Three regular articles on survival motor neuron gene copy number analysis by exome sequencing, molecular profiling of archival prostate cancer samples, and characterization of cytosine-adenine-guanine tract and flanking polymorphisms in Machado-Joseph disease were selected for the **May and June 2020 JMD CME Program in Molecular Diagnostics**. The authors of the referenced articles, the planning committee members, and staff have no relevant financial relationships with commercial interests to disclose.

Questions #1-4 are based on Chung KY, Quek JM, Neo SH, Too HP; Survival Motor Neuron Gene Copy Number Analysis by Exome Sequencing: Assisting Spinal Muscular Atrophy Diagnosis and Carrier Screening. J Mol Diagn 2020, 22: 619-628


Upon completion of this month's journal-based CME activity, you will be able to:

- Describe spinal muscular atrophy (SMA) and discuss the range of symptoms and severity of SMA.
- Understand the current standards for diagnosis and carrier screening for SMA.
- Discuss the challenges associated with stratifying patients with prostate cancer.
- Discuss the association between TMPRSS2:ERG fusion status and immune cell infiltration in prostate cancer.
- Discuss the dominantly inherited autosomal disease, polyglutamine spinocerebellar ataxia 3 (SCA3).
- Describe protocol for the characterization of cytosine-adenine-guanine tract and flanking polymorphisms in SCA3.

1. Spinal muscular atrophy (SMA) is a rare autosomal-recessive neuromuscular disorder, with an incidence of 1 in 5000 to 1 in 10,000 live births. Based on the referenced regular article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 619-628]
   
   a. The carrier frequency of SMA is approximately 1 in 20 to 1 in 50 among different ethnic groups.
   
   b. SMA is caused by degeneration and loss of motor neurons in the spinal cord and brainstem, leading to progressive muscle weakness and atrophy that impairs activities.
   
   c. SMA usually affects adults, and despite the severity of symptoms, reported outcomes preclude mortality.
   
   d. According to the age at onset, clinical severity, and the maximum muscular activity achieved, clinical subtypes of SMA have been classified into four groups, from mild to severe, as follows: types I, II, III, and IV.

2. The full-length survival motor neuron (SMN) protein is encoded by the highly homologous SMN genes: **SMN1** and **SMN2**. The SMN genes only differ by 5 bp and none of the characterized sequence variations modifies the amino acid sequence of the encoded proteins. Based on the referenced regular article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 619-628]
   
   a. **SMN1** gene status modifies the disease severity.
   
   b. The single-nucleotide polymorphism located in **SMN2** exon 7 (c.840C>T), which affects an exonic splicing enhancer, causes exon skipping by 50% to 90% and correspondingly reduce functional SMN protein.
   
   c. In most populations, approximately 95% of SMA patients show a complete absence of **SMN2** owing to gene deletion or conversion.
   
   d. Healthy individuals only display a maximum of two **SMN1** copies.
3. A few exome sequencing–based copy number variant (CNV) detection methods have been published; however, analyses for regions with highly homologous sequences such as SMN genes remain challenging for short-read technology. Since CNV is known to be a significant cause of SMA and early diagnosis or interventions are important for the disease prognosis, there is a critical need for a comprehensive analysis of SMN genes. A workflow was constructed for the SMN gene copy number analysis through uniquely mapped reads on exon 7 of SMN genes and the control region. After constructing the workflow based on a training set including 104 samples, the SMN1 copy number from 3734 individuals from the Neonatal Intensive Care Unit (NICU) was analyzed retrospectively. The cohort included 2165 males (57.98%) and 1569 females (42.02%). Among them, 95 (2.54%) patients were recorded to have abnormalities of the musculature, including muscle weakness, muscular hypotonia, amyotrophy, and electromyography abnormality. Based on the referenced regular article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 619-628]

   a. Overall, the $S_{\text{SMN1}}$ score of the cohort ranged from 0.717 to 1.327, with an average and median score of 1.024 and 1.008, respectively.
   b. Eight individuals (0.21%), who lacked clinical manifestations, were classified as SMN1 homozygous deletion, with males and females each accounting for half, with the $S_{\text{SMN1}}$ score ranging from 0.000 to 0.011.
   c. A total of 66 individuals (1.77%) were classified as SMN1 heterozygous deletion, with males favoring this deletion, with the $S_{\text{SMN1}}$ score ranging from 0.000 to 0.011.
   d. Multiple ligation-dependent probe amplification (MLPA) was used to analyze the copy number of SMN1 for 100 individuals with the highest risk of SMA ($S_{\text{SMN1}}$ score lowest) in the study cohort, including 8 individuals classified as SMN1 homozygous deletion, 66 individuals as heterozygous deletion, and 26 individuals as low risk of SMA.

4. To date, next-generation sequencing has significantly promoted the molecular diagnosis for rare diseases, covering both single nucleotide variant (SNV) and CNV. Based on the referenced regular article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 619-628]

   a. With the publication of several exome sequencing–based CNV detection methods, analyses for regions with highly homologous sequences such as SMN genes have become much easier.
   b. Because CNV is known to be a significant cause of SMA and early diagnosis or intervention is important for the disease prognosis, there is a critical need for a comprehensive analysis of SMN genes.
   c. The authors adapted an exome sequencing–based SMN gene copy number analysis workflow by calculating the copy number score independent of a decision tree for determination.
   d. MLPA was used to analyze the SMN1 copy number of the top 20 individuals with the highest risk of SMA in the cohort. The validation result showed a low recall rate in both SMN1 homozygous and heterozygous deletion situations, showing the promising potential of exome sequencing–based, high-risk SMA detection.

5. Prostate cancer (PCa) is a major global health issue and better methods for patient stratification are urgently needed. Our understanding of the molecular pathology of PCa is evolving rapidly, with advances in sequencing methods and bioinformatics. Based on the referenced regular article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 652-669]

   a. The Cancer Genome Atlas performed detailed molecular analysis on 111 primary prostate carcinomas, and 90% of tumors fell into one of the seven subtypes defined by specific gene fusions (ERG, ETV1/4, FLI1) or mutations (SPOP, FOXA1, IDH1).
   b. Recent publications by the International Cancer Genome Consortium have identified new cancer genes, routes of progression, and drug targets, sometimes affected by mutations in noncoding regions of genes, including NEAT1 and FOXA1.
   c. Immunotherapy is a treatment option for early-stage PCa, and to optimize response to immunotherapy, it is often necessary to target oncogenic driver pathways in combination with immunotherapy.
   d. In several different cancers, including prostate, colorectal, melanoma, and bladder, the correlation between T-cell infiltration and clinical outcome is currently missing.

6. The literature detailing PCa progression and inflammation is often conflicting. Based on the referenced regular article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 652-669]

   a. Previously published articles report that the radical prostatectomy cases with higher rates of biochemical progression have lower levels of systemic inflammatory markers.
   b. Consistent with literature in other cancer types, higher-grade inflammation is statistically associated with lower risk of extraprostatic extension, positive margins, and seminal vesicle invasion.
   c. PCa shows a high degree of heterogeneity at histologic and genetic levels, which poses a significant treatment challenge.
   d. Accumulation of mutations and activation of oncogenic driver pathways with cancer progression may decrease immunosuppressive cells and exhaust immune effector cells in the cancer tissue microenvironment.
7. An inverse association was shown between TMPRSS2:ERG fusion status and tumor-infiltrating lymphocyte (TIL) counts in 27 PCa tumors and a differential gene expression profile was identified in TIL-high versus TIL-low tumors. Based on the referenced regular article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 652-669]
   a. This was achieved by using a novel workflow that involved the integration of digital image analysis techniques to obtain TIL counts, which excluded gene profiling using RNA and DNA sequencing.
   b. The published literature reports an association between high TILs with TMPRSS2:ERG fusion; however, these studies used immunohistochemistry (IHC) on formalin-fixed, paraffin-embedded tissues to determine ERG status with manual annotation of region of interest (ROI).
   c. IHC detects only the expression status of ERG, which could be because of fusion or transcriptional activation by other means.
   d. Tumors with mutations that bind strongly to MHC class II molecules are positively selected, likely contributing to the low immune cell counts in TMPRSS2:ERG fusion-positive tumors.

8. There is a statistically significant association between low TILs and TMPRSS2:ERG fusion even when the analysis is performed with stratification of samples into TMPRSS2:ERG fusions producing chimeric transcripts in the coding exons versus fusion-negative or non-chimeric fusions. Based on the referenced regular article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 652-669]
   a. An intriguing possibility may be the down-regulation of immune cell infiltration (immune escape) in those samples with TMPRSS2:ERG fusions by an unknown mechanism.
   b. Findings from a mouse model of PCa suggested that the TMPRSS2:ERG fusion impedes recruitment of regulatory T cells to the tumor site.
   c. The tumors with mutations that bind poorly to MHC class I molecules are shown to be positively selected, explaining the high immune cell counts in TMPRSS2:ERG fusion–positive tumors.
   d. The mechanism is likely to involve neoantigens arising from chimeric proteins, as that would obstruct a higher immune cell infiltration in TMPRSS2:ERG positive tumor.

9. Both MHC class I and class II molecules are critical for tumor immunity in PCa cells. Based on the referenced regular article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 652-669]
   a. Using the TIL counts to stratify the samples impeded the identification of an RNA signature in TIL-high samples in this study.
   b. There was a weak signature of B-cell and antigen processing signaling pathways in TIL-high PCa samples in this study.
   c. The antitumor immune response in PCa is primarily an innate immune system response.
   d. Oncogenic gene SPINK1 was highly expressed in TIL-high samples as well as in TMPRSS2:ERG fusion-negative samples.

10. Polyglutamine spinocerebellar ataxias (SCAs) constitute a group of autosomal dominantly inherited neurodegenerative disorders of late onset. Although each of these genetic diseases has its own causative gene, they share a common etiology that consists of the abnormal expansion of the trinucleotide cytosine-adenine-guanine (CAG) in coding exons. Based on the referenced regular article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 782-793]
    a. The disease-causing proteins display an expanded polyglutamine (polyQ) tract, which tends to accumulate in specific neuronal populations, ultimately leading to dysfunction and degeneration.
    b. Symptoms frequently start in childhood and include gait ataxia, limb incoordination, speech disturbances, and oculomotor abnormalities.
    c. The establishment of a definitive diagnosis based on clinical features relies on genetic analysis solely.
    d. Sizing of CAG trinucleotide repeats in a specific causative gene has commonly involved the electrophoretic separation of PCR-amplified products by using gradient electrophoresis.

11. A single PCR and subsequent electrophoretic resolution of the PCR amplicons in a 2% agarose gel allows for the rapid identification of healthy individuals and patients with disease. A total of 6 unaffected control samples and 13 Machado-Joseph disease (MJD)/SCA type 3 (SCA3) samples were PCR amplified by using the previously optimized PCR protocol. The length of each PCR amplicon was estimated by comparison with a DNA ladder, after electrophoretic resolution on a 2% agarose gel. Based on the referenced regular article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 782-793]
    a. Healthy individuals exhibited a band that corresponds to the expanded allele (500 bp approximate size) and were genotyped by Sanger sequencing.
    b. Amplicons from patients with disease (heterozygous in all analyzed cases) exhibit three bands with considerably different molecular weights.
    c. The band with smaller size corresponds to the expanded allele (approximately 500 bp), whereas the second, with an approximate size of 900 bp, corresponds to a non-expanded allele.
d. The complete characterization of the amplified region in patients with MJD/SCA3 (normal and expanded alleles) was accomplished through the combination of two complementary methods that involve PCR cloning (method I) and PCR-capillary electrophoresis (CE) techniques (method II).

12. Accurate sizing of cytosine-adenine-guanine (CAG) trinucleotide repeats in the context of polyQ SCAs is critical for the confirmation/exclusion of a clinical diagnosis in presymptomatic testing and prenatal diagnosis. However, it is known that some genotyping errors and inaccurate interpretation of results in SCA testing can occur, which led to the implementation of laboratory best practice guidelines for these disorders. Based on the referenced regular article, select the ONE best TRUE statement: [J Mol Diagn 2020, 22: 782-793]

a. Repeat numbers found in the expanded allele of each MJD/SCA3 sample varied between 14 and 29 CAG units.
b. The number of CAG repeats in the non-expanded allele for the 13 MJD/SCA3 samples ranged from 64 to 76.
c. In accordance with the currently accepted guidelines, the detection of 30 to 43 CAG repeats in the ATXN3 gene means that a given patient has, or is predisposed to develop, MJD/SCA3.
d. Apart from technical difficulties, the limited precision of CAG sizing in alleles within the pathogenic range is partially due to the PCR amplification of a mosaic template.