Tier 2 Molecular Pathology Procedures

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➲ Instructions for Use

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Coverage Rationale

Overview

According to the American Medical Association (AMA) Current Procedural Terminology (CPT®) manual, molecular pathology procedures are medical laboratory procedures involving the analyses of nucleic acid to detect variants in genes that may be indicative of germline (e.g., constitutional disorders) or somatic (e.g., neoplasia) conditions, or to test for histocompatibility antigens (e.g., HLA). Code selection is typically based on the specific gene(s) that is being analyzed.

Codes that describe tests to assess for the presence of gene variants use common gene variant names. Typically, all of the listed variants would be tested. However, these lists are not exclusive. If other variants are also tested in the analysis, they would be included in the procedure and not reported separately. Full gene sequencing should not be reported using codes that assess for the presence of gene variants unless the CPT code specifically states full gene sequence in the code descriptor. In other words, only assign the CPT code that is described as “full gene sequence” if the test assay performed was a full gene sequence.

There are Tier 1 and Tier 2 molecular pathology procedure codes. Tier 1 codes generally describe testing for a specific gene or HLA locus. Tier 2 molecular pathology procedures represent procedures that are generally performed in lower volumes than Tier 1 molecular pathology procedures (e.g., the incidence of the disease being tested is rare). They are arranged by level of technical resources and interpretive work by the physician or other qualified healthcare professional.

Use the appropriate molecular pathology procedure level code that includes the specific analyte listed after the code descriptor. If the analyte/gene tested is not listed under one of the Tier 2 codes or is not represented by a Tier 1 code in CPT, use of the Not Otherwise Classified (NOC) CPT code is required.

Tier 2 individual biomarker CPT codes should not be used for a single gene or any combination of genes when testing is performed as part of a Next-Generation Sequencing (NGS) or other multiplexing technology panel.

Molecular pathology procedures have broad clinical and research applications. The following examples of applications may not be relevant to a Medicare member or may not meet a Medicare benefit category and/or reasonable and necessary threshold for coverage. Such examples include genetic testing and genetic counseling (when applicable) for:

- Disease risk;
- Carrier screening;

- Hereditary cancer syndromes;
- Gene expression profiling for certain cancers;
- Prenatal diagnostic testing; and
- Diagnosis and monitoring non-cancer indications.

CMS National Coverage Determinations (NCDs)

Medicare does not have an NCD for tier 2 molecular pathology procedures.

CMS Local Coverage Determinations (LCDs) and Articles

Local Coverage Determinations (LCDs)/Local Coverage Articles (LCAs) exist, and compliance with these policies is required where applicable. For specific LCDs/LCAs, refer to the Medicare Coverage Database and/or the table in this policy under CMS Related Documents.

For coverage guidelines for states/territories with no LCDs/LCAs, Tier 2 molecular pathology procedures are reasonable and necessary when **all** of the following criteria are met:

- Alternative laboratory or clinical tests to definitively diagnose the disorder/identify the condition are unavailable or results are clearly equivocal; **and**
- Availability of a clinically valid test, based on published peer reviewed medical literature; **and**
- Testing assay is Food and Drug Administration (FDA) approved/cleared, or LDT (lab developed test), or FDA modified test supports assay analytical validity and clinical utility; **and**
- Results of the testing must directly impact treatment or management of the member; **and**
- For testing panels, including but not limited to, multiple genes or multiple conditions, and in cases where a tiered approach/method is clinically available, testing is reasonable and necessary **only** for the number of genes or test that are reasonable and necessary to establish a diagnosis; **and**
- Individual has not previously received genetic testing for the disease/condition. (In general, diagnostic genetic testing for a disease should be performed once in a lifetime.)

A specific genetic test may only be performed once in a lifetime per member for inherited conditions; however, when reasonable and necessary, genetic testing may be done on acquired conditions such as malignancies (including separate malignancies developing at different times) as they are treated and are being followed, in order to assess response or other relevant clinical criteria. Likewise, there are situations where serial testing can be reasonably predicted to provide additional clinically useful information, such as when confirmed response to current therapy is likely to assist in modifying treatment.

Gene Identification

Covered

Specific diagnosis criteria for services that are reasonable and necessary can be found in the Applicable Codes section.

- **For CPT Code 81404**
  - CDKN2A (cyclin-dependent kinase inhibitor 2A).
  - FGFR2 and FGFR3 are reasonable and necessary for patients who would otherwise be candidates for erdafitinib based on the FDA label.
  - MEN1 (multiple endocrine neoplasia 1) (e.g., multiple endocrine neoplasia type 1, Werner syndrome), duplication/deletion.
  - PRSS1 [protease, serine, 1 (trypsin 1)].
  - RET (ret-proto-oncogene) (MEN Type 2B) is reasonable and necessary in patients with medullary CA of thyroid, multiple endocrine neoplasia, pheochromocytoma, and parathyroid tumors to guide therapeutic decision making.
  - VHL (von Hippel-Lindau tumor suppressor).
- **For CPT Code 81405**
  - MEN1 (multiple endocrine neoplasia 1) (e.g., multiple endocrine neoplasia type 1, Werner syndrome), full gene sequence is reasonable and necessary in patients with multiple endocrine neoplasia to guide therapeutic decision-making.
  - RET (ret-proto-oncogene) (MEN Type 2A) is reasonable and necessary in patients with medullary CA of thyroid, multiple endocrine neoplasia, pheochromocytoma, and parathyroid tumors to guide therapeutic decision making.
- **For CPT Code 81406**
  - ATP7B (ATPase, Cu++ transporting, beta polypeptide) is reasonable and necessary in patients with symptoms of Wilson's disease to guide therapeutic decision making.
  - RYR1 (Volatile anesthetics (class): desflurane, enflurane, halothane, isoflurane, methoxyflurane, sevoflurane, succinylcholine).

Non-Covered

The following individual Tier 2 genetic tests are unlikely to impact therapeutic decision-making or directly impact treatment, outcome, and/or clinical management in the care of the Medicare member and will be denied as not reasonable and necessary. (Please note that this list of genes that are not reasonable and necessary is not exhaustive, and the fact that a specific gene is not mentioned does not mean it is reasonable and necessary. In addition, many genes have several names that are used. The most common names have been used in this policy.)

- **For CPT Code 81404**
  - ACADS (acyl-CoA dehydrogenase).
  - AQP2 (aquaporin 2 [collecting duct]).
  - ARX (aristaless related homeobox).
  - BTD (biotinidase).
  - CAV3 (caveolin 3) (e.g., CAV3-related distal myopathy, limb-girdle muscular dystrophy type 1C), full gene sequence.
  - CLRN1 (clarin 1).
  - CYP1B1 (cytochrome P450, family 1, subfamily B, polypeptide 1).
  - EGR2 (early growth response 2) (e.g., Charcot-Marie-Tooth).
  - FKRP (Fukutin related protein).
  - FOXG1 (forkhead box G1).
  - FSHMD1A (facioscapulohumeral muscular dystrophy 1A).
  - HNF1B (HNF1 homeobox B).
  - HRAS (v-Ha-ras Harvey rat sarcoma viral oncogene homolog).
  - KCNJ10 (potassium inwardly rectifying channel, subfamily J, member 10).
  - SLC25A4 (solute carrier family 25 [mitochondrial carrier; adenine nucleotide translocation]).
  - VWF (von Willebrand factor).
- **For CPT Code 81405**
  - ACADS (acyl-CoA dehydrogenase).
  - CASR (CAR, EIG8, extracellular calcium-sensing receptor, FHH, FIH, GPRC2A, HHC, HHC1, NSHPT, PCAR1).
  - CDKL5 (cyclin-dependent kinase-like 5).
  - CYP21A2 (cytochrome P450, family 21, subfamily A, polypeptide2).
  - HNF1B (HNF1 homeobox B).
  - MPZ (myelin protein zero).
  - NF2 (neurofibromin 2 [merlin]).
  - TSC1 (tuberous sclerosis 1).
- **For CPT Code 81406**
  - ACADVL (acyl-CoA dehydrogenase, very long chain).
  - AIRE.
  - CBS (cystathionine-beta-synthase).
  - CDKL5 (cyclin-dependent kinase-like 5).
  - DLAT (dihydrolipoamide S-acetyltransferase).
  - DLD (dihydrolipoamide dehydrogenase).
  - F8 (coagulation factor VIII).
  - GALT (galactose-1-phosphate uridylyltransferase).
  - HADHA [hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein) alpha subunit].
  - HEXA (hexosaminidase A, alpha polypeptide).
  - IVD.
  - LMNA (lamin A/C).
  - NF2 [neurofibromin 2 (merlin)].
  - NSD1 (nuclear receptor binding SET domain protein 1).
  - PAH (phenylalanine hydroxylase).
  - PAX2 (paired box 2).
  - PDHA1 [pyruvate dehydrogenase (lipoamide) alpha1].
  - POLG (polymerase [DNA directed], gamma).
  - PRKAG2 (protein kinase, AMP-activated gamma 2 non-catalytic subunit).
  - PTPN11 (protein tyrosine phosphatase, non-receptor type 11).
  - RET (ret-proto-oncogene) (e.g., Hirschsprung disease), full gene sequence.
  - SLC9A6 [solute carrier family 9 (sodium/hydrogen exchanger) member 6].
  - SOS1 (son of sevenless homolog 1).
  - TAZ (tafazzin).
  - TSC1 (tuberous sclerosis 1).

- TSC2 (tuberous sclerosis 2).
- UBE3A (ubiquitin protein ligase).

- **For CPT Code 81407, level 8 Molecular Pathology Procedures are noncovered.**
- **For CPT Code 81408, level 9 Molecular Pathology Procedures are noncovered.**

Based on the Centers for Medicare & Medicaid Services (CMS) Program Integrity Manual (100 - 08), this policy addresses the circumstances under which the item or service is reasonable and necessary under the Social Security Act, §1862(a)(1)(A). For laboratory services, a service can be reasonable and necessary if the service is safe and effective; not experimental or investigational (exception: routine costs of qualifying clinical trial services which meet the requirements of the Clinical Trials NCD and are reasonable and necessary); and appropriate, including the duration and frequency that is appropriate for the item or service, in terms of whether it is furnished in accordance with accepted standards of medical practice for the diagnosis or treatment of the patient's condition or to improve the function of a malformed body member; furnished in a setting appropriate to the patient's medical needs and condition; ordered and furnished by qualified personnel; one that meets, but does not exceed, the patient's medical need; and is at least as beneficial as an existing and available medically appropriate alternative.

Nationally Non-Covered Indications

Compliance with the provisions in this policy is subject to monitoring by post payment data analysis and subsequent medical review. Title XVIII of the Social Security Act, Section 1862(a)(1)(A) states " ...no Medicare payment shall be made for items or services which are not reasonable and necessary for the diagnosis and treatment of illness or injury..." Furthermore, it has been longstanding CMS policy that ***"tests that are performed in the absence of signs, symptoms, complaints, or personal history of disease or injury are not covered unless explicitly authorized by statute".***

Screening services, such as pre - symptomatic genetic tests and services, used to detect an undiagnosed disease or disease predisposition are not a Medicare benefit and not covered. Similarly, Medicare may not reimburse the costs of tests/examinations that assess the risk of a condition unless the risk assessment clearly and directly effects the management of the patient.

Many applications of the molecular pathology procedures are not covered services given lack of benefit category (preventive service) and/or failure to reach the reasonable and necessary threshold for coverage (based on quality of clinical evidence and strength of recommendation). Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death (e.g., Canavan disease) are not usually relevant to a Medicare member.

Applicable Codes

The following list(s) of procedure and/or diagnosis codes is provided for reference purposes only and may not be all inclusive. Listing of a code in this policy does not imply that the service described by the code is a covered or non-covered health service; however, language may be included in the listing below to indicate if a code is non-covered. Benefit coverage for health services is determined by the member specific benefit plan document and applicable laws that may require coverage for a specific service. The inclusion of a code does not imply any right to reimbursement or guarantee claim payment. Other Policies and Guidelines may apply.

CPT Code	Description
81400	Molecular pathology procedure, Level 1 (short description)
81401	Molecular pathology procedure, Level 2 (short description)
81402	Molecular pathology procedure, Level 3 (short description)
81403	Molecular pathology procedure, Level 4 (short description)
81404	Molecular pathology procedure, Level 5 (short description)
81405	Molecular pathology procedure, Level 6 (short description)
81406	Molecular pathology procedure, Level 7 (short description)
81407	Molecular pathology procedure, Level 8 (short description)
81408	Molecular pathology procedure, Level 9 (short description)

Diagnosis Code	Description
CPT Code 81404 for FGFR2, FGFR3	
C67.0	Malignant neoplasm of trigone of bladder
C67.1	Malignant neoplasm of dome of bladder
C67.2	Malignant neoplasm of lateral wall of bladder
C67.3	Malignant neoplasm of anterior wall of bladder
C67.4	Malignant neoplasm of posterior wall of bladder
C67.5	Malignant neoplasm of bladder neck
C67.6	Malignant neoplasm of ureteric orifice
C67.7	Malignant neoplasm of urachus
C67.8	Malignant neoplasm of overlapping sites of bladder
CPT Code 81406 for RYR1	
T41.0X5A	Adverse effect of inhaled anesthetics, initial encounter
T41.0X5D	Adverse effect of inhaled anesthetics, subsequent encounter
T41.0X5S	Adverse effect of inhaled anesthetics, sequela
T41.0X6A	Underdosing of inhaled anesthetics, initial encounter
T41.0X6D	Underdosing of inhaled anesthetics, subsequent encounter
T41.0X6S	Underdosing of inhaled anesthetics, sequela
T41.1X5A	Adverse effect of intravenous anesthetics, initial encounter
T41.1X5D	Adverse effect of intravenous anesthetics, subsequent encounter
T41.1X5S	Adverse effect of intravenous anesthetics, sequela
T41.1X6A	Underdosing of intravenous anesthetics, initial encounter
T41.1X6D	Underdosing of intravenous anesthetics, subsequent encounter
T41.1X6S	Underdosing of intravenous anesthetics, sequela
CPT Codes 81404 and 81405 for RET – MEN Type 2	
C73	Malignant neoplasm of thyroid gland
C74.10	Malignant neoplasm of medulla of unspecified adrenal gland
C74.11	Malignant neoplasm of medulla of right adrenal gland
C74.12	Malignant neoplasm of medulla of left adrenal gland
C75.0	Malignant neoplasm of parathyroid gland
D35.1	Benign neoplasm of parathyroid gland
CPT Code 81406 for ATP7B	
E83.01	Wilson's disease

Non-Covered Diagnosis Code

Non-Covered Diagnosis Codes List

This list contains diagnosis codes that are **never covered when given as the primary reason for the test.** If a code from this section is given as the reason for the test and you know or have reason to believe the service may not be covered, call UnitedHealthcare to issue an Integrated Denial Notice (IDN) to the member and you. The IDN informs the member of their liability for the non-covered service or item and appeal rights. You must make sure the member has received the IDN prior to rendering or referring for non-covered services or items in order to collect payment.

Centers for Medicare and Medicaid Services (CMS) Related Documents

After checking the table below and searching the Medicare Coverage Database, if no NCD, LCD, or LCA is found, refer to the criteria as noted in the Coverage Rationale section above.

NCD	LCD	LCA	Contractor Type	Contractor Name
FGFR2 and FGFR3 Gene Tests				
N/A	L38586 MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	A58065 Billing and Coding: MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	Part A and B MAC	CGS
N/A	L38647 MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	A58181 Billing and Coding: MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	Part A and B MAC	Noridian
N/A	L38649 MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	A58187 Billing and Coding: MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	Part A and B MAC	Noridian
N/A	L38576 MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	A58028 Billing and Coding: MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	Part A and B MAC	Palmetto**
N/A	L38684 Prognostic and Predictive Molecular Classifiers for Bladder Cancer	A58211 Billing and Coding: MolDX: Prognostic and Predictive Molecular Classifiers for Bladder Cancer	Part A and B MAC	WPS*
Genetic Testing for Cardiovascular Disease				
N/A	L39084 Genetic Testing for Cardiovascular Disease	A58797 Billing and Coding: Genetic Testing for Cardiovascular Disease	Part A and B MAC	First Coast
N/A	L39082 Genetic Testing for Cardiovascular Disease	A58795 Billing and Coding: Genetic Testing for Cardiovascular Disease	Part A and B MAC	Novitas**
Pharmacogenomics Testing				
N/A	L38394 MolDX: Pharmacogenomics Testing	A58324 Billing and Coding: MolDX: Pharmacogenomics Testing	Part A and B MAC	CGS
N/A	L38335 MolDX: Pharmacogenomics Testing	A57384 Billing and Coding: MolDX: Pharmacogenomics Testing	Part A and B MAC	Noridian
N/A	L38337 MolDX: Pharmacogenomics Testing	A57385 Billing and Coding: MolDX: Pharmacogenomics Testing	Part A and B MAC	Noridian
N/A	L38294 MolDX: Pharmacogenomics Testing	A58318 Billing and Coding: MolDX: Pharmacogenomics Testing	Part A and B MAC	Palmetto**

NCD	LCD	LCA	Contractor Type	Contractor Name
Pharmacogenomics Testing				
N/A	L38435 MolDX: Pharmacogenomics Testing	A58395 Billing and Coding: MolDX: Pharmacogenomics Testing	Part A and B MAC	WPS*
N/A	L39073 Pharmacogenomics Testing	A58812 Billing and Coding: Pharmacogenomics Testing	Part A and B MAC	First Coast
N/A	L39063 Pharmacogenomics Testing	A58801 Billing and Coding: Pharmacogenomics Testing	Part A and B MAC	Novitas**
General Molecular Diagnostic Tests				
N/A	L35062 Biomarkers Overview	A52986 Billing and Coding: Biomarkers for Oncology	Part A and B MAC	Novitas**
		A58917 Billing and Coding: Molecular Pathology and Genetic Testing		
N/A	L34519 Molecular Pathology Procedures	A57451 Billing and Coding: Molecular Pathology Procedures	Part A and B MAC	First Coast
		A58918 Billing and Coding: Molecular Pathology and Genetic Testing		
N/A	L35000 Molecular Pathology Procedures	A56199 Billing and Coding: Molecular Pathology Procedures	Part A and B MAC	NGS

Medicare Administrative Contractor (MAC) With Corresponding States/Territories	
MAC Name (Abbreviation)	**States/Territories**
CGS Administrators, LLC (CGS)	KY, OH
First Coast Service Options, Inc. (First Coast)	FL, PR, VI
National Government Services, Inc. (NGS)	CT, IL, ME, MA, MN, NH, NY, RI, VT, WI
Noridian Healthcare Solutions, LLC (Noridian)	AS, AK, AZ, CA, GU, HI, ID, MT, NV, ND, Northern Mariana Islands, OR, SD, UT, WA, WY
Novitas Solutions, Inc. (Novitas)	AR, CO, DC, DE, LA, MD, MS, NJ, NM, OK, PA, TX, VA**
Palmetto GBA (Palmetto)	AL, GA, NC, SC, TN, VA**, WV
Wisconsin Physicians Service Insurance Corporation (WPS)*	IA, IN, KS, MI, MO, NE
Notes	
*Wisconsin Physicians Service Insurance Corporation: Contract Number 05901 applies only to WPS Legacy Mutual of Omaha MAC A Providers. **For the state of Virginia: Part B services for the city of Alexandria and the counties of Arlington and Fairfax are excluded for the Palmetto GBA jurisdiction and included within the Novitas Solutions, Inc. jurisdiction.	

CMS Benefit Policy Manual

Chapter 15; §§ 80.1 - 80.1.3, 80.6 Clinical Laboratory Services

CMS Claims Processing Manual

Chapter 12; § 60 Payment for Pathology Services
Chapter 16, § 10.2 General Explanation of Payment; § 20 Calculation of Payment Rates - Clinical Laboratory Test Fee Schedules; § 40 Billing for Clinical Laboratory Tests

Other(s)

CMS IOM 100-08, Medicare Program Integrity Manual, Chapter 13, Section13.5.4 Reasonable and Necessary Provisions in LCDs
CMS Clinical Laboratory Fee Schedule, CMS Website
DHHS Office of Inspector General: Report in Brief; Dated June 2023, Report No. A-09-22-03010
Palmetto GBA MolDx Website
Palmetto GBA MolDx Manual, Palmetto GBA MolDx Website
L39017 MolDX: Lab-Developed Tests for Inherited Cancer Syndromes in Patients with Cancer
A58734 Billing and Coding: MolDX: Lab-Developed Tests for Inherited Cancer Syndromes in Patients with Cancer
L38972 MolDX: Lab-Developed Tests for Inherited Cancer Syndromes in Patients with Cancer
A58679 Billing and Coding: MolDX: Lab-Developed Tests for Inherited Cancer Syndromes in Patients with Cancer
L38974 MolDX: Lab-Developed Tests for Inherited Cancer Syndromes in Patients with Cancer
A58681 Billing and Coding: MolDX: Lab-Developed Tests for Inherited Cancer Syndromes in Patients with Cancer
L38966 MolDX: Lab-Developed Tests for Inherited Cancer Syndromes in Patients with Cancer
A58652 Billing and Coding: MolDX: Lab-Developed Tests for Inherited Cancer Syndromes in Patients with Cancer
L36021 MolDX: Molecular Diagnostic Tests (MDT)
A56973 Billing and Coding: MolDX: Molecular Diagnostic Tests (MDT)
L35025 MolDX: Molecular Diagnostic Tests (MDT)
A56853 Billing and Coding: MolDX: Molecular Diagnostic Tests (MDT)
L35160 MolDX: Molecular Diagnostic Tests (MDT)
A57526 Billing and Coding: MolDX: Molecular Diagnostic Tests (MDT)
L36256 MolDX: Molecular Diagnostic Tests (MDT)
A57527 Billing and Coding: MolDX: Molecular Diagnostic Tests (MDT)
L36807 MolDX: Molecular Diagnostic Tests (MDT)
A57772 Billing and Coding: MolDX: Molecular Diagnostic Tests (MDT)
A54254 Billing and Coding: MolDX: ATP7B Gene Tests; Retired 04/23/2024
A55097 Billing and Coding: MolDX: ATP7B Gene Tests; Retired 04/23/2024
A55098 Billing and Coding: MolDX: ATP7B Gene Tests; Retired 04/23/2024
A53550 Billing and Coding: MolDX: ATP7B Gene Tests; Retired 04/23/2024
A55143 Billing and Coding: MolDX: ATP7B Gene Tests; Retired 05/30/2024
A54255 Billing and Coding: MolDX: BCKDHB Gene Test; Retired 05/08/2024
A55099 Billing and Coding: MolDX: BCKDHB Gene Test; Retired 05/08/2024
A55100 Billing and Coding: MolDX: BCKDHB Gene Test; Retired 05/08/2024
A53600 Billing and Coding: MolDX: BCKDHB Gene Test; Retired 05/08/2024
A55145 Billing and Coding: MolDX: BCKDHB Gene Test; Retired 05/30/2024
A54878 Billing and Coding: MolDX: CDH1 Genetic Testing; Retired 05/30/2024
A55970 Billing and Coding: MolDX: CDH1 Genetic Testing; Retired 05/30/2024
A55971 Billing and Coding: MolDX: CDH1 Genetic Testing; Retired 05/30/2024
A54835 Billing and Coding: MolDX: CDH1 Genetic Testing; Retired 05/30/2024
A55622 Billing and Coding: MolDX: CDH1 Genetic Testing; Retired 06/27/2024
A54243 Billing and Coding: MolDX: CHD7 Gene Analysis Guidelines; Retired 04/25/2024
A55085 Billing and Coding: MolDX: CHD7 Gene Analysis; Retired 04/25/2024
A55086 Billing and Coding: MolDX: CHD7 Gene Analysis; Retired 04/25/2024
A53565 Billing and Coding: MolDX: CHD7 Gene Analysis; Retired 04/25/2024
A55157 Billing and Coding: MolDX: CHD7 Gene Analysis; Retired 05/30/2024
A54262 Billing and Coding: MolDX: ENG and ACVRL1 Gene Tests; Retired 04/18/2024
A55181 Billing and Coding: MolDX: ENG and ACVRL1 Gene Tests; Retired 04/18/2024
A55182 Billing and Coding: MolDX: ENG and ACVRL1 Gene Tests; Retired 04/18/2024
A53536 Billing and Coding: MolDX: ENG and ACVRL1 Gene Tests; Retired 04/18/2024
A55159 Billing and Coding: MolDX: ENG and ACVRL1 Gene Tests; Retired 05/30/2024
A54268 Billing and Coding: MolDX: HEXA Gene Analysis; Retired 05/08/2024
A55255 Billing and Coding: MolDX: HEXA Gene Analysis; Retired 05/08/2024
A55256 Billing and Coding: MolDX: HEXA Gene Analysis; Retired 05/08/2024
A53598 Billing and Coding: MolDX: HEXA Gene Analysis; Retired 05/08/2024

A55168 Billing and Coding: MolDX: HEXA Gene Analysis; Retired 05/30/2024
A54274 Billing and Coding: MolDX: L1CAM Gene Sequencing Guidelines
A55277 Billing and Coding: MolDX: L1CAM Gene Sequencing; Retired 05/24/2024
A55278 Billing and Coding: MolDX: L1CAM Gene Sequencing; Retired 05/24/2024
A53659 Billing and Coding: MolDX: L1CAM Gene Sequencing; Retired 05/24/2024
A55192 Billing and Coding: MolDX: L1CAM Gene Sequencing; Retired 06/27/2024
A54209 Billing and Coding: MolDX: MMACHC Test; Retired 05/30/2024
A55288 Billing and Coding: MolDX: MMACHC Test; Retired 05/30/2024
A55289 Billing and Coding: MolDX: MMACHC Test; Retired 05/30/2024
A54035 Billing and Coding: MolDX: MMACHC Test; Retired 05/30/2024
A55191 Billing and Coding: MolDX: MMACHC Test; Retired 06/27/2024
A54291 Billing and Coding: MolDX: NSD1 Gene Tests; Retired 04/30/2024
A55609 Billing and Coding: MolDX: NSD1 Gene Tests; Retired 04/30/2024
A55615 Billing and Coding: MolDX: NSD1 Gene Tests; Retired 04/30/2024
A53585 Billing and Coding: MolDX: NSD1 Gene Tests; Retired 04/30/2024
A55198 Billing and Coding: MolDX: NSD1 Gene Tests; Retired 05/30/2024
A54299 Billing and Coding: MolDX: RPS19 Gene Tests; Retired 04/30/2024
A55610 Billing and Coding: MolDX: RPS19 Gene Tests; Retired 04/30/2024
A55614 Billing and Coding: MolDX: RPS19 Gene Tests; Retired 04/30/2024
A53587 Billing and Coding: MolDX: RPS19 Gene Tests; Retired 04/30/2024
A55205 Billing and Coding: MolDX: RPS19 Gene Tests; Retired 05/30/2024
A54284 Billing and Coding: MolDX: STAT3 Gene Testing; Retired 04/24/2024
A55480 Billing and Coding: MolDX: STAT3 Gene Testing; Retired 04/24/2024
A55481 Billing and Coding: MolDX: STAT3 Gene Testing; Retired 04/24/2024
A53562 Billing and Coding: MolDX: STAT3 Gene Testing; Retired 04/24/2024
A55209 Billing and Coding: MolDX: STAT3 Gene Testing; Retired 05/30/2024

Clinical Evidence

CDKN2A

Mukherjee et al. (2023) conducted a study of the data from family members of CDKN2A mutation carriers to calculate the relative and lifetime risks of non- melanoma cancers. Relative risks (RRs) of all non-melanoma cancers among first-degree relatives (FDRs) of melanoma patients with CDKN2A mutations (n = 65) and FDRs of melanoma patients without mutations (n = 3537) were calculated. The results showed that FDRs of CDKN2A mutation carriers had an increased risk for all nonmelanoma cancers, gastrointestinal cancer, and colorectal cancer. These individuals also had an increased lifetime risk of developing any cancer (excluding melanoma) compared with FDRs of noncarriers. The lifetime risk of any cancer other than melanoma among CDKN2A mutation carriers was estimated as 59.0% by age 85 years. These results provider further evidence on the association of CDKN2A gene mutations on the increased risk of several cancers and can influence cancer screening and prevention for these individuals to reduce risk and improve outcomes.

McWilliams et al. (2011) conducted a study on the prevalence of CDKN2A mutations of 1537 patients in pancreatic cancer and the implications for genetic counseling. The results showed that while germline mutations of CDKN2A among patients with pancreatic cancer are rare and carriers are more likely to report a personal or family history of melanoma, and a family history of pancreatic cancer. Age at diagnosis of pancreatic cancer may be slightly younger for mutation carriers, although it did not reach statistical significance. Penetrance of pancreatic cancer and melanoma was increased among mutation carriers, with pancreatic cancer risk estimates of 58% by age 80 and melanoma risk of 39% by age 80. Penetrance for carriers was higher among ever-smokers. Carriers of germline mutations in CDKN2A should avoid tobacco use and be targeted for prevention and screening studies.

Goldstein et al. (2006) reported on high-risk melanoma susceptibility genes and pancreatic cancer, neural system tumors, and uveal melanoma across GenoMEL. GenoMEL is comprised of major familial melanoma research groups from North America, Europe, Asia, and Australia and has created the largest familial melanoma sample available to characterize mutations in the high-risk melanoma susceptibility genes CDKN2A/alternate reading frames (ARF), which encodes p16 and p14ARF, and CDK4 and to evaluate their relationship with pancreatic cancer (PC), neural system tumors (NST), and uveal melanoma (UM). This study included 466 families (2,137 patients) with at least three melanoma patients from 17 GenoMEL centers. Overall, 41% of families had mutations; most involved p16. There were striking differences in mutations across geographic locales. Single founder CDKN2A mutations were predominant in Sweden and the Netherlands France, Spain, and Italy. Similarly, Australia and United Kingdom had the same most common mutations. As reported previously, there was a strong association between PC and CDKN2A mutations which differed by mutation.

There was little evidence for an association between CDKN2A mutations and NST or UM. There was a marginally significant association between NST and ARF. This GenoMEL study provides the most extensive characterization of mutations in high-risk melanoma susceptibility genes in families with three or more melanoma patients available.

FGFR2, FGFR3

Loriot et al. (2019) conducted an ongoing, open- label phase 2 study of 99 patients who had locally advanced and unresectable or metastatic urothelial carcinoma with at least one FGFR3 mutation or FGFR2/3 fusion and treated with erdafitinib, a pan-FGFR tyrosine kinase inhibitor. All participants had a history of disease progression during or after at least one course of chemotherapy or within 12 months after neoadjuvant or adjuvant chemotherapy. Prior immunotherapy was allowed. The primary end point was the objective response rate. Secondary end points included progression-free survival, duration of response, and overall survival. The results showed a 40% response rate after continuous daily treatment with erdafitinib and an acceptable safety profile, emerging as a first-in-class treatment for patients with surgically unresectable or metastatic urothelial cancer (mUC) harboring FGFR mutations/fusions.

Zieger et al. (2005) conducted a study on banked bladder tumors from 85 patients with initial superficial disease to study the biological differences in those that progress and those that do not. Patients were followed prospectively with control cystoscopies every 4 months in the first year, every 6 months in the second, and yearly until a recurrence-free period of 5 years. A total of 55 FGFR3 mutations were found in 115 tumors and at least one tumor showed an activating mutation in 43 of the 85 patients The results showed that FGFR3-mutated superficial tumors progress and retain their mutation and chromosomal instability patterns during progression. The decreasing frequency of FGFR3 mutations in later stages of tumor development is caused by the emergence of tumors following a different molecular pathway with no FGFR3 mutations. FGFR3-mutated tumors have malignant potential, but FGFR3 mutations do not appear to be directly involved in progression. The authors concluded that FGFR3 has a central role in the development of papillary bladder tumors but does not impact the progression.

MEN1

Faggiano et al. (2023) evaluated the predictive role of specific clinical factors for the diagnosis of Multiple Endocrine Neoplasia type-1 (MEN1) and type-4 (MEN4) in patients with an initial diagnosis of gastrointestinal, bronchial, or thymic neuroendocrine tumor (NET). The study included patients referred to the NET Unit with a diagnosis of NET and at least one clinical criterion of suspicion for MEN1 and MEN4 and underwent molecular analysis of the MEN1 and CDKN1B genes. Phenotypic criteria were: (1) age ≤ 40 years; (2) NET multifocality; (3) MEN1/4-associated manifestations other than NETs; and (4) endocrine syndrome related to NETs or pituitary/adrenal tumors. The authors reported of the 22 patients studied, 18 patients (81.8%) tested negative for the first-level genetic test (Group A), while four patients (25%) were positive for MEN1 (Group B). No patient was positive for MEN4. In Group A, 10 patients met one clinical criterion, and three patients met three criteria. In Group B, three patients had three criteria, and one met all criteria. The authors concluded that the preliminary data showing a diagnosis of NET in patients with a negative family history is suggestive of MEN1 in the presence of ≥ three positive phenotypic criteria, including early age, multifocality, multiple MEN-associated manifestations, and endocrine syndromes. This indication may allow optimization of the diagnosis of MEN in patients with NET. The study aimed to provide a molecular analysis on MEN1 due to the paucity of studies exploring the correlation between genotype and phenotype in MEN1 and MEN4 syndromes.

La Salvia et al. (2021) conducted a systematic review analyzing the safety and efficacy of somatostatin analogue (SSA) treatment in patients affected by multiple endocrine neoplasia type 1 (MEN1)-related pancreatic neuroendocrine neoplasms (pNENs). MEN1 syndrome is the result of a germline mutation of the MEN1 tumor suppressor gene. The MEN1 gene is linked to the chromosomal locus 11q13, composed of 10 exons encoding the protein Menin, and plays a key role in cell division, proliferation, and epigenetic regulation. Twenty studies were selected consisting of 105 patients between the ages of 18—73 with a diagnosis of one or more well-differentiated pNENs associated with MEN1 syndrome. The authors reported the tumor (T), node (N), and metastasis (M) (TNM) stage at diagnosis was stage I–II in 84.8% and stage IV in 15.2% of the patients. The overall response rate (stable disease + partial response + complete response) was achieved in 88.3% of cases, with stable disease in 75.6% and objective response in 12.7% of patients. The safety profile was favorable with both somatostatin analogue agents: 78.2% of patients reported no SSA-related side effects with only a minority of patients showed low-grade and manageable toxicities.

In 2012, Thakker et al. reported on specific guidelines for evaluation, treatment, and genetic testing for multiple endocrine neoplasia type1 (MEN1), reviewed by a group of researchers comprised of physicians, surgeons, and geneticists from international centers. MEN1 is an autosomal dominant disorder that is due to mutations in the tumor suppressor gene MEN1, which encodes a 610-amino acid protein, Menin. Thus, the finding of MEN1 in a patient has important implications for family members because first-degree relatives have a 50% risk of developing the disease and can often be identified by MEN1 mutational analysis. Some patients may also develop carcinoid tumors, adrenocortical tumors, meningiomas,

facial angiofibromas, collagenomas, and lipomas. Patients with MEN1 have a decreased life expectancy, and the outcomes of current treatments, which are generally similar to those for the respective tumors occurring in non-MEN1 patients, are not as successful because of multiple tumors, which may be larger, more aggressive, and resistant to treatment, and the concurrence of metastases. The prognosis for MEN1 patients might be improved by presymptomatic tumor detection and undertaking treatment specific for MEN1 tumors. Thus, it is recommended that MEN1 patients and their families should be cared for by multidisciplinary teams comprising relevant specialists with experience in the diagnosis and treatment of patients with endocrine tumors.

ClinGen Actionability Assertion for the MEN1 gene and multiple endocrine neoplasia type 1 was reported to have a strong actionability as computed based on the Semi-quantitative Metric (SQM) scoring rubric (ClinGen, 2024).

PRSS1 [Protease, Serine, 1 (Trypsin 1)]

Girodon et al. (2021) conducted a systematic review of the published literature related to the effect of the PRSS1 gene in idiopathic chronic and recurrent acute pancreatitis, and the impact of PRSS1 variants on PRSS1 experimental models. Eighteen PRSS1 gene molecular studies were included. The results shown 56 different PRSS1 variants identified in patients with hereditary, familiar, and sporadic pancreatitis, and the two most prevalent variants are found in more than 80% of hereditary pancreatitis world-wide. The authors concluded that while PRSS1 testing cannot determine if asymptomatic individuals will develop disease, or predict onset and severity, early-onset pancreatitis is strongly associated with PRSS1 variants. Furthermore, progression to chronic pancreatitis is faster in children with PRSS1 variants. Chronic pancreatitis has been shown to be a risk factor for pancreatic cancer.

In a 2018 systematic review, Zhan et al. identified germline variants associated with pancreatic cancer and summarized which ones are pathogenic or likely to be pathogenic using multiple criteria that included cell injury, cell growth and cycle control, DNA repair, and cell mobility and adhesion. Twenty were identified as pathogenic, and 46 were identified as likely pathogenic with the remainder classified as “variants of uncertain significance”. The PRSS1 was categorized as likely pathogenic due to cell injury. The risk of pancreatic cancer appears to be higher in patients with long-standing chronic pancreatitis, especially when genetic mutations in pancreatitis susceptibility genes cause early onset chronic pancreatitis. While these mutations increase the risk of pancreatic cancer in patients who develop chronic pancreatitis, most pancreatic cancer patients do not have commonly recognized mutations in PRSS. Patients with PRSS1 gain-of-function variants are at high risk of hereditary pancreatitis, which is associated with a first attack around age 10–12 years and a high risk for progression to CP in the 2nd or 3rd decade. Estimates for cumulative risk for pancreatic cancer at age 70 range from 7.2–40% which is believed to be a consequence of lifetime exposure of the pancreas to inflammation. However, PRSS1 mutations are an uncommon cause of pancreatitis (~ 1%), and only 5% of patients with CP (all etiologies) will develop pancreatic cancer over a 20 year period. The authors concluded that no single gene is the primary contributor to pancreatic cancer.

RET (Ret-Proto-Oncogene) (MEN Types 2A, 2B)

Milićević et al. (2021) performed a retrospective analysis to determine the frequency and type of rearranged during transfection (RET) mutation in individuals with medullary thyroid cancer (MTC) and estimated the crude annual incidence. The study included 186 individuals diagnosed with MTC between 1995 and 2015 and their relatives who underwent genetic counseling and testing. The study results revealed the average crude annual incidence rate of MTC in Slovenia was 0.34/100,000. The estimated crude incidence rate was noted to have increased significantly, with an annual percentage change of 3.6%. A germline mutation in the RET proto-oncogene was identified in 25.9% of individuals diagnosed with MTC. The authors concluded the annual incidence increase and nation-specific frequency of RET mutations justified the future use of genetic counseling and testing of individuals diagnosed with MTC in Slovenia.

Mishra et al. (2019) conducted a comprehensive risk association of 13 single nucleotide polymorphisms (SNPs) and a meta-analysis of RET SNPs. The analysis included 438 patients with histologically diagnosed MTC and 489 gender and ethnicity matched healthy controls. Germline RET mutation analysis was performed on peripheral blood samples. A multivariate logistic regression analysis identified a protective risk association of CDKN1ASer31Arg SNP with both hereditary and sporadic MTC. An increased risk association was also identified for NAT2Y94Y SNP and CDKN2A3'UTR SNP with sporadic MTC, and RET S904S with hereditary MTC. The meta-analysis of RET SNPs identified increased risk association of all four RET SNPs with MTC. The authors concluded a significant protective risk association of CDKN1ASer31Arg SNP with MTC was shown for the first time. Additionally, the meta-analysis identified a significant risk association for all four RET SNPs.

Elisei et al. (2019) evaluated the prevalence of germline RET mutations in a series of individuals diagnosed with MTC over 25 years and to reappraise their clinical significance. The study included 2,031 individuals who presented with sporadic (n = 1264) or hereditary (n = 117) MTC, plus 650 relatives. All individuals underwent RET genetic screening. The

study results revealed a RET germline mutation in 115/117 (98.3%) hereditary cases and in 78/1264 (6.2%) apparently sporadic cases. In total, 42 distinct germline variants were found. The V804M mutation was the most prevalent, especially in cases that presented as sporadic. Mutations affecting cysteine residues were the most frequent in the group of clinically hereditary cases. All M918T mutations were considered de novo and exclusively associated with multiple endocrine neoplasia type 2B (MEN2B). Several variants of unknown significance (VUS) were also found. The authors concluded RET genetic screening was informative in both hereditary and sporadic MTC. The prevalence of different mutations varied, with V804M being the most frequent. Additionally, while RET screening identified some VUS, the pathogenic role of these variants must be demonstrated before screening the family.

Vuong et al. (2018) performed a systematic review and meta-analysis to investigate the clinical and prognostic significance of RET and rat sarcoma (RAS) mutations in individuals with sporadic MTC. The review included 23 studies, including 964 individuals with sporadic MTC. The study results revealed the presence of RET mutation was associated with an elevated risk for lymph node metastasis, distant metastasis, advanced tumor stage, tumor recurrence, and patient mortality. However, the presence of RAS mutation had no significant prognostic value in predicting tumor aggressiveness. The authors concluded that RET mutation is a reliable molecular biomarker to identify a group of individuals with highly aggressive sporadic MTC. It can also aide clinicians to better assess prognosis and select appropriate treatment decisions.

Martins-Costa et al. (2018) conducted a study to determine the need for earlier diagnoses and detection in relatives at risk for MTC. RET screening was performed in 60 individuals referred to the Brazilian Research Consortium for Multiple Endocrine Neoplasia who were diagnosed with MTC and 187 at-risk family members. Prior to RET screening, 54/60 (90%) individuals were diagnosed with apparent sporadic disease and 6/60 (10%) individuals were diagnosed with hereditary disease. After RET screening, 31/60 (52%) individuals were determined to have sporadic disease, and 29/60 (48%) individuals were determined to have hereditary disease. For at-risk relatives, 73/187 individuals were mutation carriers. Mutations in RET codon 804 and the rare p.M918V mutation were the most prevalent. The authors concluded that performing RET screening allowed the identification of a different mutation profile in this region compared with other areas. RET screening also enabled the diagnosis of a significant number of individuals with hereditary MTC who were initially diagnosed as individuals with sporadic disease. Additionally, RET testing benefited relatives, who were unaware of the risks and the consequences of the RET mutation.

Raue et al. (2018) analyzed long-term MEN2B outcomes and defined prognostic factors in a retrospective, comparative study. The study included 75 individuals diagnosed with MEN2B. Individuals diagnosed and treated before and after 2000 were compared for demographic, biochemical, surgical, and outcome parameters. The main outcome measure was long-term survival. The study results identified seven familial and 68 de novo cases of MEN2B. Of those, 61 cases exhibited the RET M918T genotype (2 others exhibited A883F and E768D/L790T mutations). Surgery was performed at a mean age of 16.4 ±11.2 years. The tumor stages at diagnosis for 71 individuals were stage I, 15%; stage II, 6%; stage III, 35%; and stage IV, 44%. The mean follow-up was 9.6 ±9.0 years. The outcomes were 15 (20%) cured, 9 (12%) with minimal residual disease, 19 (25%) with metastatic disease, and 10 (13%) unknown. MTC caused 22 deaths (29%) 7.3 ±6.2 years after diagnosis (mean age, 22.9 ±10.7 years). The overall survival rates at 5, 10, and 20 years were 85%, 74%, and 58%, respectively. After 2000, versus before 2000, significantly more patients had stage I and II (32% versus 11%) and more were cured (43% versus 20%), with a higher survival trend. The authors concluded individuals with MEN2B developed MTC at an early age with wide ranging aggressiveness, but the outcome was generally better after 2000 than before 2000. This was determined to be due to earlier diagnoses with molecular and biochemical screening, greater awareness of the phenotype, and increasing use of comprehensive operative techniques.

Romei et al. (2015) described a 20-year study regarding RET genetic screening for MTC and the clinical impact that this investigation had on diagnosis and treatment. The study included a total of 1,556 individuals. Of the total, 1,007 individuals presented with an apparent sporadic MTC, and 95 individuals presented with a hereditary form of MTC. The remaining 454 individuals were relatives of individuals with MTC who tested positive for RET. All of these individuals underwent RET genetic screening using blood and / or tumor tissue. The study results revealed an unsuspected germline RET mutation in 68 of 1,007 (6.7%) individuals who presented with an apparent sporadic MTC. These individuals were reclassified as having a hereditary form of MTC. Within this group, 61 familial MTC and seven multiple endocrine neoplasia type 2A (MEN2A) syndromes were also identified. The remaining 939 individuals with MTC were found to be negative for germline RET mutations, confirming their sporadic nature. Of the 454 individuals who were relatives of individuals with MTC, who tested positive for RET, 137 individuals were identified as gene carriers. These individuals underwent clinical evaluation for the diagnosis of MTC and other endocrine neoplasia. None of these individuals were affected by MTC, at the time of the genetic screening, or during their follow-up examinations. A total of 139 multiple endocrine neoplasia type 2 (MEN2) families were discovered. In this group, the prevalence of familial MTC was higher (94/139, 67.6%) than the MEN2A (33/139, 23.7%) and MEN2B prevalences (12/139, 8.7%). The authors concluded that RET genetic screening is highly specific and sensitive. It allowed for the reclassification of individuals who initially

presented with apparent sporadic MTC and the identification of gene carriers who require adequate follow-up. Familial MTC was also determined to be the most prevalent MEN2 syndrome and that it is strongly correlated with non-cysteine RET mutations. The authors suggested these findings should be considered in the follow-up approach for individuals with hereditary MTC in the future.

Burnichon et al. (2011) investigated 202 pheochromocytomas / paragangliomas, including 75 hereditary tumors, using expression profiling, bacterial artificial chromosome array comparative genomic hybridization, and somatic mutation screening. Tumor and blood samples were obtained from 190 individuals with pheochromocytoma/paraganglioma, which was histologically confirmed. The study results revealed somatic mutations in von Hippel Lindau (VHL) or RET genes in 14% of sporadic pheochromocytomas / paragangliomas. Additionally, a germline or somatic genetic alteration was found in 45.5% of pheochromocytomas / paragangliomas. The authors concluded these findings suggested germline and somatic VHL and RET mutation analysis is likely to yield important clues for personalizing molecular targeted therapies for individuals with metastatic pheochromocytoma / paraganglioma.

Neumann et al. (2002) used molecular tools to classify a large cohort of individuals with nonsyndromic pheochromocytoma with respect to the presence or absence of gene mutations and to investigate the relevance of genetic analyses to clinical practice. The study included blood samples from 271 unrelated individuals with histologically confirmed pheochromocytoma, without a family history of the disease. Samples underwent molecular genetic analyses for mutations of RET, VHL, succinate dehydrogenase subunit D (SDHD), and succinate dehydrogenase subunit B (SDHB). Samples from 300 anonymous, healthy blood donors matched with the registry patients for race and region were also analyzed as controls. The study results revealed that 66 individuals (24%) were found to have mutations. Of these individuals, 30 had mutations of VHL, 13 of RET, 11 of SDHD, and 12 of SDHB. Younger age, multifocal tumors, and extraadrenal tumors were significantly associated with the presence of a mutation. However, only 21 individuals had multifocal pheochromocytoma. Twenty-three individuals (35%) presented with nonsyndromic pheochromocytoma after the age of 30 years and 17 individuals (8%) presented after the age of 40 years. Sixty-one individuals (92%) were identified solely by molecular testing and had no associated signs and symptoms at presentation. The study authors concluded that almost 25% of individuals with apparently sporadic pheochromocytoma may be carriers of mutations. The routine analysis for RET, VHL, SDHD, and SDHB mutations is indicated to identify pheochromocytoma-associated syndromes that would otherwise be missed.

ClinGen Actionability Assertion for the RET gene and multiple endocrine neoplasia type 2a (MEN2A) has a final assertion of strong actionability as computed based on the Semi-quantitative Metric (SQM) scoring rubric (ClinGen, 2024).

VHL (Von Hippel-Lindau Tumor Suppressor)

Binderup et al. (2022) developed a guideline based on evidence from the international vHL literature and extensive research of genotypic and phenotypic characteristics, disease progression and surveillance in the national Danish vHL cohort. Also included were the consensus among Danish experts which were compared with international recommendations. The objectives were to outline the diagnostic strategy for suspected vHL and surveillance of patients with or predisposed to vHL. The consensus in the literature is that in individuals with a first-degree relative with vHL, a clinical diagnosis can be made when the individual has at least one vHL manifestation, and in a patient without a family history of vHL, the diagnosis of vHL can be confirmed in patients with two different vHL manifestations, of which one is a hemangioblastoma. If a variant in VHL has previously been identified in a relative, predictive testing for this variant is performed. If an appropriate genetic work-up has previously been performed in the family, no clinically actionable variant has been detected in VHL, and the diagnosis of vHL was made using the clinical criteria, the patient is counselled according to his/her relation to the affected relative. In all other cases a genetic work-up is performed. Predictive testing is recommended to all first-degree relatives to a carrier of a VHL variant, regardless of age.

Wolters et al. (2022), as part of a multidisciplinary panel from five Dutch University medical centers, convened to develop a care pathway for patients with VHL syndrome. This was developed using a modified Delphi consensus making process, and panel members included internists, urologists, neurosurgeons, ophthalmologists, geneticists, medical oncologists, neurologists, gastroenterologists, pediatricians, and ear-nose-throat specialists. As part of the care pathway, it was recommended that when VHL is suspected based on clinical presentation, patients should be referred for genetic testing. If the genetic testing confirms a pathogenic variant, family members should be offered DNA testing.

ATP7B

Gao et al. (2018) conducted a systematic review of known disease-causing pathogenic variants in ATP7B and calculated the global genetic prevalence of Wilson disease to be 13.9 per 100,000 or 1 per 7194. The genetic prevalence of Wilson disease is much higher than epidemiological estimates, potentially indicating underdiagnosis. The authors reported 787 unique variants in ATP7B were identified. 569 were classified as disease-causing variants, and 539 of them were

categorized as pathogenic after excluding missense mutations likely to be tolerant by SIFT/Polyphen-2 analysis. There were 155 mutations identified in gnomAD. East Asian ethnicity had the highest estimated prevalence (37.56 per 100,000) compared to only 1.75 per 100,000 (95% CI 1.00–3.00) in the African-American population. The authors concluded their findings have an implication for genetic counselling and clinical suspicion of Wilson disease across ethnicities.

Hunter et al. (2016) developed a practical, standardized, evidence-based protocol explaining ClinGen (sponsored by the NIH), and its collaborative effort of researchers and clinicians to assess the clinical actionability of genetic disorders associated with genomic variation. Clinical actionability is part of the effort to create a central resource of information for the clinical relevance of genomic variation. The authors developed a standardized protocol to identify evidence and generate summary reports of actionability with an applied metric for an actionability score as an effort aimed at improving patient health outcomes. The intent is to identify genetic disorders with greater clinical utility when identified by testing in previously undiagnosed adults. Clinical actionability is a clinically prescribed intervention specific to the genetic disorder under consideration, effective for prevention or delay of clinical disease, lowered clinical burden, or improved clinical outcome in a previously undiagnosed adult. Intervention included patient management and ways to improve outcomes for at-risk family members; however, genetic testing recommendations for at-risk family members alone did not meet the criteria for actionability. ClinGen provides a summary of scoring for severity of disease, likelihood of disease, effectiveness of specific interventions, nature of intervention, and state of the knowledge base (level of evidence). The summary is publicly available to all clinicians and patients and provides a structure to enable research and clinical communities to make clear, streamlined, and consistent determinations of clinical actionability based on transparent criteria to guide analysis and reporting of genomic variation from clinical genome-scale sequencing.

ClinGen Actionability Assertion for the ATP7B gene and Wilson Disease was reported to have a strong actionability as computed based on the Semi-quantitative Metric (SQM) scoring rubric (ClinGen, 2024).

RYR1

Johnston et al. (2021) reported on the ClinGen Expert Panel adopted of the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) pathogenicity criteria for classification of RYR1 variants as related to autosomal dominantly inherited malignant hyperthermia (MH). The panel specified ACMG/AMP criteria for variant classification for RYR1 and MH. Proposed rules were piloted on 84 variants. The panel applied quantitative evidence calibration for several criteria using likelihood ratios based on the Bayesian framework. The authors reported that seven ACMG/AMP criteria were adopted without changes, nine were adopted with RYR1-specific modifications, and ten were dropped. The in silico (PP3 and BP4) and hotspot criteria (PM1) were evaluated quantitatively. The revised ACMG/AMP criteria were applied to 44 recognized MH variants, of which 29 were classified as pathogenic, 13 as likely pathogenic, and 2 as variants of uncertain significance. The authors concluded that curation of these variants will facilitate classification of RYR1/MH genomic testing results, as it is especially important for secondary findings analyses. The panel's approach to quantitatively calibrating criteria is generalizable to other variant curation expert panels.

ClinGen Actionability Assertion for the RYR1 gene and malignant hyperthermia of anesthesia has a final assertion of strong actionability as computed based on the Semi-quantitative Metric (SQM) scoring rubric (ClinGen, 2024).

In a 2018 review, Riazi et al. summarized the evidence on the genetics of MH susceptibility, and its connection to non-anesthesia-related disorders. The evidence shows that the phenotypic variability shown in recent studies of RYR1-related disorders, that abnormalities in this gene may confer not only MH, but may also be linked to individuals with myopathies, metabolic derangements, exertional heat illness, exertional rhabdomyolysis possibly bleeding disorders. Anesthesiologists should insist on a genetic work-up for RYR1 variants in patients with a previous history of recurrent rhabdomyolysis or those with congenital myopathies without a genetic diagnosis before administration of triggering anesthetics.

This clinical evidence review focuses on Tier 2 molecular pathology procedures and whether the current available evidence is sufficient to draw conclusions about improved health outcomes for the Medicare population. Due to low quality evidence, the clinical evidence reviewed is insufficient to conclude that the following individual Tier 2 genetic tests impact therapeutic decision-making or directly impact treatment, outcome, and/or clinical management in the care of the Medicare member:

ACADS (Acyl-CoA Dehydrogenase)

Studies substantiating the value of ACADS are limited to at this time. Further studies with a larger number of patients and longer follow-up are needed to determine if the use of this ACADS gene provides clinical utility to individuals.

Breilyn et al. (2023) conducted a retrospective chart review in an attempt to identify if the biallelic variation in the ACADS gene is an isolated finding in short-chain acyl CoA dehydrogenase deficiency (SCADD), which has been a contested

debate. Clinically ascertained individuals have a range of reported metabolic and physical symptoms. Conversely, individuals identified through newborn screening remain overwhelmingly asymptomatic. Two common ACADS variants, c.511C > T (p.Arg171Trp) and c.625G > A (p.Gly209Ser) are known to reduce enzymatic activity with undetermined clinical correlate. The authors used exome sequence data thru electronic medical records to identify clinically relevant ACADS variants along with ICD codes corresponding to eight SCADD-associated phenotypes in 27,447 ancestrally diverse and unrelated adults. Phenotypes included intellectual disability, behavioral disorders with onset in childhood, epilepsy or seizure disorders, hypoglycemia, muscle weakness, metabolic acidosis, fatty liver, and a diagnosis of SCADD or disorder of fatty acid oxidation. The results were as follows: 1 in 10,000 BioMe participants were homozygous for rare pathogenic variants (PVs) in ACADS, 1 in 20 were homozygous or presumed compound heterozygous for common variants (CVs), and 1 in 300 harbored both a PV and a CV. Of the 2035 variant positive individuals, none had a documented diagnosis of SCADD. They identified five PV/PV positive individuals, none of whom had evidence of symptomatic SCADD on manual chart review. CV/CV positive and CV/PV positive individuals did not have increased odds of any of the eight ACADS phenotypes evaluated compared to variant negative individuals. The frequency of clinically relevant ACADS variants in an unselected population was higher than previously reported SCADD occurrence of 1 in 35,000 in the United States. Study limitations included the following: an electronic record was use which could result in inaccurate or lack of information; use of ICD codes may be inconsistent amongst providers; use of sequence variants rather than copy variants; and a lack of biochemical profiling. This was the first evaluating clinically relevant ACADS variants in a diverse and unselected population which revealed a high frequency of common variants and little evidence of disease among individuals considered to be variant positive. This indicates that SCADD is unlikely to represent a clinically significant disease entity in adulthood, when caused by the presence of one or two common variants. These findings support prior claims that SCADD is a biochemical entity without clinical correlate.

AQP2 (Aquaporin 2 [Collecting Duct])

Studies substantiating the value of AQP2 are limited to at this time, especially for adults in the Medicare population. Further studies with a larger number of patients and longer follow-up are needed to determine if the use of this AQP2 gene provides clinical utility to individuals.

Watanabe et al. (2023) in a case study in 3 pediatric individuals with congenital nephrogenic diabetes insipidus (CNDI). Clinical symptoms of CNDI, such as dehydration and electrolyte disturbances (hypernatremia and hyperchloremia) can lead to a developmental delay without prompt treatment. In approximately 90% of cases, CNDI is an X-linked disease caused by mutations in the arginine vasopressin receptor 2 (AVPR2) gene. In approximately 9% of cases, CNDI is an autosomal recessive disease caused by mutations in the water channel protein aquaporin 2 (AQP2), and 1% of cases are autosomal dominant. The authors report a case of CNDI caused by a novel AVPR2 nonsense mutation, c.520C > T (p.Q174X), and cases of siblings in another family who had a different AVPR2 nonsense mutation, c.852G > A (p.W284X). Both cases responded well to treatment with hydrochlorothiazide and spironolactone. If a diagnosis of CNDI is suspected, especially in carriers and neonates, genetic testing of the AVPR2 gene and prompt treatment may decrease growth disorders and prevent permanent central nervous system disorders and developmental delays. Presently, there is no definitive treatment of CNDI.

ARX (Aristaless Related Homeobox)

Homeobox genes are a large and diverse group of genes, many of them play an important role in embryonic development. Approximately 300 homeobox points have been identified in the human genome (Holland, 2007).

In a 2005 article, Suri presented the different mutations in the ARX gene including phenotypes associated with severe brain malformations and less severe phenotypes associated with syndromic or non-syndromic forms of X-linked mental retardation (XLMR). There seems to be a consistent genotype-phenotype correlation and both interfamilial and intrafamilial variability of expression of some of the mutations, particularly the common 428-451dup(24 bp) mutation. Premature termination mutations in the first four segments of the gene are associated with the most severe phenotypes: X-linked lissencephaly with ambiguous genitalia (XLAG) and hydranencephaly with ambiguous genitalia. Non-conservative missense mutations in the homeodomain result in a milder form of XLAG and conservative missense mutations in this region can cause either Proud syndrome or X-linked myoclonic epilepsy with spasticity and intellectual disability. Familiarity with the phenotypic spectrum of ARX mutations is helpful in determining when to request ARX mutation analysis.

In a 2003 review, Sherr conducted a review of the many disorders caused by mutations in the ARX gene. This provides genetic information and may ultimately offer treatment options for these patients. Research has shown that mutations in ARX cause X-linked West syndrome, X-linked myoclonic epilepsy with spasticity and intellectual disability, Partington syndrome (mental retardation, ataxia, and dystonia), as well as nonsyndromic forms of mental retardation. Patients with these diseases and ARX mutations were not reported to have brain imaging abnormalities. In contrast, mutations in ARX

mutations have also been found in X-linked lissencephaly with abnormal genitalia, which typically includes severe brain malformations (lissencephaly, agenesis of the corpus callosum, and midbrain malformations), intractable seizures, and a severely shortened lifespan. It was concluded that mutations in the homeobox gene, ARX, cause a diverse spectrum of disease that includes cognitive impairment, epilepsy, and in another group of patient's severe cortical malformations. Although the precise prevalence of ARX mutations is unclear, it may be a leading cause of mental retardation and epilepsy in males.

PAX2 (Paired Box 2)

In a 2021 paper, Lv et al. introduced the expression and role of PAX2 in neurodevelopment and discusses the neurodevelopmental disorders associated with Pax2 mutations. Mutations in the human Pax2 gene are associated with abnormalities in multiple systems which can result in neurodevelopmental disorders such as intellectual disability, epilepsy, and autism spectrum disorders. The structure of Pax2 gene and PAX2 protein, as well as the function of Pax2 gene in neural development. Pax2-related diseases are inherited in an autosomal dominant manner and patients with mutations show renal and eye abnormalities, such as renal dysplasia or optic nerve coloboma, as well as neurodevelopmental disorders including autism, intellectual disability, epilepsy, and developmental delays. There are 334 public variants of the PAX2 gene and it is unclear how these individually lead to neurodevelopment disorders. Studies in humans on the PAX2 gene in human embryo development are limited.

Panneerselvam et al. (2019) presented a review on the role of pancreatic specific PAX regulators in the development of the pancreas and its related disorders. Three members of the PAX family; PAX2, PAX4 and PAX6 are critical at multiple steps of pancreatic development and differentiation and also play a pivotal role in the regulation of pancreatic islet hormones synthesis and secretion. The PAX2 gene is required for the development of the central nervous system, eye, ear, kidney, and mammary glands. Research shows that the PAX2 is a critical regulator in determining the endocrine-exocrine function and the loss of which leads to the expansion of endocrine cells during embryonic development, however the role of PAX2 in pancreatic development and function and pathophysiology is still uncertain, and further research is needed.

In a 2017 review, Capone et al. presented the current research on the genetic basis of congenital anomalies of the kidney and urinary tract (CAKUT). The pathogenesis of these anomalies is based on the disturbance of normal nephrogenesis, secondary to environmental and genetic causes. CAKUT may be the first clinical manifestation of a complex systemic disease, and early molecular diagnosis can help in the identification of other subtle clinical manifestations which can significantly affect the management and prognosis. The number of sporadic CAKUT cases explained by highly penetrant mutations in a single gene may have been overestimated over the years and a genetic diagnosis is missed in most cases, illustrating the importance of identifying new genetic approaches which can help identify unexplained CAKUT cases.

HNF1B (HNF1 Homeobox B)

Ferrè and Igashari (2019) presented new insights into the role of the HNF1B Hepatocyte nuclear factor (HNF-1β) in the regulation of the development and function of epithelia in the kidney, liver, pancreas, and genitourinary tract. In the embryonic kidney, HNF-1β is required for ureteric bud branching, initiation of nephrogenesis, and nephron segmentation, and people who carry HNF1B mutations develop heterogeneous renal abnormalities, including multicystic dysplastic kidneys, glomerulocystic kidney disease, renal agenesis, renal hypoplasia, and renal interstitial fibrosis. In the adult kidney, HNF-1β controls the expression of the genes required for metabolism within the kidney and solute transport by tubular epithelial cells. Tubular abnormalities observed in HNF-1β nephropathy include hyperuricemia with or without gout, hypokalemia, hypomagnesemia, and polyuria. Recent studies have identified novel post-transcriptional and post-translational regulatory mechanisms that control HNF-1β expression and activity. Further understanding of the molecular mechanisms of HNF-1β may lead to the development of new therapeutic approaches in cystic kidney disease and other HNF1B-related renal diseases.

In a 2016 review article, Bockenhauer and Jaureguiberry presented the clinical phenotypes associated with HNF1B mutations outside of renal malformations, and the potential management of these conditions. Since it was first identified as a gene for diabetes in 1997 multiple associated phenotypes have been reported, including genital malformations, autism, epilepsy, gout, hypomagnesaemia, primary hyperparathyroidism, liver and intestinal abnormalities and a rare form of kidney cancer. No published guidelines exist on screening for potential associated extrarenal abnormalities in HNF1B mutation carriers, and the author presented the following statements based on current evidence systematic screening for all potential abnormalities should likely be restricted to research cohorts to better determine the frequency and characteristics of these complications. Routinely, clinicians should be aware of these potential implications and assess patients with suspicious symptoms on an individual basis.

In a 2016 review article, El-Khairy and Vallier discussed the role of HNF1B in human pancreas and liver development, summarizes the disease phenotypes and identifies areas for future investigations in HNF1B-associated diabetes and liver disease. HNF1B-associated disease is a multi-system disorder with an extremely variable and expanding clinical phenotype. The pathophysiology of diabetes in HNF1B mutation carriers includes ß-cell dysfunction and hepatic insulin resistance. However, further studies are required to precisely define the gene regulatory networks and signaling pathways controlled by HNF1B and the role of HNF1B at different stages of pancreas and liver development, and in adult tissues in humans.

BTD (Biotinidase)

Biotinidase Deficiency (BD) screening has been incorporated into all state newborn screening tests for decades, and as such most individuals with (BD) are diagnosed as newborns. Untreated adolescents and adults usually exhibit myelopathy and optic neuropathy and are often initially diagnosed with multiple sclerosis. The onset is usually in the 20s and 30s (Wolf 2000). Research in adult-onset BD consists of a very few case reports (Deschamps et al., 2018; Wolf et al.,1997; Desai et al., 2008).

CAV3 (Caveolin 3) (e.g., CAV3-Related Distal Myopathy, Limb-Girdle Muscular Dystrophy Type 1C), Full Gene Sequence

In 2023, Berling et al. retrospectively reassessed the clinical and paraclinical data of 23 patients with CAV3 symptomatic mutations, from 16 different families with caveolinopathy. Clinical and functional data were collected during the mean follow-up of 24 years. The results of muscle imaging, electroneuromyography, muscle histopathology, immunohistochemistry, and caveolin-3 Western blot analysis were compiled. The results showed that the main symptom was myalgia, which often presented as exercise intolerance. Rippling muscle, a signature feature of the disease, was found in two thirds of the patients, a finding similar to other cohorts. Calf hypertrophy was found in 80% of patients. Combined with exercise intolerance and elevated CK level, this may point toward CAV3 mutation, especially in patients with an absence of weakness. Nine patients had lower limb muscle weakness, and only two patients of the 23 required a walking aid, which indicates caveolinopathy is a benign myopathy. There was no respiratory or cardiac involvement in this cohort. Creatinine kinase (CK) levels were consistently elevated with half of the patients having CK levels more than three times the upper limit of normal. It was concluded that caveolinopathy is overall a benign or mild myopathy.

Gazzero et al. (2010) presented a review on the functions of CAV3 in muscle cells and the muscle and heart disease phenotypes associated with mutations. Caveolin-3 deficiency leads to four skeletal muscle phenotypes: Limb Girdle Muscular Dystrophy (LGMD) 1C, Isolated HyperCKemia, Rippling Muscle Disease, Distal Myopathy. CAV3 mutations were reported also in one case of Familial Hypertrophic Cardiomyopathy, 4 patients affected by Long QT Syndrome (LTQS) and 3 infants died from Sudden Infant Death Syndrome. Many patients show an overlap in symptoms of the affected skeletal muscles and studies have shown that the same mutation can lead to varied disease presentation. CAV3 mutations are associated with distal myopathy, and the majority of CAV3 mutations do not cause cardiac phenotypes. The two single reports available in the literature suggests different roles in the skeletal and cardiac muscle tissues. The role of CAV3 regulation on cardiac ion channels and LTQS requires addition research.

Vatta et al. (2006) reported the results of a study of a CAV3 mutations in 905 unrelated patients referred for LQTS genetic testing. CAV3 mutations were engineered by site-directed mutagenesis and the molecular phenotype determined by transient heterologous expression into cell lines that stably express the cardiac sodium channel hNa(v)1.5. 4 novel mutations in CAV3-encoded caveolin-3 that were absent in > 1000 control alleles were identified. Electrophysiological analysis of sodium current in HEK293 cells stably expressing hNa(v)1.5 and transiently transfected with wild-type and mutant caveolin-3 demonstrated that mutant caveolin-3 results in a 2- to 3-fold increase in late sodium current compared with wild-type caveolin-3. This study reports the first CAV3 mutations in subjects with LQTS and provides functional data demonstrating disruption in ion channel function, which may be associated with a genetic susceptibility for LQTS.

CLRN1 (Clarin 1)

Nisenbaum et al. (2022) conducted a review of the clinical evidence regarding the genes and mutations associated with Usher Syndrome (USH) with a focus on genotype-phenotype relationships. The review notes CLRN1 has been associated with Usher Syndrome type III and non-syndromic RP. The author noted the current treatment of USH is entirely symptom directed and a better understanding of the genotype-phenotype relationships is needed to predict USH gene mutation outcomes and develop future treatments.

Jouret et al. (2019) conducted a systematic study and meta-analysis of Usher syndrome (US) genetics (a rare genetic disorder), to define the genetic and phenotypic range of US after six years of studies by next-generation sequencing (NGS). The study was threefold: a meta-analysis of 11 NGS studies in 684 Usher patients to establish an Usher gene classification; a meta-analysis of 21 NGS studies in 2,476 individuals with seemingly isolated deafness to evaluate the

Usher genotype in non-syndromic hearing loss and proportion of individuals at high risk of retinitis pigmentosa (RP); and a statistical analysis of differences between parts one and two. Only cohort studies published between January 2000 and August 2017 were considered for inclusion in the review. In patients with both visual and hearing impairments, the biallelic disease-causing mutation rate was assessed for each Usher gene to propose a classification by frequency: USH2A: 50% (341/684) of patients, MYO7A: 21% (144/684), CDH23: 6% (39/684), ADGRV1: 5% (35/684), PCDH15: 3% (21/684), USH1C: 2% (17/684), CLRN1: 2% (14/684), USH1G: 1% (9/684), WHRN: 0.4% (3/684), PDZD7 0.1% (1/684), CIB2 (0/684). In patients with seemingly isolated sensorineural deafness, 7.5% had disease causing mutations in Usher genes, and are therefore at high risk of developing RP. These new findings provide evidence that usherome dysfunction is the second cause of genetic sensorineural hearing loss after connexin dysfunction. The authors concluded the study supports early molecular screening for US in deaf children and recommend future studies of genotype-phenotype correlations with prospective longitudinal clinical screening and follow-up of individuals with mutations.

CYP1B1 (Cytochrome P450, Family 1, Subfamily B, Polypeptide 1)

In 2022, Selvan et al. conducted a literature review on the multiple genetic variants that impact juvenile onset open-angle glaucoma (JOAG). The CYP1B1 gene has been implicated in primary congenital glaucoma (PCG), JOAG and adult onset primary open angle glaucoma (POAG), with the prevalence in JOAG varying widely across the world. The role of this mutation is not completely understood.

EGR2 (Early Growth Response 2) (e.g., Charcot-Marie-Tooth)

In a 2023 observational retrospective study, Echaniz-Laguna et al. presented the clinical, molecular, and electrophysiological characteristics in 14 patients with heterozygous EGR2 mutations. The mean age was 44 years, 10 patients were female and mean disease duration was 28 years. Disease onset was before age 15 years in nine patients, after age 35 years in four patients and one patient (aged 26 years) was asymptomatic. All symptomatic patients had pes cavus and distal lower limbs weakness, with distal lower limbs sensory symptoms present in 86%. Hand atrophy was exhibited in 71%, and scoliosis in 21%. Nerve conduction studies showed a predominantly demyelinating sensorimotor neuropathy in all cases, and five patients needed walking assistance after a mean disease duration of 50 years. Three patients were misdiagnosed as chronic inflammatory demyelinating polyneuropathy (CIDP) and treated with immunosuppressive drugs for years before the correct diagnosis was made. Two patients presented with additional neurologic disorders, namely Steinert's myotonic dystrophy and spinocerebellar ataxia. Eight EGR2 gene mutations were found, including four previously undescribed. The authors concluded that this study shows EGR2-gene related inherited neuropathies are rare demyelinating neuropathies that present with two main clinical pictures, an early-onset subtype and an adult-onset subtype which may be misdiagnosed as chronic inflammatory CIDP. Outcome is mild in a majority of cases, with only five patients needing walking assistance after a mean disease duration of 50 years.

Baets et al. (2011) conducted a study of 77 unrelated patients that presented with symptoms of motor and sensory neuropathy within the first year of life, to estimate mutation frequencies and to gain detailed insights into the genetic and phenotypic heterogeneity of early onset hereditary neuropathies. Systematic mutation screening by means of direct sequencing of the coding regions of 11 genes: MFN2, PMP22, MPZ, EGR2, GDAP1, NEFL, FGD4, MTMR2, PRX, SBF2 and SH3TC2. In addition, screening for the Charcot–Marie–Tooth type 1A duplication on chromosome 17p11.2-12 was performed. The results showed mutations in MPZ, PMP22 and EGR2 in 35 patients and were found most frequently in patients presenting with early hypotonia and breathing difficulties. Several patients that displayed congenital foot deformities, but otherwise normal early development carried the Charcot–Marie–Tooth type 1A duplication. The authors concluded that genetic testing for this cohort shows heterogeneity in genetic mutations and making a correct diagnosis is challenging. Parallel sequencing may be a more efficient way to make a definitive diagnosis.

FKRP (Fukutin Related Protein)

Studies substantiating the value single-gene testing of the FKRP gene is limited at this time. Further studies with a larger number of patients and longer follow-up are needed to determine if the use of this FKRP gene provides clinical utility to individuals.

Vajsar et al. (2006) conducted a review of Walker-Warburg Syndrome (WWS). This is a rare form of autosomal recessive congenital muscular dystrophy associated with brain and eye abnormalities. WWS has a worldwide distribution. The overall incidence is unknown but a survey in North-eastern Italy has reported an incidence rate of 1.2 per 100,000 live births. Most children die before the age of three years when diagnosed with this rare disease. WWS presents at birth with generalized hypotonia, muscle weakness, developmental delay with mental retardation and occasional seizures. It is associated with type II cobblestone lissencephaly, hydrocephalus, cerebellar malformations, eye abnormalities and congenital muscular dystrophy characterized by hypoglycosylation of alpha-dystroglycan. Several genes have been implicated in the etiology of WWS, and others are as yet unknown. Several mutations were found in the Protein O-Mannosyltransferase 1 and 2 (POMT1 and POMT2) genes, and one mutation was found in each of the fukutin and FKRP

genes. There is usually elevated creatine kinase, myopathic/dystrophic muscle pathology and altered alpha-dystroglycan. Prenatal diagnosis is possible in families with known mutations. Prenatal ultrasound may be helpful for diagnosis in families where the molecular defect is unknown. No specific treatment is available. Management is only supportive and preventive. At the time of the review, there was no known candidate genes for 80-90% of children with WWS. The exact pathophysiology of this disorder is not fully understood. The P0MT1 gene involved in WWS indicates a defect in O-mannosylation of α-dystroglycan. As new genetic defects associated with WWS are reported, a better genotype-phenotype correlation should be established.

FOXG1 (Forkhead Box G1)

No relevant evidence found for adults in the Medicare population. This is a rare mutation, and babies are often diagnosed based on clinical presentation.

Pejhan et al. (2021) reported on Rett Syndrome (RTT), which is a severe, rare, and progressive developmental disorder with patients displaying neurological regression and autism spectrum features, affecting individuals who are primarily young females. While the majority of RTT patients have MECP2 mutations (classical RTT), a small fraction of the patients (atypical RTT) may carry genetic mutations in other genes such as the cyclin-dependent kinase-like 5 (CDKL5) and FOXG1.

FSHMD1A (Facioscapulohumeral Muscular Dystrophy 1A)

Studies substantiating the value of FSHMD1A are limited to at this time. Further studies with a larger number of patients and longer follow-up are needed to determine if the use of this gene provides clinical utility to individuals in the Medicare population.

In 2020, Greco et al. conducted a review on the consequences of epigenetic derepression in facioscapulohumeral muscular dystrophy (FSHD), which is a hereditary myopathy caused by either by contraction of the D4Z4 macrosatellite repeat at the distal end of chromosome 4q to a size of 1 to 10 repeat units (FSHD1) or by mutations in D4Z4 chromatin modifiers such as Structural Maintenance of Chromosomes Hinge Domain Containing 1 (FSHD2). Both genotypes (FSHD1 and FSHD2) typically present with progressive and asymmetric muscle weakness and atrophy and epigenetic alterations of the D4Z4 repeat. The epigenetic changes join both genetic forms into one disease and explain the derepression of the DUX4 gene, which is silent in skeletal muscle is consistently transcriptionally upregulated in FSHD1 and FSHD2 skeletal muscle cells. Generally, the disease manifests in the second decade of life. Cardiac and respiratory muscles involvement are rare, and general life expectancy is not reduced for FSHD patients. The authors reported that FSHD is a complex disorder and expect trials with new and existing drugs that target DUX4 and its regulation or damaging effects in skeletal muscle.

HRAS (V-Ha-Ras Harvey Rat Sarcoma Viral Oncogene Homolog)

In a 2010 study, Gripp et al. analyzed the MRI imaging of 28 patients with a documented HRAS mutation enrolled in an ongoing hospital study of Costello syndrome (CS), which is a type of rasopathy caused by germline mutations in the proto-oncogene HRAS. The results showed in 27 patients, there was a consistent assemblage of abnormalities that included accelerated postnatal brain growth in relation to body size, prominent or bossed forehead, ventriculomegaly, and posterior fossa crowding with cerebellar tonsillar herniation. Twenty two of this patient cohort share the same mutation resulting in a heterozygous p.G12S amino acid change. Three individuals had a p.G12A change, and three other mutations were seen in one person each. This distribution of mutations reflects the mutation spectrum and frequency reported in Costello syndrome individuals. Limitations of the study include a small sample size.

Kerr et al. (2006) presented the results of an analysis of HRAS genetic tests in 43 individuals with a clinical diagnosis of Costello syndrome. The results showed mutations were found in 37 of patients. Parental DNA samples were analyzed in16 cases for both parents and in three cases for one parent, and confirmed mutations were identified for all of the cases. Three novel mutations (G12C, G12E, and K117R) were found in five cases. The authors concluded that these findings confirm that in most cases, CS is caused by heterozygous missense mutations in the proto-oncogene HRAS. Analysis of the major phenotypic features by mutation suggests a potential correlation between malignancy risk and genotype, which is highest for patients with an uncommon (G12A) substitution and that mutation testing for HRAS is a reliable diagnostic test for CS. The authors considered the data as preliminary requiring testing on a larger sample size, and the need to analyze more cases is required due to the broad genotype-phenotype correlation.

In 2013, the National Cancer Institute (NCI) at the National Institutes of Health (NIH) established the RAS Initiative to develop ways to understand and target cancers driven by RAS oncogene mutations, innovative approaches for attacking the proteins encoded by mutant forms of RAS genes and to ultimately create effective, new therapies for RAS-related cancers. More than 30% of all human cancers (including nearly all pancreatic cancers and 45 percent of colorectal

cancers) are caused by mutations in the RAS family of genes. Furthermore, these cancers are known to be resistant to chemotherapies and due to the structure of mutant RAS proteins, effective therapies are difficult to target.

KCNJ10 (Potassium Inwardly Rectifying Channel, Subfamily J, Member 10)

Jaing et al. (2021) conducted a systematic review and meta-analysis to investigate the genetic association between 3 polymorphisms of the KCNJ10 gene and the susceptibility for epilepsy. Eight articles were included and showed inconclusive results with regard to the association between the KCNJ10 polymorphisms. Further research is required to draw definitive conclusions on mutations in this gene and epilepsy.

In a 2015 study, Dai et al. investigated the associations between idiopathic generalized epilepsy syndromes and the subtypes of childhood and juvenile absence epilepsy, juvenile myoclonic epilepsy, and epilepsy with generalized tonic-clonic attacks and KCNJ10 gene polymorphisms specifically rs2486253 and rs61822012. Two hundred participants with epilepsy and 200 healthy controls were enrolled. The results showed that rs2486253, but not rs61822012, polymorphism of the KCNJ10 gene was associated with childhood idiopathic generalized epilepsy and that G/T genotype and T allele frequencies were high in the generalized tonic-clonic subgroup. These results support the role of the KCNJ10 gene as a likely candidate gene for seizure susceptibility.

SLC25A4 [Solute Carrier Family 25 (Mitochondrial Carrier; Adenine Nucleotide Translocation)]

Yang et al. (2024) identified the SLC25A4 gene as one of several potential markers associated with cancer and membranous nephropathy; the gene may represent a possible therapeutic target for membranous nephropathy as well as several types of cancers. Other research of the SLC25A gene includes a few case reports related to several different medical conditions such as: muscle weakness, myopathy, cardiomyopathy, Kearns-Sayre syndrome, chronic progressive external ophthalmoplegia (CPEO), and mitochondrial disease (King, 2018; Thompson, 2016; von Renesse, 2019; Zhao, 2022). Based on this limited evidence, analysis of SLC25A4 for mutations is not supported.

VWF (Von Willebrand Factor)

VWF (von Willebrand factor) gene testing is unlikely to directly impact treatment or clinical management in the care of the patient. While a VWF genetic test can be helpful in diagnosing von Willebrand disease, patient clinical presentation and other standard coagulation tests are sufficient to make a diagnosis and determine necessary treatment, making the additional information from a Tier 2 VWF test less impactful on the clinical management in the care of the patient.

James et al. (2021) reported the evidence-based guidelines on the diagnosis of von Willebrand disease developed by the American Society of Hematology (ASH), the International Society on Thrombosis and Haemostasis (ISTH), the National Hemophilia Foundation (NHF), and the World Federation of Hemophilia (WFH). The panel concluded that there is low-certainty evidence from diagnostic accuracy studies to suggest using either VWF:FVIIIB or genetic testing for patients with suspected type 2N VWD. Though, the panel agreed that the tests can be complementary in the diagnostic workup of patients. Identifying a reference standard for type 2N VWD was identified as a research priority.

CASR (CAR, EIG8, Extracellular Calcium-Sensing Receptor, FHH, FIH, GPRC2A, HHC, HHC1, NSHPT, PCAR1)

In a 2017 review article, Vahe et al. presented the diseases associated with mutations of the CASR gene. The diseases caused by an abnormality of the CASR are genetically determined consist of hypercalcemia or hypocalcemia disorders. Hypercalcemia disorders are related to inactivating mutations of the CASR gene either heterozygous (autosomal dominant familial benign hypercalcemia) or homozygous (severe neonatal hyperparathyroidism). Variants of the CASR gene are associated with higher serum calcium levels than in the general population. Hypocalcemia disorders, which are more rare, are related to heterozygous activating mutations of the CASR gene (type 1), consisting of autosomal dominant hypocalcemia disorders, sometimes with a presentation of pseudo-Bartter's syndrome. The acquired diseases are related to the presence of anti-CaSR antibodies, which can cause hyper- or especially hypocalcemia disorders. The role of CaSR in digestive, respiratory, cardiovascular, and neoplastic diseases is slowly progressing. Two types of CASR modulators are known: CASR agonists and calcilytic antagonists. CASR agonists, such as cinacalcet, are shown in secondary and primary hyperparathyroidism. Calcilytics have no efficacy in osteoporosis, but could be useful in the treatment of hypercalciuric hypocalcemia syndromes.

In a 2016 systematic review and meta-analysis, Wang et al. evaluated the correlations between polymorphisms of calcium-sensing receptor (CASR) gene and the risk of primary hyperparathyroidism (PHPT). Six studies were included and were comprised of 693 PHPT patients and 1252 healthy controls. The results showed that single nucleotide

polymorphisms (SNPs) of CASR gene A986S and R990G, but not Q1011E may increase the risk of PHPT. The authors concluded that these polymorphisms can potentially be used as important biological markers for early diagnosis of PHPT.

In 2014 systematic review, Besiroglu et al. examined the relationship between mutations in the CASR and kidney stones. The findings of individual studies indicate that CaSR gene polymorphisms are implicated in the development of nephrolithiasis through different mechanisms. However, representing a cause and effect relationship for a suspected gene is not the only factor for development of kidney stones, as nutrition and environment are also contributing factors. Well-designed studies in which nutritional and lifestyle factors are described are needed.

CDKL5 (Cyclin-Dependent Kinase-Like 5)

In a 2022 review on CDKL5 deficiency disorder, Leonard et al. states that genetic testing in epilepsy is now largely done through epilepsy gene panels and exome sequencing, in which CDKL5 is one of the high-yield genes. The genetic differential diagnosis of early-onset epileptic encephalopathy is broad but early features suggestive of CDD include seizures with multiple motor phases, prominent hypotonia, cerebral visual impairment, and progressively worse encephalopathy on EEG. In childhood and beyond, symptoms of CDD might overlap with those of Rett syndrome or one of the related developmental epileptic encephalopathies. Research is ongoing on the pathophysiology of CDD, including the role of CDKL5 in microtubular dynamics. However, due to the rarity of the condition and genetic heterogeneity, progress is limited.

This disorder typically manifest in infancy, and the genes associated with it would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

CYP21A2 (Cytochrome P450, Family 21, Subfamily A, Polypeptide2)

21-hydroxylase deficiency (21-OHD) is the most common cause of congenital adrenal hyperplasia (CAH), an autosomal recessive disorder, and its diagnosis of classic 21-OHD CAH is established in newborns (Nimkarn et al., 2016). This disorder would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

MPZ (Myelin Protein Zero)

Pisciotta et al. (2023a) performed a study on data collected from the Italian CMT Registry to provide an overview of CMT epidemiology across Italy and to report clinical characteristics and different CMT subtypes. A pathogenic mutation was identified in 711 out of 805 individuals with the gene being the third most frequently reported (82 patients -10.2%). Pisciotta et al. (2023b) notes CMT disease has no effective drug treatment and management currently entails rehabilitation therapy, surgery for skeletal deformities, and symptomatic treatment. A systematic review summarizing the available data on CMT in Africa found CMT-associated variants were reported in 11 genes: LMNA, GDAP1, GJB1, MPZ, MTMR13, MTMR2, PRX, FGD4/ FRABIN, PMP22, SH3TC2, and GARS; but globally PMP22, GJB1, MFN2, MPZ genes explain at least 90% of CMT cases (Yalcouyé, 2021). Other research of the MPZ gene includes several case reports related to medical conditions such as: inherited neuropathies and CMT (Fernandez-Garcia, 2021; Hao, 2022). Based on this limited evidence, analysis of MPZ for mutations is not supported as clinically useful.

Mascaró et al. (2024) developed a consensus guideline through a multidisciplinary panel to provide recommendations for the diagnosis, prognosis, follow-up, and treatment of CMT in Spain. The guideline states the diagnosis of CMT is clinical, with patients usually presenting with a common or classical phenotype, an appropriate neurophysiological study should follow the clinical assessment, then a genetic diagnosis should be performed sequentially. The authors recommend the following order: duplication of the PMP22 gene in patients with demyelinating or undetermined forms, once ruled out; Next-generation sequencing study including genes associated with CMT and related diseases (GJB1, MPZ, PMP22, SH3TC2, MFN2, GDAP1, MME, and HSPB1); consider complete sequencing of GJB1, SORD, MTATP6, etc., as well as copy number variants (CNV) analysis in other genes.

NF2 (Neurofibromin 2 [Merlin])

Lassaletta et al. (2024) developed a guideline for the management of vestibular schwannoma (VS) through a systematic literature review. The guideline states an absence of sufficient prospective studies accounts for the level of evidence being generally medium or low. The levels of evidence are graded from 1 to 5, with 1 being the highest level of evidence obtained from meta-analyses of randomized clinical trials or at least one high quality randomized clinical trial, and 5 being evidence obtained from expert opinion or expert committee reports. The guideline notes (not all inclusive):

- Inactivation of both alleles of the NF2 tumor suppressor gene plays a significant role in the development of sporadic VS associated with neurofibromatosis type 2.
- Most VS are associated with a dysfunction of the merlin protein, encoded by the NF2 tumor suppressor gene located on the long arm of chromosome 22; and both sporadic VS and neurofibromatosis type 2-associated VS are caused by

the inactivation of both alleles of the NF2 gene. In a significant percentage of VS, in addition to NF2-specific alterations, abnormalities in other molecular pathways have been discovered, especially in the case of cystic or radioresistant tumors. The use of drug combinations targeting these molecular alterations could potentially offer future personalized therapeutic strategies.
- The gold standard test for the diagnosis of a VS is Magnetic Resonance Imaging (MRI) with gadolinium focusing on the internal auditory canal; and pontocerebellar angle. It does not require histological confirmation because its sensitivity and specificity are close to 100%.

Hiltbrunner et al. (2022) conducted a study to determine the prevalence of targetable alterations in pleural and peritoneal mesothelioma in a large cohort of cases by reporting the mutational profile of 1468 mesothelioma patients. The study found alterations in NF2 occurred with a prevalence of at least 10%.

Foss-Skiftesvik (2022) performed a population-based study to evaluate genetic predisposition in children (n = 43) with ependymoma due to rare pathogenic germline variants both in and outside known cancer genes. Additionally, the practicality of performing germline whole genome sequencing (WGS) and tumor DNA methylation profiling in a combined, retro/prospective cohort was assessed. Children in Denmark, less than 18 years old and diagnosed with ependymoma from 2000 to 2016 were identified and included in the retrospective cohort. In the prospective cohort, children less than 18 years old, diagnosed with cancer in Denmark were offered germline WGS through the STAGING study from 2016 to 2021. Thirty-seven children with normal tissue for sequencing were assessed with single nucleotide and structural germline variants in 457 cancer related genes and 2986 highly evolutionarily constrained genes. DNA methylation profiling for 39 children was used to perform molecular ependymoma classification. In known cancer predisposition genes, pathogenic germline variants were detected in 11% (4/37; NF2, LZTR1, NF1 & TP53). However, DNA methylation profiling resulted in revision of the histopathological ependymoma diagnosis to non-ependymoma tumor types in 8% (3/39). This included the two children with pathogenic germline variants in TP53 and NF1 whose tumors were reclassified to a diffuse midline glioma and a rosette-forming glioneuronal tumor, respectively. Therefore, 50% (2/4) of children with pathogenic germline variants had other tumor types. A meta-analysis that combined this study with pediatric pan-cancer germline sequencing studies showed an overall frequency of pathogenic germline variants of 3.4% (7/207) in children with ependymoma. The authors concluded that genetic predisposition plays a role in less than 4% of childhood ependymoma, essentially confined to pathogenic variants in NF2 and NF1. The authors recommend diagnostic reconsideration in children with non-molecularly classified ependymoma with cancer predisposition syndromes other than neurofibromatosis type 2. Limitations include small sample size, and the tumor and germline tissue were unavailable for four and six patients, respectively.

TSC1 (Tuberous Sclerosis 1) And TSC2 (Tuberous Sclerosis 2)

Variants in the TSC1 and TSC2 genes can cause tuberous sclerosis complex, which is considered a rare genetic disorder of childhood onset (Northrup et al, 2021). The genes associated with it would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

ACADVL (Acyl-Coa Dehydrogenase, Very Long Chain)

Bleeker et al. (2019) notes that most infants with very-long-chain acyl-CoA dehydrogenase deficiency (VLCADD) identified by newborn screening (NBS) are asymptomatic at the time of diagnosis and remain asymptomatic. Therefore, a 10-year longitudinal national cohort study of genetically confirmed VLCADD patients born before and after the start of NBS in the Netherlands to evaluate the effect of NBS on the genetic and biochemical characteristics and clinical outcomes was conducted. Clinical outcome parameters, acyl-CoA dehydrogenase very long chain gene analysis, VLCAD activity, and overall capacity of long-chain fatty acid oxidation (LCFAO flux) in lymphocytes and cultured skin fibroblasts were the primary outcomes. Median VLCAD activity in lymphocytes of 54 patients, 21 diagnosed pre-NBS and 33 by NBS was, respectively, 5.4% and 12.6% of the reference mean. The median LCFAO flux was 33.2% and 41% of the control mean, respectively. Clinical characteristics in 23 pre-NBS and 37 NBS patients revealed hypoglycemic events in 12 versus two patients, cardiomyopathy in five versus four patients and myopathy in 14 versus three patients. All patients with long-chain fatty acid oxidation (LC-FAO flux) < 10% developed symptoms. Of the patients with LC-FAO flux > 10% seven out of 12 diagnosed pre-NBS versus none by NBS experienced hypoglycemic events of LC-FAO flux in lymphocytes and cultured skin fibroblasts. The authors concluded there was a beneficial effect on prevention of hypoglycemic events in those with some residual enzyme activity. However, in those with very low residual enzyme activity, NBS did not prevent cardiac complications or hypoglycemia and the effect of NBS remains unclear on prevalence and prevention of myopathy-related complications. The authors recommend future long-term studies to evaluate the benefits of NBS on VLCADD.

This deficiency typically manifest in childhood. The associated ACADVL gene would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

AIRE

De Martino et al. (2016) conducted a review to summarize recent evidence on the AIRE gene and its functioning in the development of APECED and the clinical implications. Evidence shows that mutations of AIRE can lead to various degrees of autoimmunity, ranging from classic APECED to specific autoimmune conditions, which were not previously known to be related. Partial alterations of AIRE could play a role in common autoimmune diseases; however Aire needs to be sequenced in large cohorts of healthy individuals and autoimmune patients to characterize in-depth all mutant alleles. Furthermore, in the last decade, knowledge of AIRE's function and regulation has been significantly expanded leading to the identification of several partners and regulators of AIRE. These molecular insights open new perspectives in understanding the phenotypic variability related to AIRE mutations and may provide new targets for novel therapeutic approaches.

In a 2016 prospective observational natural history study, 35 American patients from nonconsanguineous families with a clinical and/or genetic diagnosis of APECED were enrolled and the genetic, clinical, autoantibody, and immunological characteristics were examined. The results showed there were previously unknown early manifestations of the syndrome, including organ specific manifestations. The authors found that patients were likely to have been seen by dermatologists, gastroenterologists, dentists, ophthalmologists, pulmonologists, and hepatologists early in the course of their disease, often before they developed the widely recognized endocrine and fungal manifestations. In this patient cohort, incorporating urticarial eruption, intestinal dysfunction, and enamel hypoplasia into the diagnostic criteria of APECED would result in earlier recognition and diagnosis. Earlier recognition and diagnosis could have major implications for the prevention of a life-threatening adrenal crisis and hypocalcemic seizures, which are the chief presentations of undiagnosed patients. Furthermore, early diagnosis would provide for timely recognition and development of treatments for life-threatening autoimmune complications, including hepatitis and pneumonitis. Early diagnosis would allow the consideration of preemptive immunomodulation before multiorgan autoimmunity develops, and avert autoimmune complications, including those that have long-term adverse effects on bone metabolism, renal function, and fertility. These findings redefine the clinical features and diagnostic criteria of APECED, promoting its broader recognition and earlier diagnosis. More research is required to gain more insight into the organ-specific flaws in AIRE-dependent immune tolerance; to develop novel screening, diagnostic, and prognostic tools; and to implement targeted preventive and therapeutic strategies.

CBS (Cystathionine-Beta-Synthase)

In 2017, Morris et al. reported on a guideline developed by a group consisting of pediatricians, adult physicians, dietitians, biochemists, a clinical geneticist and a statistician convened as part of the European Network and Registry for Homocystinurias and Methylation Defects (E HOD) to conduct a systematic review of the literature to make recommendations on the diagnosis and management of cystathionine beta-synthase deficiency. With regard to diagnosis, it was recommended that plasma total homocysteine (tHcy) should be the frontline test for diagnosis of CBS deficiency. Deficiency should then be confirmed by measurement of cystathionine synthase activity in fibroblasts or plasma and/or by mutation analysis of the CBS gene. Neither technique can be relied on to demonstrate abnormalities in all cases The gold standard for confirming CBS deficiency is generally considered to be the determination of cystathionine production from Hcy and serine in cultured fibroblasts using radioactive or deuterium labelled substrate. Furthermore, molecular genetic analysis of the CBS gene is helpful carrier and prenatal testing.

Homocystinuria typically manifest in childhood. The associated CBC gene would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

DLAT (Dihydrolipoamide S-Acetyltransferase)

DLAT is currently not supported by the peer-reviewed, published evidence. Additional studies are necessary to investigate variables which may impact results and provide support for clinical utility.

The DLAT gene provides instructions for making the E2 enzyme (also known as dihydrolipoamide acetyltransferase), which is part of a large group of proteins called the pyruvate dehydrogenase complex (PDHC). This complex comprises multiple copies of three enzymes, including E2, and several related proteins. The E2 enzyme is the core to which the other proteins attach to form the complex. Mutations in the DLAT gene lead to an abnormal E2 enzyme and reduced activity of the pyruvate dehydrogenase complex, although the mechanism is unclear. With decreased activity of this complex, pyruvate builds up and is converted, in another chemical reaction, to lactic acid, causing lactic acidosis. In addition, the production of cellular energy is diminished. The brain, which is especially dependent on this form of energy, is severely affected, resulting in the neurological problems associated with pyruvate dehydrogenase deficiency. (MedlinePlus 2020) It is also thought that Leigh syndrome is associated with the DLAT gene. DLAT is on of many genes associated with PDHC. While 1-4% of cases are related to the DLAT gene, the other genes include DLD, PDHB, PDHX, PDP1, PDK1 and the most common associated gene is PDHA1. Molecular genetic testing approaches can include a

combination of gene-targeted testing (multigene panel or serial single-gene testing) and comprehensive genomic testing (exome sequencing or genome sequencing). Gene-targeted testing requires the clinician to hypothesize which genes are likely involved, whereas genomic testing does not (Ganetzky et al., 2021). PDHA1 is an X-chromosome gene, and PDHA1 mutation is the main reason for Pyruvate dehydrogenase complex deficiency (PDCD), and about 25% of PDCD can cause LS (DeBrosse et al., 2012; Patel et al., 2012). Although there is currently no effective treatment, the continuation of relevant research and the emergence of new technologies, genetic diagnostic tools, and gene therapy approaches may also provide a cure for pyruvate dehydrogenase deficiency and LS in the future.

DLD (Dihydrolipoamide Dehydrogenase)

The diseases and deficiencies linked to the DLD gene typically manifest in infancy (Quinonez et al., 2021). The associated DLD gene would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

F8 (Coagulation Factor VIII)

Tier 2 molecular pathology procedures represent procedures that are generally performed in lower volumes than Tier 1 molecular pathology procedures (e.g., the incidence of the disease being tested is rare). F8 gene testing billed with a high level tier 2 code (e.g., 81406, 81407) is not supported.

GALT (Galactose-1-Phosphate Uridylyltransferase)

Galactosemia is linked to the GALT gene and typically manifests in infancy (Berry, 2021). The associated GALT gene would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

HADHA [Hydroxyacyl-Coa Dehydrogenase/3-Ketoacyl-Coa Thiolase/Enoyl-Coa Hydratase (Trifunctional Protein) Alpha Subunit]

Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) and mitochondrial trifunctional protein (MTP) deficiencies are recessive autosomal disorders caused by mutations in the genes coding for MTP. Deficiencies in LCHAD and MTP arise as a result of mutations in the HADHA gene. Their clinical presentations are variable, and premature death is common. They are included in newborn blood spot screening programs (Stinton et al., 2021). The associated HADHA gene would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

HEXA (Hexosaminidase A, Alpha Polypeptide)

Tay-Sachs disease (TSD) and Sandhoff disease (SD) are recessively inherited and caused by mutations in HEXA and HEXB genes, respectively. Infantile patients typically have < 0.1% normal enzyme activity and are diagnosed in the first 12–14 months of life (Flotte et al., 2022). The associated HEXA gene would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

IVD

Isovaleric acidemia (IVA) is an autosomal recessive inborn error of leucine metabolism caused by a deficiency of isovaleryl-CoA dehydrogenase (IVD) that is detectable by newborn screening (Ensenauer et al., 2004). The associated IVD gene would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

LMNA (Lamin A/C)

Hershberger et al. (2022) reviewed LMNA-related dilated cardiomyopathy (DCM), which usually presents in early to mid-adulthood. The diagnosis is established in a proband with suggestive findings and a heterozygous pathogenic variant in LMNA identified by molecular genetic testing. Single-gene testing (sequence analysis of LMNA, followed by gene-targeted deletion/duplication analysis) for suspected LMNA-related DCM is rarely useful and typically not recommended.

In a 2020 review article, Castro et al. provided an update on the studies on cardiac disease caused by mutations in LMNA gene (LMNA-CMP) and the relationship of clinical manifestations with molecular mechanisms. Consensus has yet to be reached on the pathogenetic mechanism of cardiolaminopathy and researchers are still trying to understand the molecular role of A-type lamins in the heart and to establish clear genotype/phenotype correlations. The results indicate that cardiolaminopathy is a complex disease and patients exhibit extremely variable phenotypes, regarding specific clinical manifestations or the severity and progression of the disease. The authors reported that so far there is still lack of knowledge explaining the link between specific LMNA mutations and a defined phenotype.

NSD1 (Nuclear Receptor Binding SET Domain Protein 1)

Multiple monogenic overgrowth syndromes result from variants in epigenetic regulators: variants in the histone methyltransferase NSD1 cause Sotos syndrome, an overgrowth syndrome with prenatal onset. A modest increase in cancer risk (about 3% prevalence) has been report for neuroblastoma and teratoma in patients with Sotos syndrome (Lui, 2024). Murali and Saloura (2022) summarized the available literature on the functions of NSD1, NSD2, and NSD3 in head and neck squamous cell carcinoma (HNSCC) to evaluate their role as oncogenic drivers and possible therapeutic targets. The study found NSD1-mutant HPV-negative HNSCC tumors represent a subset of tumors that may be more sensitive to chemoradiotherapy, although more resistant to immunotherapeutic interventions compared to NSD1-wild-type tumors. The authors recommend prospective clinical studies to establish the chemoradiosensitizing effect of NSD1 mutations in HPV-negative HNSCC. Foster et al. (2019) investigated the phenotype of 44 adults with Sotos syndrome and NSD1 pathogenic variants; they concluded that adults with Sotos syndrome are generally healthy with few medical issues. Although the study noted that lymphedema, poor dentition, hearing loss, contractures, and tremor have developed in a small number of individuals. Other research of the NSD1 gene includes small case studies related to Sotos syndrome (Lui, 2023; Ren, 2024).

These diseases/syndromes typically manifest in childhood, and the genes associated with them would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

PAH (Phenylalanine Hydroxylase)

Elhawary et al. (2022) reviewed the genetic etiology and clinical challenges of phenylketonuria (PKU), an autosomal recessive disease, which is an inborn error of phenylalanine metabolism caused by pathogenic variants in the phenylalanine hydroxylase (PAH) gene. Early diagnosis and intervention must start shortly after birth to prevent major cognitive and neurological defects.

PAH gene testing is unlikely to directly impact treatment or clinical management in the care of a patient in the general Medicare population.

PDHA1 [Pyruvate Dehydrogenase (Lipoamide) Alpha1]

The mean age of diagnosis of primary pyruvate dehydrogenase complex deficiency (PDCD) is 45 months. Presentation may be as early as prenatal with routine prenatal ultrasound detecting microcephaly, ventriculomegaly, paraventricular pseudocysts, cerebellar hypoplasia, delayed gyration, and/or dysgenesis of the corpus callosum. Fetal MRI may also show cerebral volume loss and/or periventricular T2-weighted hyperintensity. Newborns may present with a history of intrauterine growth restriction, and lactic acidosis. Children may present in late infancy or early childhood with chronic neurologic symptoms. Rarely, individuals present later in childhood with intermittent ataxia paroxysmal dystonia or dyskinesia and other atypical clinical findings such as alternating hemiplegia or episodic limb paralysis (Ganetzky et al., 2021).

This disease typically manifest in late infancy and early childhood, and the genes associated with it would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

POLG [Polymerase (DNA Directed), Gamma]

POLG-related disorders are an overlapping spectrum of disease presenting from early childhood to late adulthood.

Individuals with early-onset disease (prior to age 12 years), liver involvement, feeding difficulties, seizures, hypotonia, and muscle weakness are the most common clinical features. This group has the worst prognosis. In the juvenile/adult-onset form (age 12-40 years), disease is typically characterized by peripheral neuropathy, ataxia, seizures, stroke-like episodes, and, in individuals with longer survival, progressive external ophthalmoplegia (PEO). This group generally has a better prognosis than the early-onset group. Late-onset disease (after age 40 years) is characterized by ptosis and PEO, and peripheral neuropathy, ataxia, and muscle weakness. This group overall has the best prognosis. POLG pathogenic variants are associated with a continuum of features – encompassing and transcending previously defined clinical designations – in which almost any organ system can be involved (Cohen et al., 2024).

The published literature with regard to late onset presentation of POLG mutation disorders is limited to case reports (Mancuso et al., 2004; Spracklen et al., 2021; Meira et al., 2019; Ng et al., 2017).

PRKAG2 (Protein Kinase, Amp-Activated Gamma 2 Non-Catalytic Subunit)

Tier 2 molecular pathology procedures represent procedures that are generally performed in lower volumes than Tier 1 molecular pathology procedures (e.g., the incidence of the disease being tested is rare). PRKAG2 gene testing billed with a high level tier 2 code (e.g., 81406) is not supported.

PTPN11 (Protein Tyrosine Phosphatase, Non-Receptor Type 11)

Baldo et al. (2022) performed a retrospective observational study which included 32 participants with clinical and/or genetic diagnosis of Noonan syndrome (NS) or variants of NS. Missense mutations in PTPN11 gene on chromosome 12 are responsible for this condition in over half of the instances. The authors reported that the study validates the significant occurrence of NS and clinical findings not described in this condition before could be helpful in leading to diagnosis of NS. Limitations of this study include small sample size, retrospective study, and exclusion of subjects from the cohort due to absence or incompleteness of medical documentation.

Noonan disease is usually apparent at birth or early infancy. The genes associated with it would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

RET (Ret-Proto-Oncogene) (e.g., Hirschsprung Disease), Full Gene Sequence

Hirschsprung's disease (HSCR) is diagnosed in the first year of life in over 50% of the cases and in 90% of the cases by age 13. Typically, HSCR is diagnosed with a combination of imaging tests, anorectal manometry, and rectal biopsies. The use of genetic testing of the RET gene has not been established as effective for the diagnosis of HSCR. The genes associated with it would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

Xiao et al. (2023) performed a retrospective analysis of 53 articles that included 129 families with 416 cases of Hirschsprung's disease (HSCR) to analyze the penetrance and transmission patterns of the RET gene to assess familial recurrence, penetrance and genetic characteristics. The authors stated that there are more than 20 genes that have been linked to HSCR and that, while most cases of HSCR are sporadic, some families with two or more family members with HSCR are classified as HSCR families. The authors noted that familial HSCR does not follow Mendelian inheritance, and that the RET and PHOX2B genes showed incomplete penetrance in members of familial HSCR. The authors reported that the male-to-female ratio and the percentage of short segment-HSCR in familial HSCR were much lower than in sporadic HSCR. When the authors looked at the 62 families with familial HSCR that had detailed gene information, RET was associated with 65% (40/62) of the cases while, in 40 RET-associated families, 30% (12/40) showed dominant inheritance and 58% (23/40) showed incomplete dominance. The study was limited by the retrospective design and the small number of cases available for review.

Jiang et al. (2018) evaluated the frequency of RET mosaicism in HSCR to evaluate if it had been underestimated and to assess its contribution to HSCR risk. The study included 152 patients, 83 of which were diagnosed with HSCR based on surgical reports and pathological examination, and blood DNA from an additional 69 patients. The study population included eight de novo HSCR families that the authors reported finding somatic mosaicism in 75% of the cases, in either the patient or an asymptomatic parent with six of the eight being pathogenic mosaic mutations, including two that were somatic mosaics with mutations detected in blood, colon and saliva. The authors also reported that germline mosaicism was identified in four clinically unaffected subjects, each with an affected child, in multiple tissues. The authors concluded that somatic mutations of the RET gene are under-recognized in HSCR and that molecular investigation of the parents of affected children with seemingly sporadic mutations was essential to determine recurrence risk in their families. Limitations of the study include the lack of a control group and the single center design.

SLC9A6 [Solute Carrier Family 9 (Sodium/Hydrogen Exchanger) Member 6]

The SLC9A6 gene can have mutations that lead to Christianson syndrome, characterized by neurological problems, including intellectual disabm ilities, seizures, and an inability to walk or speak. This syndrome becomes apparent in infancy. The gene mutation associated with it would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

SOS1 (Son Of Sevenless Homolog 1)

SOS1-associated Noonan syndrome has a higher prevalence of ectodermal abnormalities but less intellectual disability, short stature, and atrial septal defect than PTPN11-associated Noonan syndrome (Roberts et al., 2013). Mutations in the SOS1 gene have also been associated with hereditary gingival fibromatosis type 1, a rare benign, slowly progressive over

growth of gingival tissue (Hart et al., 2002). Clinical indications are present in childhood as teeth are erupting (Almiñana-Pastor et al., 2017).

The genes associated Noonan's disease and hereditary gingival fibromatosis would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

TAZ (Tafazzin)

Barth syndrome (BS) is an X-linked infantile-onset disease caused by mutations in the TAZ gene and is characterized by cardiomyopathy, hypotonia, growth delay, neutropenia and 3-methylglutaconic aciduria (Ferri et al., 2013).

The gene associated with this disease would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

UBE3A (Ubiquitin Protein Ligase)

Angelman syndrome (AS) is a neurodevelopmental disorder, and most children with AS have delayed developmental milestones. The cause of this syndrome is a variation of genetic abnormalities involving the UBE3A gene (Clayton-Smith et al, 2003).

Angelman syndrome presents in childhood. The genes associated with this syndrome would not generally be tested for in the Medicare population, which is predominantly 65 years of age and older (OIG, 2023).

Level 8 and 9 Tier 2 Molecular Pathology Lab Tests

Tier 2 molecular pathology procedures are generally performed in lower volumes than Tier 1 molecular pathology procedures (e.g., the incidence of the disease being tested is rare). They are arranged by level of technical resources and interpretive work by the physician or other qualified healthcare professional. Tier 2 individual genetic tests listed under level 8 are identified by CPT code 81407 and level 9 by CPT code 81408. The majority of the examples of diseases associated with the genes listed for CPT code 81408 in AMA's CPT are rare diseases that manifest in childhood, such as Duchenne and Becker muscular dystrophy, Joubert syndrome, and Marfan syndrome, as well as those listed for 81407 (e.g., primary microcephaly, X-linked hydrocephaly). Therefore, the genes associated with these diseases would not generally be tested for in the Medicare population, which is predominantly 65 years old and older (OIG, 2023).

Clinical Practice Guidelines

American Academy of Neurology (AAN), American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM), and American Academy of Physical Medicine and Rehabilitation (AAPMR)

In a joint practice parameter on the role of laboratory and genetic testing, the AAN, AANEM and AAPMR make the following evidence -based recommendations for the evaluation of distal symmetric polyneuropathy (England et al.):

- Genetic testing should be conducted for the accurate diagnosis and classification of hereditary neuropathies. (Level A).
- Genetic testing may be considered in patients with a cryptogenic polyneuropathy and classic hereditary neuropathy phenotype. (Level C).
- There is insufficient evidence to support or refute the usefulness of routine genetic testing in cryptogenic polyneuropathy patients without a classic hereditary phenotype. (Level U).

American Academy of Ophthalmology (AAO)

In the consensus guidelines for ocular surveillance of von Hippel-Lindau Disease, the AAO states that patients at risk of VHL disease, including first-degree relatives of patients with known VHL disease, or any patient with single or multifocal retinal hemangioblastomas (RHs), should undergo genetic testing for pathologic VHL disease gene variants as part of an appropriate medical evaluation. (AAO, 2024)

In the 2022 preferred practice pattern on primary open-angle glaucoma (POAG), the AAO states that the understanding of the complex genetic contribution to the development of POAG is rapidly expanding. The genetic variants require further investigation to determine if these factors are protective, are associated with disease progression, or represent potential new therapeutic targets. However, routine genetic testing for glaucoma risk alleles is not recommended for patients with POAG. (AAO, 2020)

American College of Gastroenterology (ACG)

In the 2020 clinical guidelines on chronic pancreatitis, ACG states that patients with idiopathic chronic pancreatitis should be evaluated for PRSS1, SPINK1, CFTR, and CTRC gene mutation analysis (ACG).

American College of Medical Genetics and Genomics (ACMG)

In the 2014 guidelines for the diagnosis and management of phenylalanine hydroxylase deficiency (Vockley et al.), the ACMG makes the following recommendations for diagnosis and therapy of this disorder:

- Quantitative blood amino acids should be performed as part of the diagnostic testing for follow-up of a positive newborn screening (NBS);
- Additional testing is needed to define the cause of elevated blood phenylalanine (PHE) and should include analysis of pterin metabolism;
- Phenylalanine hydroxylase (PAH) genotyping is indicated for improved therapy planning.

American Association for the Study of Liver Diseases (AASLD)

Diagnostic criteria for Wilson Disease (WD) include KF rings, low serum ceruloplasmin concentration (in most patients) and measurement of copper concentration in percutaneous liver biopsy specimens. Most patients with cirrhosis, neurological manifestations, and Kayser-Fleischer (KF) rings are easily diagnosed as having WD. However, approximately 50% of patients presenting with liver disease lack two of these criteria and pose a diagnostic challenge. Genetic testing for disease-specific ATP7B mutations is a newer modality and not universally available. The AASLD recommends this test if available as part of a routine evaluation. It was noted genetic testing is most beneficial in patients for which the diagnosis is uncertain and for targeted mutational analysis in first-degree relatives. Untreated, asymptomatic patients with evidence of organ damage typically progress to symptomatic WD. The timely identification is critical. WD is confirmed by molecular genetic studies in patients in their early 70s and 80s. In older individuals displaying concurrent neurological or psychiatric symptoms and biochemical or histological findings suggesting WD, further evaluation is warranted. WD is an uncommon but eminently treatable genetic disorder due to abnormalities of hepatocellular copper disposition, associated with dysfunction of the P-type ATPase ATP7B. It may present at any age. Genetic evaluation of ATP7B can expedite diagnosis. Medical therapy remains highly effective.

Clinical Pharmacogenetics Consortium (CPIC)

In 2019, the CPIC published guidelines based on a systematic review of the literature for the use of potent volatile anesthetics and depolarizing succinyl choline in patients with RYR1 or CACNA1S genotypes (Gonsalves et al). The guideline states that any of the potent volatile anesthetics and succinylcholine can trigger a malignant hyperthermia (MH) reaction in susceptible individuals. The true incidence of MHS is difficult to establish, as screening for the susceptibility is challenging and the majority of susceptible individuals are phenotypically normal unless exposed to an MH triggering agent, and not all exposures to a triggering agent in an individual with MHS will lead to an MH reaction.

The diagnosis of MHS is made by one of two criteria:

- Positive response to an in vitro muscle bioassay, such as the in vitro contracture test (IVCT), or the caffeine-halothane contracture test (CHCT), or
- The presence of a pathogenic variant in RYR1 or CACNA1S found by molecular genetic testing.

Also noted in this guideline, the American College of Medical Genetics and Genomics has included RYR1 and CACNA1S in its list of genes for which pathogenic variants should be returned as secondary findings. (A secondary finding is defined as a genomic variant of potential medical value that is unrelated to the primary reason for testing.)

European Malignant Hyperthermia Group

In the first update to the 2001 guidelines for the laboratory diagnosis of malignant hyperthermia, Hopkins et al. states that advances in genetic technology resulted in a change in the recommendations for the diagnostic pathway of patients referred for investigation of MH susceptibility. The previous genetic testing recommended muscle biopsy and IVCT as the primary diagnostic modalities, with mutation screening when the IVCT confirmed MH susceptibility. They now consider DNA screening to be a viable alternative primary diagnostic approach.

Lawson Wilkins Pediatric Endocrine Society (LWPES) and The European Society for Pediatric Endocrinology (ESPE)

In 2002, the Lawson Wilkins Pediatric Endocrine Society (LWPES) and The European Society for Pediatric Endocrinology (ESPE) met to develop a consensus statement regarding CAH caused by 21-hydroxylase deficiency and does not address the other rarer forms of CAH. The panel included 40 participating physicians, psychologists, scientists, and

surgeons from 12 countries on 4 continents agreed with the following consensus statement. With regards to genetic testing, they indicated the following:

- Molecular genetic analysis is not essential for the diagnosis but may be helpful to confirm the basis of the defect, to aid in genetic counseling, and to establish the diagnosis in uncertain cases. Ten mutations account for 90–95% of the affected alleles, but molecular genetic analysis is complicated by multiple copies of the genes and the possibility of multiple mutations on one allele.
- Preimplantation genetic diagnosis for CAH is possible, but further research is required to determine its utility. Gene therapy is currently not possible in humans with this disorder.

There remain important deficits in our knowledge about this disorder; and again, these have been highlighted. New therapeutic strategies are emerging but, as yet require longer evaluation before being introduced into routine practice. In the meantime, we should focus on early diagnosis, optimal medical and surgical treatment, and attention to compliance.

National Comprehensive Cancer Network (NCCN)

In the clinical practice guidelines for bladder cancer, the NCCN states the following with regard to molecular/genomic testing as part of an additional work up:

- For Stage IIIB (cT1-cT4a, N2,3) molecular/genomic testing in a Clinical Laboratory Improvement Amendments (CLIA) approved laboratory should be considered.
- For IVA, (cT4b, Any N, M0; Any T, Any N, M1a) molecular/genomic testing in a Clinical Laboratory Improvement Amendments (CLIA) approved laboratory is recommended.
- For Metastatic (Stage IVB Any T, Any N, M1b) molecular/genomic testing in a Clinical Laboratory Improvement Amendments (CLIA) approved laboratory is recommended. (NCCN Bladder Cancer, v4.2024)

In the clinical practice guidelines for cutaneous melanoma, the NCCN states the following with regard to CDKN2A genetic testing:

- Consider genetic counseling referral for p16/CDKN2A mutation testing in the presence of three or more invasive cutaneous melanomas, or a mix of invasive melanoma, pancreatic cancer, and/or astrocytoma diagnoses in an individual or family.
- Multigene panel testing that includes CDKN2A is recommended for patients with invasive cutaneous melanoma who have a first-degree relative diagnosed with pancreatic cancer. (NCCN Melanoma: Cutaneous, v4.2024)

In the clinical practice guidelines for uveal melanoma, the NCCN states the following for the workup for ocular recurrence and distant metastatic disease:

- For patients with metastasis who are considering treatment with targeted therapy, tissue should be obtained for genetic analysis (screening for mutations that may be potential targets for treatment or to determine eligibility for a clinical trial) from either biopsy of the metastasis (preferred) or archival material.
- Broader genomic profiling may be considered if the results could inform future treatment decisions or eligibility for clinical trials. (NCCN Melanoma: Uveal, v1.2024)

In the clinical practice guidelines for uveal melanoma, the NCCN states that genetic testing for inherited mutations is recommended for any patient with confirmed pancreatic cancer, using comprehensive gene panels for hereditary cancer syndromes. Genetic counseling is recommended for patients who test positive for a pathogenic mutation (ATM, BRCA1, BRCA2, CDKN2A, MLH1, MSH2, MSH6, PALB2, PMS2, STK11, and TP53) or for patients with a positive family history of cancer, especially pancreatic cancer, regardless of mutation status. (NCCN Pancreatic Adenocarcinoma, v2.2024)

In the clinical guidelines for genetic/familial high-risk assessment: Breast, Ovarian, and Pancreatic, testing for pancreatic cancer susceptibility genes, specifically ATM, BRCA1, BRCA2, CDKN2A, and Lynch syndrome genes is clinically indicated for the following:

- All individuals diagnosed with exocrine pancreatic cancer and first-degree relatives of individuals diagnosed with exocrine pancreatic cancer.
    - For patients with germline variants in CDKN2A, consider pancreatic cancer screening beginning at age 40 years (or 10 years younger than the earliest exocrine pancreatic cancer diagnosis in the family, whichever is earlier).
- Neuroendocrine pancreatic tumors. (NCCN Breast, Ovarian, and Pancreatic; v3.2024)

In the clinical guidelines for Neuroendocrine and Adrenal Tumors, NCCN recommends genetic risk evaluation and genetic testing for hereditary endocrine neoplasia syndromes for patients with a clinical suspicion for MEN1 due to 2 or more of the following, or 1 AND a family history of 1 or more of the following: Primary hyperparathyroidism; duodenal/pancreatic NET; Pituitary adenoma; foregut carcinoid (lung, thymic, or gastric). (NCCN Neuroendocrine and Adrenal Tumors; v1.2024)

National Comprehensive Cancer Network (NCCN) guidelines for neuroendocrine and adrenal tumors state:

- Tumor / somatic molecular profiling should be considered for patients with locoregional unresectable / metastatic disease who are candidates for anticancer therapy to identify actionable alterations. Actionable somatic findings include RET fusions for extrapulmonary, poorly differentiated, neuroendocrine carcinoma, large or small cell carcinoma, and mixed neuroendocrine-non-neuroendocrine neoplasm.
- Genetic counseling and genetic testing (including RET, when appropriate) are recommended in patients with a diagnosis of pheochromocytoma or paraganglioma and in those individuals with a family history of these tumors.
- Genetic counseling and RET genetic testing should be offered to individuals with MTC, primary C-cell hyperplasia, or a clinical diagnosis of MEN2.
- All patients with MTC should be tested for germline mutation of the RET oncogene, even if the family history is not suggestive of a hereditary syndrome. (NCCN, Neuroendocrine and Adrenal Tumors; v1.2024)

NCCN guidelines for thyroid carcinoma state:

- For advanced, progressive, or threatening structurally persistent / recurrent locoregional or distant metastatic disease, not amenable to radioactive iodine therapy, somatic testing to identify actionable mutations (including RET gene fusions) is indicated for papillary carcinoma, follicular carcinoma, and oncocytic carcinoma.
- Genetic counseling and RET genetic testing should be offered to individuals with MTC.
- For stage IVC anaplastic carcinoma, molecular testing (including RET) for actionable mutations is indicated.
- Germline testing for RET with genetic counseling is recommended for all patients with newly diagnosed MTC or clinically suspected sporadic MTC. If a germline RET mutation is found, then mutation testing should also be done for family members.
- If MTC is diagnosed after thyroid surgery, workup including RET is indicated to determine whether additional surgery is needed. (NCCN, Thyroid Carcinoma; v3.2024)

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Policy History/Revision Information

Date	Summary of Changes
02/01/2025	**Applicable Codes** ***Non-Covered Diagnosis Codes*** • Added Z59.71 and Z59.72 • Added notation to indicate Z59.7 was “deleted Sep. 30, 2024” • Removed Z11.52 **Supporting Information** • Archived previous policy version MMP381.19

Instructions for Use

The Medicare Advantage Policy documents are generally used to support UnitedHealthcare coverage decisions. It is expected providers retain or have access to appropriate documentation when requested to support coverage. This document may be used as a guide to help determine applicable:

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- Medicare coding or billing requirements.

Medicare Advantage Policies are applicable to UnitedHealthcare Medicare Advantage Plans offered by UnitedHealthcare and its affiliates. This Policy is provided for informational purposes and does not constitute medical advice. It is intended to serve only as a general reference and is not intended to address every aspect of a clinical situation. Physicians and patients should not rely on this information in making health care decisions. Physicians and patients must exercise their independent clinical discretion and judgment in determining care. Treating physicians and healthcare providers are solely

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Benefit coverage for health services is determined by the member specific benefit plan document and applicable laws that may require coverage for a specific service. The member specific benefit plan document identifies which services are covered, which are excluded, and which are subject to limitations. In the event of a conflict, the member specific benefit plan document supersedes this policy. For more information on a specific member's benefit coverage, please call the customer service number on the back of the member ID card or refer to the Administrative Guide.

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UnitedHealthcare follows Medicare coverage guidelines found in statutes, regulations, NCDs, and LCDs to determine coverage. The clinical coverage criteria governing certain items or services referenced in this Medical Policy have not been fully established in applicable Medicare guidelines because there is an absence of any applicable Medicare statutes, regulations, NCDs, or LCDs setting forth coverage criteria and/or the applicable NCDs or LCDs include flexibility that explicitly allows for coverage in circumstances beyond the specific indications that are listed in an NCD or LCD. As a result, in these circumstances, UnitedHealthcare applies internal coverage criteria as referenced in this Medical Policy. The internal coverage criteria in this Medical Policy was developed through an evaluation of the current relevant clinical evidence in acceptable clinical literature and/or widely used treatment guidelines. UnitedHealthcare evaluated the evidence to determine whether it was of sufficient quality to support a finding that the items or services discussed in the policy might, under certain circumstances, be reasonable and necessary for the diagnosis or treatment of illness or injury or to improve the functioning of a malformed body member.

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For members in UnitedHealthcare Medicare Advantage plans where a delegate manages utilization management and prior authorization requirements, the delegate’s requirements need to be followed.