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ABSTRACT & COMMENTARY

Plasma Phospho-Tau217 Is a Promising Alzheimer's Disease Biomarker

By Michael T. Lin, MD

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Dr. Lin reports no financial relationships relevant to this field of study.

SYNOPSIS: Validation and replication of findings in the use of the blood biomarker phospho-tau 217 for the diagnosis of Alzheimer's disease holds out the possibility for a new era in the early diagnosis and treatment of this debilitating illness.

SOURCE: Palmqvist S, Janelidze S, Quiroz YT, et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. *JAMA* 2020; July 28. doi:10.1001/jama.2020.12134. [Online ahead of print].

The diagnosis of Alzheimer's disease (AD) still is primarily clinical. Although approved biomarker tests are commercially available, they either are prohibitively expensive (amyloid PET imaging) or invasive (CSF Aβ42 and phospho-tau levels), and commonly are not obtained outside of research settings. A blood test potentially would be much more useful. Brain and cerebrospinal fluid (CSF) proteins do cross the blood-brain barrier and arachnoid granulations to enter the blood, but their usefulness depends on the degree of dilution and whether there are central nervous system (CNS) isoforms distinct from peripheral isoforms. Several recent publications and conference presentations show that plasma phospho-tau217 (Ptau217, tau phosphorylated at threonine 217) has impressive ability to discriminate AD from normal and non-AD neurodegenerative control subjects, as measured by area under receiver operating characteristic curve (AUC) of approximately 0.9.[1-3] Intuitively, the AUC is the probability that a random target subject will have a more abnormal test value than a random control subject.

Palmqvist and colleagues used an immunoassay to measure levels of plasma Ptau217 in 1,402 subjects from three separate cohorts, comparing performance with other biomarkers.[1] Cohort 1 consisted of 81 subjects (34 AD, 47 non-AD) who had subsequent autopsy verification of diagnosis. In this cohort, the AUC was 0.89 for distinguishing intermediate or high probability AD from non-AD, and 0.98 for distinguishing high probability AD from non-AD. These AUC values were significantly higher than those for other plasma

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biomarkers (Ptau181, neurofilament light chain [NfL]). Plasma Ptau217 levels also correlated significantly with brain tangle density in AD cases, but not in non-AD cases.

Cohort 2 consisted of 699 subjects from the Swedish BioFINDER-2 study. These subjects were classified by recognized clinical criteria into cognitively unimpaired, mild cognitive impairment (MCI), AD dementia, and other neurodegenerative diseases. All subjects had CSF biomarker measurements. In this cohort, the AUC was 0.96 for distinguishing AD dementia from other neurodegenerative diseases. This outperformed plasma Ptau181, plasma NfL, magnetic resonance imaging (MRI) volumetric measures, and even CSF Ptau181; only CSF Ptau217 (AUC 0.99) and brain tau positron emission tomography (PET) (AUC 0.98) performed better. Plasma Ptau217 also correlated well with amyloid pathology, with AUC of 0.87 for distinguishing positive from negative amyloid PET. By comparison, the CSF Aβ42:Aβ40 ratio had an AUC of 0.97, and CSF Ptau217 had an AUC of 0.93 for distinguishing amyloid PET status. Of note, plasma Ptau217 was not elevated in neurodegenerative disease with non-AD tau pathology, such as progressive supranuclear palsy or corticobasal syndrome.

Cohort 3 consisted of 622 subjects from the Colombian presenilin-1 E280A kindred. In this cohort, mean plasma Ptau217 levels were lowest in noncarriers, and then progressively higher in cognitively unimpaired carriers, and cognitively impaired carriers. Levels increased with age in carriers, and by age 24.9 years (about 20 years before median age of MCI onset), there was a significant difference from noncarriers. Plasma Ptau217 also correlated significantly with Mini-Mental State Exam (MMSE) scores and memory performance. Of note, this study was cross-sectional, not longitudinal.

Barthélemy and colleagues used a different, mass spectrometry approach to measure levels of plasma Ptau217, also comparing performance with other biomarkers.[2] They previously had reported that CSF Ptau217 was closely associated with amyloid plaques, and Ptau217 was better than tau phosphorylation at other sites (T181, S202, T205) at predicting amyloid plaques. In the current study, plasma Ptau217 correlated with CSF Ptau217 (Spearman rho = 0.78) better than plasma Ptau181 with CSF Ptau181 (Spearman rho = 0.68). Moreover, when normalized to total tau, plasma Ptau217/tau217 had a greater magnitude of change (+230% to 340%) from controls to amyloid-positive groups than plasma Ptau181/tau181 (+60% to 80%). Also, CSF and plasma Ptau217 were able to distinguish amyloid-positive, tau-PET-negative individuals from controls, suggesting that fluid Ptau biomarkers are changed before detectable tau aggregation, and that they reflect abnormalities in soluble tau occurring concomitantly with amyloid pathology. In a discovery cohort (n = 36), plasma Ptau217 had an AUC of 0.991 for distinguishing Aβ+ from Aβ- cases. In a validation cohort (n = 92), the AUC was 0.925, possibly lower because of a different plasma extraction method or lowering of plasma Ptau levels by concomitant CSF drainage. For both cohorts, Ptau217 outperformed Ptau181.

Thijssen and colleagues previously had shown that plasma Ptau181, measured by an immunoassay, was 3.5-fold higher in AD than in controls, and in a series of 404 cases could distinguish AD from clinically diagnosed frontotemporal dementia (FTD) (AUC 0.894) and autopsy-confirmed FTD (AUC 0.878).[3] At the recent Alzheimer's Association International Conference, they reported the results of plasma Ptau217 in a series of 210 cases (healthy controls, clinical AD spectrum, MCI, and FTD).[4] Plasma Ptau217 was increased 5.7-fold in clinical AD relative to controls (AUC 0.92) and 5.0-fold relative to clinical FTD (AUC 0.95). Plasma Ptau217 was increased 3.3-fold in autopsy-confirmed AD relative to FTD (AUC 0.86). Again, Ptau217 was superior to Ptau181. Thus, multiple independent groups, using different biochemical methods, examining different populations, have shown diagnostic utility for Ptau217 as a plasma biomarker discriminating brain AD pathology from other pathology. Availability of such a test clearly would be a huge advance, making accurate diagnosis of AD much easier for both clinical and research purposes.

■ COMMENTARY

A number of issues remain to be worked out. The methodology should be standardized and replicable. With both immunoassays and mass spectrometry, a substantial proportion of samples were below the limit of detection. The discovery and validation cohorts in Barthélemy's work may have given

slightly different results because of a change in extraction method. All of the studies noted here used highly selected research populations, and they should be extended to real world clinical populations, more ethnically diverse subjects, and subjects with multiple pathologies. The study designs were cross-sectional, and inferences about the progression of Ptau217 levels over time should be validated by longitudinal studies. Plasma Ptau217 can detect brain AD pathology decades before symptoms develop. This raises several conundrums. Should testing be done in asymptomatic subjects, especially given that there currently is no effective disease-modifying intervention? Even in subjects who are symptomatic, should testing be done if there are explanatory comorbidities (depression, medications, sleep disorders, etc.), as is common? If multiple pathologies coexist, can the testing be used to judge the relative contribution of AD pathology? Can the testing be used to predict the rate of progression? It is worth reconsidering appropriate use criteria for AD biomarker testing, expanding to include use of blood biomarkers, which surely are coming soon.[5] ■

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ABSTRACT & COMMENTARY

Randomized Diagnostic Trial of First-Line Genome Sequencing in Pediatric White Matter Disorders

By Eric Mallack, MD

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Dr. Mallack reports no financial relationships relevant to this field of study.

SYNOPSIS: The results of this study indicate that first-line genome sequencing in pediatric patients with suspected genetic white matter disease is more diagnostically efficient, defined as higher diagnostic efficacy and shorter time to diagnosis, than current standard of care approaches.

SOURCE: Vanderver A, Bernard G, Helman G, et al. Randomized clinical trial of first-line genome sequencing in pediatric white matter disorders. *Ann Neurol* 2020; April 28. doi:10.1002/ana.25757. [Online ahead of print].

Genetic, non-acquired, pediatric white matter disorders are caused by mutations in genes that code for the structural and metabolic components vital to the development and maintenance of cerebral white matter. Despite a growing understanding of these disorders, diagnosis has remained a significant challenge. Next-generation sequencing has been shown to be effective in resolving persistently undiagnosed cases. Genome sequencing (GS) is a particularly promising diagnostic approach given its ability to capture large copy number variants across both the nuclear and mitochondrial genomes in addition to small indels and single nucleotide variants typically detected by exome sequencing. This study prospectively compared the diagnostic yield and time to diagnosis between GS and current standard of care (SoC) approaches in pediatric patients newly identified to have a white matter disorder without a definitive diagnosis by clinical assessment and magnetic resonance imaging (MRI). One-third of the recruited patients were randomized to immediate GS plus SoC (treatment arm). Two-thirds were randomized to SoC followed by a four-month delayed GS (control arm). SoC was defined as routine clinical testing, which included enzymatic and metabolic testing, and non-GS genetic testing, which included chromosomal, targeted gene sequencing, or gene panel testing.

Eighty-four patients met inclusion criteria and 34 ultimately were enrolled. Nine patients were randomized to immediate-GS and 23 to delayed GS. Two cases were resolved by SoC metabolic testing prior to randomization and were not included in the analysis. In the treatment

arm, five of nine patients received a diagnosis by immediate GS, whereas zero of nine patients were diagnosed by SoC ($P < 0.005$). Five of 23 patients were diagnosed by SoC vs. 14 of 23 patients diagnosed by GS introduced after a four-month delay in the control arm ($P < 0.005$). The diagnostic yield was superior for GS vs. SoC (59% vs. 16%, $P < 0.005$). Time to diagnosis was significantly shorter in the immediate-GS group ($P = 0.04$). Overall, 76.5% of the cases were resolved in the study, 73% of which were achieved by GS.

■ COMMENTARY

This study by Vanderver et al indicates that first-line GS is a superior diagnostic approach to SoC testing in pediatric white matter disorders. Patients who underwent first-line, agnostic GS received a diagnosis more often, and in less time, than those who received the SoC approach, which included more focused, traditionally first-line genetic testing methodologies (i.e., chromosomal analysis, targeted testing, gene panel testing). The authors placed their findings in the context of imaging and metabolic testing, offering an updated diagnostic algorithm for patients with a suspected white matter disorder. Increasing the efficiency of disease diagnosis has important downstream implications. It addresses the "diagnostic odyssey" characteristic of many children affected by neurogenetic disease. Accordingly, first-line GS may prove to be a viable strategy to streamline resources and healthcare use. Clinically, decreasing the time to accurate diagnosis, especially in presymptomatic or minimally symptomatic patients, leads to the timely delivery of therapy. This is especially important in diseases where invasive treatments are most successful when instituted early in the disease course.[1] The molecular insights gained from accurate genetic diagnoses, and potential gene discovery in previously uncharacterized diseases, are the initial steps crucial to the development of novel therapies, including gene therapy.[2-5]

Important limitations exist in the study. Limited patient enrollment decreases the power of the overall results and limits the generalizability of the conclusions to other diseases. This is a difficulty common to all rare disorders. Additionally, two patients were resolved by SoC metabolic testing after study enrollment but prior to randomization and therefore were excluded from the primary analysis. If those two patients were added back into the study and were randomized to the immediate-GS treatment arm, the results may not have reached statistical significance (5/11 diagnosed by GS vs. 2/11 diagnosed by SoC). Importantly, as noted by the authors, the diagnostic yield by GS would remain superior to SoC despite the addition of the two enrolled cases resolved by SoC (56% [19/34] vs. 21% [7/34], $P < 0.001$). Given the findings from this study, and the overall progress in the field of pediatric neurogenetics, one can reasonably expect the continued adoption of next-generation sequencing as a diagnostic method for adult-onset neurological disorders. Additionally, as more pediatric patients are tested, more healthy family members will be identified during family screening to have risk alleles for adult-onset neurological disease. This has the potential to evolve the molecular understanding of these diseases and increase the specificity of targeted preventive strategies for pre-symptomatically identified patients. ■

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ABSTRACT & COMMENTARY

Dermatomyositis Sine Dermatitis Is an Important Clinical Entity

By Daniel MacGowan, MD, MRCPI

Assistant Professor of Clinical Neurology, Weill Cornell Medical College, Cornell University

Dr. MacGowan reports no financial relationships relevant to this field of study.

SYNOPSIS: Dermatomyositis is an autoimmune disorder of skeletal muscle associated with a variety of auto-antibodies and specific muscle pathology but it may not have a skin rash. Muscle pathology and antibody determinations are important for accurate diagnosis and treatment.

SOURCE: Inoue M, Tanboon J, Hirakawa S, et al. Association of dermatomyositis sine dermatitis with anti-nuclear matrix protein 2 autoantibodies. *JAMA Neurol* 2020;77:1-6.

Inoue et al reported an 8% rate of dermatomyositis without skin or nail involvement (heliotrope rash, Gottron's sign, shawl or V-sign, Gottron's papules, or any kind of rash) in a cohort of 182 patients with muscle biopsies consistent with dermatomyositis, drawn from the primary referral center for muscle biopsies in Japan.

These 14 patients all were diagnosed incorrectly with polymyositis until muscle biopsy. Clinical information (skin findings, presence of muscle weakness, myalgia, dysphagia, interstitial lung disease or malignancy), serum creatine kinase level and serum results for the myositis specific antibodies (MSAs), anti-transcription intermediary factor 1-gamma (anti-TIF1-γ), Mi-2, melanoma differentiation-associated protein 5 (MDA5), nuclear matrix protein (NXP-2), and small ubiquitin-like modifier-1 activating enzyme (SAE) also were provided. Twelve of these 14 (86%) patients without rash were found to be positive for anti-NXP-2, compared with only 28% of the 168 patients with rash ($P < 0.001$).

One of the 14 patients without rash was positive for anti-TIF1-γ, and another for anti-Mi-2, compared with 38% who were TIF1-γ positive and 15% who were Mi-2 positive in those patients with rash. None of the patients without rash were positive for MDA5 or SAE antibodies. Of those patients with rash, 22 (13%) were positive for MDA5 antibodies and four (2%) were positive for SAE antibodies. All of the 182 muscle biopsies showed evidence of sarcolemmal expression of the myxoma virus resistance protein A (MxA), an interferon 1-induced antiviral product of the Mx1 gene on chromosome 21. Autoimmune diseases that are associated with strong activation of interferon 1-induced proteins include dermatomyositis, Sjögren's syndrome, systemic sclerosis, and rheumatoid arthritis.

This same Japanese group recently demonstrated 71% sensitivity and 98% specificity of MxA expression for a diagnosis of dermatomyositis in 34 biopsies from 12 patients with definite, 18 with probable, and four with possible diagnoses of dermatomyositis, based on The European Neuromuscular Criteria for dermatomyositis. The criteria define definite as myositis patients with typical rash and perifascicular atrophy (PFA) on biopsy; probable as those with typical rash but no PFA; and possible as those with PFA but no rash.

The 71% sensitivity and 98% specificity of MxA expression for a diagnosis of dermatomyositis compared with 47% and 98% for PFA, and 35% and 93% for capillary endothelial C5-9b membrane attack complement deposition (MAC), represents a significant improvement in the sensitivity of muscle pathology for the diagnosis of dermatomyositis. In the 14 biopsies from patients without rash, all but one (93%) showed MAC positive capillaries and 9/14 (64%) showed PFA. The malignancy rate in the 168 patients with rash was 21%, and the rate was 2% in the 14 without rash.

■ COMMENTARY

This study represents yet another nail in the coffin for the diagnostic entity "polymyositis," an increasingly rare disease entity that should be reserved for cases of inflammatory myopathy without MSA or anti-tRNA synthetase antibody, and pathological features of endomysial inflammation with invasion of non-necrotic MHC-1 expressing myofibers by CD-8 T cells without PFA, MAC, or rimmed vacuoles. The diagnosis of polymyositis carries with it a frequently false impression of less response to treatment and lower risk of malignancy than dermatomyositis.

Dermatomyositis usually responds to glucocorticoids and/or intravenous immune globulin, with refractory cases recently demonstrating responsiveness to tofacitinib, an oral agent currently approved to treat moderate to severe rheumatoid arthritis and ulcerative colitis. Tofacitinib is a disruptor of interferon 1-induced activation of the Janus kinase/signal transducer and activator of the transcription pathway (JAK/STAT), which is crucial for cytokine signaling and transcription. Tofacitinib has been promising in case reports of anti-MDA5 dermatomyositis with severe interstitial lung disease. Recently, in an open-label, 12-week trial of 10 patients with refractory dermatomyositis treated with tofacitinib, all reached the end point of an at least 20% improvement in a core set of measures derived from physician and patient impression, global activity, muscle strength, CK level, and health assessment questionnaire.

This study further reinforces the importance of performing muscle biopsy and antibody testing in all but the most obvious cases of dermatomyositis with classic rash and Gottron's papules, since cases may occur without rash and without weakness.

Coexisting specific antibodies often are clinically significant — more than half of TIF1-γ cases will have concurrent or future malignancy; MDA5 cases involve a hypomyopathic presentation associated with rash and severe interstitial lung disease; NXP-2 cases are associated with subcutaneous edema, calcinosis, risk of contractures, and malignancy; and Mi-2 is associated with classic presentation and very good treatment response.

Signal recognition particle antibody (SRP) and HMG-CoA reductase antibody positive cases may present with severe, rapidly progressive necrotizing myopathy requiring treatment with intravenous immunoglobulin and/or rituximab, and anti-tRNA synthetase cases may present with interstitial lung disease, arthritis, mechanic's hands, Raynaud's phenomenon, and overlap with scleroderma/CREST syndrome. ■

ABSTRACT & COMMENTARY

Nerve Disorders from Mutations in the BSCL2 Gene

By Michael Rubin, MD

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Dr. Rubin reports he is a consultant for Merck Sharp & Dohme Corp.

SYNOPSIS: The neuromuscular syndromes caused by BSCL2 gene mutations may mimic several other disorders, including Charcot-Marie-Tooth disease and amyotrophic lateral sclerosis.

SOURCE: Fernandez-Eulate G, Fernandez-Torron R, Guisasola A, et al. Phenotypic correlations in a large single-center cohort of patients with BSCL2 nerve disorders: A clinical, neurophysiological and muscle magnetic resonance imaging study. *Eur J Neurol* 2020;27: 1364-1373.

Distal hereditary motor neuropathy (dHMN), or distal spinal muscular atrophy, comprises a genetically and phenotypically heterogeneous group of disorders, characterized by almost exclusive degeneration of motor nerve fibers, predominantly in the distal parts of the limbs. Several forms are linked to the Berardinelli-Seip congenital lipodystrophy (BSCL2) gene, of which two mutations comprise a majority of the patients. In this study, 26 patients from five families carrying the p.N88S mutation were studied to form a description of the full phenotypic spectrum of this entity.

Electronic medical records provided clinical data, including age of onset, age at diagnosis, mutation, phenotype, and limb onset. Muscle strength was graded using the Medical Research Council (MRC) scale and disability was ranked using the modified Rankin Scale (mRS) score. Nineteen patients had undergone nerve conduction studies, and 18 underwent whole body muscle magnetic resonance imaging (MRI), including the hands and feet. Patients underwent either whole-exome sequencing for diagnosis (n = 2) (which also excluded other genetic causes of distal weakness, such as Charcot-Marie-Tooth [CMT] disease) or Sanger-sequencing of exon 3 of the BSCL2 gene, with mutation carriers identified using BigDye chemistry in AB13130 (Life Technologies) equipment. Statistical analysis included the mean and standard deviation of symmetric quantitative variables, and the median and interquartile range of asymmetric quantitative variables, with parametric and non-parametric tests applied as needed, and statistical significance considered at $P < 0.05$.

All patients came from a small region on the coast of the Basque Country in northern Spain and were confirmed carriers of the p.N88S mutation. Ages ranged from 20 to 99 years, males comprised 53.8% of the patient group (n = 26), the mean age of onset was 24.4 years, and the mean age of diagnosis was 51.4 years. Pure dHMN was the most common presentation, seen in 13 patients (50%), with spastic paraplegia 17 (Silver syndrome) present in five patients (19%), and CMT2 in three patients (11%). Five patients (19%) remain asymptomatic. Limb onset was seen in 18 patients (68%), affecting the legs in 12, the arms in four, and both arms and legs in two. Sensory symptoms were present in only three (11%), and respiratory insufficiency in two (8%), but pes cavus and pyramidal signs were present in 46% and 50%, respectively. Slow progression was seen in all patients, with low mean disability — two patients walked with aid, one required help with activities of daily living, and none used a wheelchair. Axonal neuropathy was seen in 17 of 19 patients, with slowing of nerve conduction velocities rare and mild if present, but dispersed compound muscle action potentials in the legs were found in seven patients (37%). Of eight patients who underwent cervical spine MRI, one showed disc herniation with signs of myelopathy, which resolved with surgery. No signs of myelopathy were appreciated otherwise.

Whole body MRI in 18 patients showed generally symmetric proximal to distal gradient fat replacement in each muscle in 15, with the most frequently affected muscles being the soleus, tibialis anterior, and thenar eminence (61%, 56%, and 50%, respectively), followed by the extensor digitorum longus, medial gastrocnemius, peroneus, and flexor digitorum longus (in 44%, 39%, 33%, and 33%, respectively). Proximal limb and axial muscle abnormalities were unusual. Patients with the p.N88S BSCL2 mutation can be phenotypically variable, but most frequently present as a slowly progressive dHMN. However, all phenotypes demonstrate a consistent muscle MRI pattern of fat replacement, which may be helpful in differential diagnosis and as an outcome measure in future clinical trials.

■ COMMENTARY

Among 407 Japanese patients clinically suspected of having CMT disease, but screened for BSCL2 mutations by exome sequencing, five were identified with heterozygous BSCL2 mutations, of which three had known mutations (p.N88S and p.S90l) and two had novel mutations

(p.N88T and p.S141A). Early onset and significant vocal cord paresis were present in the p.N88T mutation, and the p.S141A mutation showed features typical of CMT type 1 based on electrodiagnostic studies. BSCL2 mutation may rarely mimic the demyelinating form of CMT.[1] ■

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ABSTRACT & COMMENTARY

Molecular and Cellular Correlates of Human Nerve Regeneration

By Mary L. Vo, MD, PharmD

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Dr. Vo reports no financial relationships relevant to this field of study.

SYNOPSIS: Specific and objective measures of improvement after carpal tunnel decompression (i.e., electrodiagnostic testing, somatosensory function, and intraepidermal nerve fiber density) also correlated with upregulation of ADCYAP1/PACAP genes. These genes may be potential therapeutic targets in the future.

SOURCE: Baskozos G, Sandy-Hindmarch O, Clark AJ, et al. Molecular and cellular correlates of human nerve regeneration: ADCYAP1/PACAP enhance nerve outgrowth. *Brain* 2020; July 1. doi: 10.1093/brain/awaa163. [Online ahead of print].

Despite extensive preclinical studies highlighting potential targets of peripheral nerve regeneration, human studies elucidating molecular and cellular factors important to nerve regeneration and clinical outcomes are severely limited. In recent years, skin biopsies have been used to gain perspective into the regeneration of human sensory nerves, myelination of large fiber nerves, and histological changes during nerve regeneration.[1]

Preclinical literature has highlighted the function of adenylate cyclase-activating polypeptide 1 (ADCYAP1)/pituitary adenylate cyclase-activating peptide (PACAP) in neural regeneration, but its effect in human neurites is not understood. PACAP is a highly conserved protein involved in neuronal survival following injury and neurite outgrowth. PACAP binds with high affinity to the polypeptide type 1 (PAC1) receptor, expressed in human sensory afferents both in human skin as well as in human-induced pluripotent stem cell-derived (hiPSCd) sensory neurons in vitro. In this study, the authors combined histological and molecular analysis with traditional clinical outcome measures to evaluate the role of the ADCYAP1/PCAP in human nerve regeneration.

Baskozos et al studied 60 patients with clinically and electrodiagnostically confirmed carpal tunnel syndrome (CTS) from the Oxford University Hospitals NHS Foundation Trust prior to and six months following surgical decompression. Twenty matched healthy controls also were included. Patients with osteoarthritis, cervical radiculopathy, trauma, pregnancy, diabetes, and prior CTS surgery were excluded. All subjects underwent electrodiagnostic testing, quantitative sensory testing, and completed a Boston Carpal Tunnel Questionnaire. Skin biopsies were taken from the ventrolateral aspect of the proximal phalanx of the index finger at baseline and were repeated six months following surgical decompression.

Samples were evaluated for measures of myelinated fiber integrity, including Meissner corpuscles per millimeter, dermal nerve bundles, and nodal length. Histological analysis included measurements of intraepidermal nerve fiber density and subepidermal plexus nerve fiber length. Molecular analysis of target tissue entailed ribonuculeic acid (RNA) sequencing validated with droplet digital polymerase chain reaction (PCR). hiPSCd sensory neurons were derived from two control lines and differentiated into primary sensory neurons, where they were treated with different doses of PACAP protein.

Pre- and postsurgical data were analyzed using two-sided independent *t*-tests or Mann Whitney U-tests. Spearman's correlation analysis was used to determine the magnitude of clinical recovery and objective measures of neural regeneration. Clinical recovery was determined as a two-point improvement on a global rating scale focused on hand symptoms and function. Following surgery, 83.3% of subjects reported significant improvement in symptoms (≥ 5 points on a global rating scale), including improvements in both symptom subscore (pre: 2.8 [0.7], post: 1.5 [0.5], t [59] = 13.8, $P < 0.0001$) and functional subscore (pre: 2.2 [0.8], post: 1.5 [0.5], t [59] = 7.00, $P < 0.00001$). Although the majority of patients had notable improvement, 46.6% of postoperative subjects continued to have pain and 38.3% had paresthesia, albeit much milder than baseline.

Similarly, the majority of subjects were found to have objective improvements on electrodiagnostic testing, somatosensory function, and intraepidermal nerve fiber

density during postoperative testing, but they failed to reach the levels of healthy controls. Follow-up electrodiagnostic testing showed improvement measured by a graded neurophysiological CTS rating scale (2.0 [2.0], z [55] = -5.62, $P < 0.0001$). Postoperative somatosensory function measured by thermal and mechanical thresholds also showed improvement relative to the control group (t [78] > 2.30, $P < 0.015$). Intraepidermal nerve fiber density improved following surgery relative to controls (preoperative mean: 4.20 [2.83]; controls: 8.03 [2.08], t [77] = 5.3, $P < 0.00001$; post: 5.35 [3.34], t [57] = 3.5, $P = 0.001$). No differences in nerve fiber length density in subepidermal plexus were seen at follow-up. Median nodal length and percent of elongated nodes increased in patients and remained higher in healthy controls.

RNA sequencing from 47 patients showed 31 differentially expressed genes in the CTS group, with ADCYAP1 showing the most significant degree if dysregulation. ADCYAP1 encodes for PACAP protein, which was increased in postoperative studies. Further, in vitro hiPSCd sensory neurons exposed to different levels of PACAP protein resulted in dose-dependent neurite outgrowth.

■ COMMENTARY

The authors demonstrate a histologic and molecular signature in nerve recovery, including strong upregulation of ADCAP1/PACAP that parallels recovery of intraepidermal nerve fibers and correlates with traditional symptomatic and functional outcome metrics. These findings provide valuable insight into the complex determinants of sensory nerve regeneration. Additional research is needed to assess the role of PACAP as a therapeutic target in nerve regeneration. ■

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CME QUESTIONS

1. **Which of the following statements regarding blood biomarkers for the diagnosis of Alzheimer's disease (AD) is true?**
   a. Blood tests to diagnose AD have been approved by the Food and Drug Administration.
   b. The blood biomarker phospho-tau217 has a high degree of accuracy in diagnosing AD.
   c. Blood tests to diagnose AD are commercially available.
   d. Blood tests to diagnose AD are part of the standard clinical evaluation.
2. **In the evaluation of pediatric leukodystrophies, first-line genome sequencing has which of the following benefits?**
   a. Genome sequencing reduces the overall costs of diagnostic studies.
   b. First-line genome sequencing results in a more rapid diagnosis.
   c. Genome sequencing can prevent the progression of some diseases.
   d. Genome sequencing is easy to analyze and interpret.
3. **Which of the following is *not* a presentation of the BSCL2 mutation?**
   a. Charcot-Marie-Tooth disease
   b. Distal spinal muscular atrophy
   c. Multifocal motor neuropathy with conduction block
   d. Distal hereditary motor neuropathy
4. **Which of the following is *not* one of the criteria for diagnosing dermatomyositis?**
   a. Elevated creatine kinase
   b. Muscle pain
   c. Muscle weakness
   d. Skin rash
5. **Which of the following outcome measures improves after successful median nerve decompression for carpal tunnel syndrome?**
   a. Improved electrophysiologic measures
   b. Improved sensory and motor function
   c. Increased intraepidermal nerve fiber density
   d. All of the above

[IN FUTURE ISSUES]

Stroke Alert