physiology, and pathology, including heart stress (Sorge and Brancaccio, 2016). This article deals with heart muscle, and brings into focus a protein, melusin, which apparently has chaperoning functions. The main points are: 1) Melusin is a muscle specific chaperone protein interacting with Hsp90; 2) Melusin activates AKT and ERK1/2 cardioprotective pathways, inducing adaptive hypertrophy and cardiomyocyte survival in the heart subjected to stress conditions, as demonstrated in different animal models of heart pathologies; 3) The possibility to upregulate melusin expression in cardiomyocytes by gene therapy represents a promising approach for future therapies; and 4) It is likely that sooner or later, mutations in melusin will be identified causing heart disease; researcher should be alert to this possibility.

Article 10. This article deals with chaperonopathies caused by mutation in the *HSP60* (*HSPD1*) gene, which are among the first to have been described (Bross and Fernandez-Guerra, 2016). This gene resides in the nucleus but its product, HSP60, goes to the mitochondrion, which is its canonical place of residence. However, nowadays it is well established that HSP60 occurs in many other places, beyond mitochondria, intra- and extracellularly. Therefore, when abnormal, HSP60 can cause lesions, and thereby symptoms and signs throughout the body. The main points of the article are: 1) Mutations with deleterious or strong dominant negative effects in the genes encoding the HSP60/HSP10 complex are not compatible with life; 2) The few disease-associated gene variations in the *HSPD1* encoding HSP60 that have been found in patients so far impair function of the variant HSP60 proteins; 3) Depending on the mechanism and degree of HSP60 function impairment conferred

by the mutations, neuro-muscular diseases with different phenotype and severity are caused; 4) The impact of the mutations on the HSP60 architecture and on its intrinsic properties and chaperoning functions are clearly discussed and presented in Figure 1 and Table 1, which could constitute useful material for teaching purposes; and 5) HSP60-associated chaperonopathies are good candidates for positive chaperonotherapy, using gene replacement for instance. Therefore, this article should be of value to those interested in developing novel therapeutic agents for treating genetic diseases.

Article 11. This article is about superoxide dismutase 1 or SOD1 (Superoxide Dismutase [Cu-Zn]), its role in Amyotrophic Lateral Sclerosis (ALS) and, more specifically, the article describes efforts to find chemical compounds for counteracting the effects of mutations in SOD1 that cause ALS (Anzai et al., 2016). Molecules of mutant SOD1 tend to aggregate and accumulate in the cell and in doing so, cause cell death, a phenomenon that in ALS affects predominantly the spinomuscular motor neurons. Therefore, a number of laboratories are trying to find chemical compounds that would prevent aggregate formation and/or would dissolve aggregates already formed. The authors tested more than 600 drugs already approved by the pertinent USA regulatory agency, namely the Federal Drug Administration (FDA), and found some that actually inhibited SOD1 aggregation. These compounds cannot yet be used in clinics but provide key information as to what chemical structures are good candidates for further testing and chemical modification to improve their efficacy and reduce secondary undesirable effects. A recent paper describes a compound, Ebselen, which reacts with cysteine and promotes intra-subunit disulphide bond formation in SOD1 (Capper et al., 2018). It is known that in addition to a mutant SOD1 other destabilizing factors must concur to aggravate ALS pathology and, one of these factors is the failure of SOD1 to mature and reach a functional native status. This maturation process needs chaperones and includes correct folding, incorporation of the metal atoms, intra-subunit disulphide bond formation, and dimerization. If this multistep maturation process is incomplete, the resulting immature SOD1 molecules form toxic oligomers and aggregates within the spinomuscular

neurons. Ebselen seems to reinstate disulphide bond formation and, thus, reboot the maturation process avoiding accumulation of mutant SOD1.

Conclusions and perspectives for the future

Knowledge about diseases affecting predominantly muscles and nerves has expanded considerably in the last decades and now we have a great deal of information on their signs and symptoms. We also have information on the genetic variations and mode of inheritance that characterize many neuromuscular diseases. However, molecular mechanisms are still poorly understood and specific treatments are still wanted for most of these pathologic conditions. A promising road toward elucidating mechanisms and inventing novel therapies has been opened with the realization that many neuromuscular diseases can be produced by failure of certain molecular chaperones. These are molecules that play crucial roles in muscle and nerve development and in the maintenance of their functional structure throughout life. By identifying the pathogenic chaperone or chaperones in any given disease, several options open up to new investigations on the use of the pathogenic chaperone as biomarker to monitor disease progression and response to treatment. Furthermore, identification of a chaperone as a pathogenic factor provides a solid basis to begin developing chaperonotherapy. The tenets for this type of research are as follows: The pathogenic chaperone can be targeted for elimination, or to block it (i.e., negative chaperonotherapy), if it contributes to the disease with its unchecked activity. If, on the other hand, the pathology is caused by a deficient chaperone, positive chaperonotherapy is in order; compounds should be found that on interaction with the sick chaperone molecule will boost its activity, or methods should be standardized for chaperone replacement using the chaperone gene or its protein product.

Although these options may seem still a long time away, the reality teaches us that it may not be so -- suffice it to check the new publications on molecular chaperones that appear in considerable number every day. To accelerate progress in the study, diagnosis, prevention, and treatment of many neuromuscular diseases the scientific community must become aware of current advances in the study of chaperones and their abnormalities. These molecules are

central to muscle and nerve physiology and, when abnormal, play a determinant role in many neuro- and myopathies.

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