November 21, 2019

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Re: MolDX: Repeat Germline Testing (DL38288)

Dear Dr. Loveless,

Thank you for the opportunity to review and comment on CGS' proposed coverage policy for Repeat Germline Testing DL38288 (hereafter 'draft LCD'). Members of the Association for Molecular Pathology (AMP) and the College of American Pathologists (CAP) are submitting joint comments at this time because both organizations share the same perspective regarding this draft LCD.

The AMP is an international medical and professional association representing approximately 2,300 physicians, doctoral scientists, and medical technologists who perform or are involved with laboratory testing based on knowledge derived from molecular biology, genetics, and genomics. Membership includes professionals from the government, academic medicine, private and hospital-based clinical laboratories, and the in vitro diagnostics industry.

The CAP is the world's largest organization of board-certified pathologists and the leading provider of laboratory accreditation and proficiency testing programs. The CAP serves patients, physicians, hospitals and healthcare systems worldwide, fostering and advocating excellence in the practice of pathology and laboratory medicine.

We commend CGS for recognizing the vital importance of providing coverage to Medicare beneficiaries for germline testing and for your thoughtful approach to repeat testing of panels that contain non-duplicative test components that demonstrate clinical utility. We agree that repeated testing of an individual's genome for inherited diseases is generally not reasonable and necessary. However, improved testing methodologies and knowledge base continue to expand and improve information that can be returned from a genetic test. Therefore, we offer the following comments and recommendations for CGS' consideration.

1. dLCD statement: "Germline testing using gene panels that contain some genetic content that has already been tested in the same Medicare beneficiary may be considered reasonable and necessary provided that there is established clinical utility present in the remaining, non-duplicative genetic components of the test."

Comment: Next Generation Sequencing technology has allowed for simultaneous testing of multiple genes in panel tests, greatly expanding the possibility of finding a disease-associated variant to explain predispositions to inherited cancer and germline variants that can affect treatment choices such as the use of PARP inhibitors in breast and ovarian cancer. The LCD's current statement correctly provides for scenarios to repeat germline testing to identify inherited breast and ovarian cancer syndromes. For example, tests for inherited breast cancer used to only examine BRCA1/2, but use of a larger cancer panel has increased the diagnostic rate significantly from 2.5% (BRCA1/2 alone) to 6.3% using a guideline panel (11 genes) and 9.4% using a large cancer panel (80 genes) in a cohort of 959 patients (Beitsch et al. 2019).

Although Next Generation Sequencing (NGS) methods have become widely employed for diagnosis and treatment, rapid gains in methodology and gene data base are still being made. Modifications to protocols,

techniques and instruments for NGS may provide significant improvements in detection of germline variants associated with inherited cancer today compared to earlier tests. Methods and knowledge will continue to improve, hence re-testing with newer methods or re-analysis of existing sequencing data may be needed to successfully detect the following clinically relevant variants:

a. *Mosaic variants:* Genetic testing is typically performed on DNA extracted from either blood or saliva samples. These sample types are most convenient for providers to collect and least invasive for patients. For a subset of disorders where mosaicism occurs, repeat testing with a biopsy sample from an affected tissue and /or sequencing to a greater depth of coverage is clinically indicated as mosaicism may not be well represented in blood alone.

   Note, each of these disorders has clinical features that overlap other genetic syndromes diagnosable by routine testing or are themselves often diagnosed by routine testing; therefore, methods developed for detection of mosaicism would not be a first-line genetic test.

   Detecting mosaicism increases diagnostic yield in several diseases including:

   - Epilepsy: Most genetic causes of epilepsy are inherited in an autosomal dominant or X-linked manner. Although most cases are either inherited or occur *de novo* as heterozygous or hemizygous variant, in a study of ~1,000 cases, 1.4%-3.4% were found to be mosaic (Stosser et al. 2018, Burgess et al. 2019). There are many identified genetic causes of epilepsy and a molecular diagnosis is valuable in guiding treatment (Mei et al. 2017, Orsini et al. 2018).

   - Tuberous sclerosis: Tuberous sclerosis complex (TSC) is caused by pathogenic variants in *TSC1* or *TSC2*. Although 85-90% of individuals with a clinical diagnosis of TSC have a pathogenic variant identified by routine genetic testing, 10-15% do not. A significant proportion of those individuals are mosaic for a pathogenic variant that is detectable by testing of a different sample type or sequencing to a greater than typical depth of coverage (Tyburczy et al. 2015, Byers et al. 2018).

   - Vascular and overgrowth disorders: CLOVES and Klippel-Trenaunay syndromes, caused by mosaic pathogenic variants in *PIK3CA*, and Proteus syndrome, caused by mosaic pathogenic variants in *AKT1*, may require NGS testing on different samples before low level mosaicism is identified (Lindhurst et al. 2011, Keppler-Noreuil et al. 2015, Luks et al. 2015).

b. *Copy number variants:* Similarly, detecting copy number variants (CNVs) has improved diagnosis in several disease types such as inherited cancer syndromes (8.3%) (Truty et al. 2019), pediatric disorders (7.7%) (Truty et al. 2019), cardiovascular disease (4.7%) (Truty et al. 2019), neurologic disorders (35%) (Truty et al. 2019), muscular dystrophy (0.8%, 7/793) (Valipakka et al. 2017), and various genetic disorders associated with CNVs detected by exome sequencing (15%, 8/54; 1.6% 12/693)(Marchuk et al. 2018, Gao et al. 2019).

   Please note, these methods continue to improve. Recently reported methods attain the best result when sequence read depth is greater than typically used for routine testing (Ellingford et al. 2017, Kerkhof et al. 2017, Yao et al. 2017). Based on the limitations identified by these studies, older NGS data will be of insufficiently consistent quality to call CNVs; therefore, resequencing of previously tested genes will be the technically superior and most economical way to detect CNVs.

c. *Pathogenic tandem repeat expansions:* Expansions of repetitive base-pair motifs are known to cause a number of inherited neurological or muscular disorders such as Fragile X syndrome, Huntington disease, myotonic dystrophy and ataxias (Paulson 2018). Making a specific diagnosis requires molecular testing due to overlapping clinical features of these disorders. A specific diagnosis is important to patient care when specific treatments are indicated or contraindicated, as is the case with certain cerebellar motor dysfunction and ataxia disorders (Zesiewicz et al. 2018). Repetitive regions are difficult to sequence and to align to reference sequences with current methods. Technical improvements in short read sequencing quality (Dolzhenko et al. 2017) or in long read sequencing (Liu et al. 2017) generate such data. Improved informatic techniques allow such variants to be detected and characterized (Tankard et al. 2018). Such technological advances will be the result of improved methods and, therefore, require re-testing of patient samples rather than reanalysis of existing data.

As expanded panel tests become more widely adopted it is imperative that coverage policies keep pace to reflect quality health care for Medicare beneficiaries.

d. *Pathogenic variants in an expanded reportable range:* As advanced sequencing methodologies improve they provide higher accuracy and greater reportable range for regulatory and deep-intronic gene variants and the pathogenic splicing alterations that result from them. Many labs do not report intronic variants >5bp from the exon, but pathogenic variants may exist in deeper intronic areas and have been shown to lead to alternative splicing patterns and/or disrupt normal splicing and lead to a truncated or absent protein.

   Examples of genes with clinically relevant promoter or deep intronic variants include:

   - *GJB1*, pathogenic promoter and non-coding exon variants are associated with Charcot-Marie-Tooth neuropathy X type 1 (Tomaselli et al. 2017)

   - *FDFT1*, a pathogenic promoter variant is associated with squalene synthase deficiency (Kulshrestha et al. 2017, Coman et al. 2018).

   - *DMD*, multiple deep intronic pathogenic variants have been associated with Becker / Duchenne muscular dystrophy (Trabelsi et al. 2014, Zaum et al. 2017).

   It is reasonable to conclude that a pathogenic variant in a typically untargeted region could be described in any gene associated with disease, prompting resequencing of the gene.

e. *Complex variants:* As sequencing technology improves, disease associated sequence variants not detected by current methods, such as reciprocal translocation breakpoint sequence, inversions, insertions, and complex chromosomal rearrangements may be detectable (Chatron et al. 2019, Schluth-Bolard et al. 2019). The sequencing methodology required would be substantially different necessitating retesting rather than reanalysis. Although such tests are likely to fall into the category of genome sequencing, it is conceivable that gene panels or gene-specific clinical tests could be offered using such methods.

Recommendation: We recommend that CGS modify its coverage policy to reflect the following: A germline test is usually performed only once in a lifetime per beneficiary for inherited conditions. However, when medically reasonable and necessary, repeat testing may be allowed as follows: when additional implicated genes are included in the test; when the reportable range in genes already tested has been expanded to encompass pathogenic variants for which there is sufficient evidence for clinical testing; or when the analytic sensitivity has improved since the time of the previous test to allow the detection of difficult variants such as mosaic variants, copy number variants or triplet repeat alleles.

2. dLCD statement:" Providers should take reasonable measures to be aware of what, if any, germline testing a beneficiary has had prior to billing for germline testing so as to avoid billing Medicare for services that are not reasonable and necessary. Clinicians who order germline testing may wish to be aware of whether the test that they are ordering is covered under Medicare and may wish to verify that they are not ordering repeat germline testing."

Comment: CGS does not define what is meant by 'reasonable measures.' Most ordering providers will not know the reportable range of a genetic test, as that information is not likely to be in a patient's report. Additionally, if the original test was performed by a laboratory that is no longer in business, it may be impossible to obtain details about the methodology used and analysis done. In the absence of an original clinical report and / or consultation with the laboratory, a clinician should not make assumptions about the testing performed.

As more data accumulates over time, a patient may need a "variant of uncertain significance" (VOUS or VUS) to be re-evaluated, as doing so may obviate the need for additional testing if it is upgraded to likely pathogenic or pathogenic. Variant reinterpretation would consider the most up to date knowledge and require no additional technical services. Recent studies have shown utility in variant reinterpretation in inherited cancer syndromes, epilepsy, and cardiomyopathies (Aronson et al. 2012, Walsh et al. 2017, Mersch et al. 2018, SoRelle et al. 2019).

Recommendation: We recommend that CGS remove this requirement from its final policy. Alternatively, we recommend modifying the LCD language to reflect the following: **"**Providers should take reasonable measures to

be aware of what, if any, germline testing a beneficiary has had prior to billing for germline testing to avoid billing Medicare for services that are not reasonable and necessary. Any testing for which prior results are not reasonably discoverable by the beneficiary's treating providers cannot be considered as duplicative. Providers will not be held responsible when the original report lacks information about previous testing that is needed to prevent duplicate future tests."

Thank you again for the opportunity to review and comment on this proposed policy. If you have any questions please direct your correspondence to Tara Burke, AMP Senior Director of Public Policy, at tburke@amp.org or Nonda Wilson, CAP's Manager, Economic and Regulatory Affairs, at nwilson@cap.org.

Sincerely,

Association for Molecular Pathology
College of American Pathologists

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