Proposal form for the evaluation of a genetic test for NHS Service Gene Dossier/Additional Provider

Submitting laboratory: Exeter RGC

1. Disorder/condition – approved name and symbol as published on the OMIM database (alternative names will be listed on the UKGTN website)

If this submission is for a panel test please complete appendix 1 listing all of the conditions included using approved OMIM name, symbol and OMIM number.

This application is for a panel of genes involved in Endocrine disorders that includes the following sub-panels:

1. Congenital hypothyroidism
2. Hereditary Phaeochromocytoma and Paraganglioma
3. Hypophosphatemic Rickets
4. Combined pituitary hormone deficiency
5. Congenital Generalised Lipodystrophy
6. Familial Glucocorticoid deficiency
7. Familial Tumoral Calcinosis
8. Pseudohypoaldosteronism
9. Endocrine Neoplasia syndromes (MEN types 1, 2 and 4, FMTC, FIPA)
10. General Arterial Calcification of Infancy (GACI)
11. Chondrodysplasia Punctata
12. Familial Isolated Primary Hyperparathyroidism
13. Familial Hypoparathyroidism
14. Primary Pigmented Nodular Adrenocortical Disease

2. OMIM number for disorder/condition

If a panel test – see 1. Above

See Appendix 1

3a. Disorder/condition – please provide, in laymen's terms, a brief (2-5 sentences) description of how the disorder(s) affect individuals and prognosis.

A group of disorders of the endocrine system that result in a number of serious multi system disorders

1. **Congenital Hypothyroidism**

   Congenital hypothyroidism is a condition that affects infants from birth (congenital) and results from a partial or complete loss of thyroid function (hypothyroidism). It occurs when the thyroid gland fails to develop or function properly. If untreated, congenital hypothyroidism can lead to intellectual disability and abnormal growth. It is also associated with an increased incidence of birth defects. If treatment begins early, infants usually develop normally and severe disability is avoided. Treatment is lifelong.

2. **Familial Phaeochromocytoma and Paraganglioma**

   A phaeochromocytoma is a tumour that forms in the adrenal gland (gland located above the kidney) that causes it to make too much adrenaline. Phaeochromocytomas cause very high blood pressure, pounding headaches, heart palpitations, flushing of the face, nausea, and vomiting. Paragangliomas are identical to phaeochromocytomas but originate outside the adrenal gland.

3. Hypophosphataemic rickets

Phosphate is a mineral that is essential for the normal formation of bones and teeth. Hypophosphatemic rickets results from low levels of phosphate in the blood. In most cases, the signs and symptoms begin in early childhood with slow growth, and development of bone abnormalities that interfere with movement and cause bone pain. They may also have premature fusion of the skull bones (craniosynostosis) and dental abnormalities (abscesses). In adults, hypophosphatemia is characterized by a softening of the bones known as osteomalacia.

4. Combined pituitary hormone deficiency

Combined pituitary hormone deficiency is a condition that causes a shortage (deficiency) of several hormones produced by the pituitary gland, which is located at the base of the brain. A lack of these hormones may affect the development of many parts of the body. Patients fail to grow at the expected rate, have delayed or absent puberty and infertility. Some patients are also more susceptible to infections or have underdeveloped optic nerves (optical nerves carry visual information from the eyes to the brain).

5. Congenital Generalised Lipodystrophy

Congenital generalized lipodystrophy, which is typically evident from birth, is characterised by generalised loss of adipose tissue affecting the limbs, trunk, face and neck. Advanced bone age and prominent skeletal muscle are also seen. The liver may also become enlarged.

6. Familial Glucocorticoid deficiency

Familial Glucocorticoid deficiency is a rare, potentially lethal, disorder caused by the failure of the adrenal glands to produce glucocorticoids (hormones involved in the regulation of the metabolism of glucose) that usually presents in the neonatal period or in early childhood. The majority of patients present with hypoglycaemia (low blood glucose) in the neonatal period which, in many cases, is resolved with more frequent feeding regimes. The condition is potentially fatal if left untreated and in the long term recurrent hypoglycaemia can lead to irreversible brain damage causing learning difficulties in addition to other neurological problems.

7. Familial Tumoral Calcinosis

Hyperphosphatemic Familial Tumoral Calcinosis (HFTC) is characterised by painful growths (calcifications) caused by abnormal deposits of phosphate and calcium in the body's tissues, particularly around the hips, elbows or shoulders. Other features include eye problems such as calcium build up in the clear front covering of the eye (corneal calcification). It is caused by high serum phosphate level (hyperphosphatemia) and normal or elevated levels of vitamin D. Onset is within the first decade of life. Normophosphatemic familial tumoral calcinosis (NFTC) is characterized by calcium deposition in skin and mucous membranes and is associated with unremitting pain and life-threatening skin infections.

8. Pseudohypoaldosteronism type 2

Patients with Pseudohypoaldosteronism have problems regulating the amount of sodium and potassium in the body. Sodium and potassium play a crucial role in control of blood pressure. Patients have high blood pressure and high levels of potassium and, in many cases, high levels of chloride in their blood. Patients present with nausea, vomiting, extreme tiredness and muscle weakness.

9. Endocrine Neoplasia syndromes

Multiple Endocrine Neoplasia is a group of disorders that affect the body's network of hormone producing glands (the endocrine system).

- **Multiple Endocrine Neoplasia type 1:** The major endocrine features of MEN include parathyroid tumours and hyperparathyroidism, pancreatic tumours and pituitary tumours. A clinical diagnosis of MEN1 is made when patients have at least two of these tumour types.
- **Multiple Endocrine Neoplasia type 2A and 2B:** The most common feature of MEN2 is a form of thyroid cancer called medullary thyroid cancer. Some people with this disorder also develop a phaeochromocytoma, which is an adrenal gland tumour that can cause dangerously high blood pressure.
- **Familial Medullary Thyroid Cancer:** Medullary thyroid cancer is the only feature in patients with this disorder.
- **Multiple Endocrine Neoplasia type 4:** the features of MEN4 have signs and symptoms similar to those of type 1. Hyperparathyroidism is the most common feature but pituitary tumours are also common.
- **Familial Isolated Pituitary Adenoma:** Tumours of the pituitary gland are the only feature in patients with this disorder. These tumours can secrete growth hormone causing gigantism (overgrowth) or other hormones that can cause breast milk production without pregnancy and infertility. Some pituitary adenomas can cause weight gain, excess growth of body and facial hair whilst others can cause a fast or irregular heartbeat, fatigue, sleep problems. All of the tumours can cause headaches and visual disturbances if the tumour is large.

10. General Arterial Calcification of Infancy (GACI)

Generalized arterial calcification of infancy (GACI) is a life-threatening disorder in young infants. Patients usually present with heart problems in the first month of life. The symptoms are caused by the accumulation of calcium in the arteries. GACI is often fatal within the first 6 months of life due to heart failure.

11. Chondrodysplasia Punctata

Chondrodysplasia punctata is a group of disorders that share the features of stippled epiphyses (end of long bones) and skeletal changes.

12. Familial Isolated Primary Hyperparathyroidism

Hyperparathyroidism is a condition in which the parathyroid glands, located in the neck, secrete too much parathyroid hormone (PTH). Parathyroid hormone regulates the amount of calcium and phosphorus in the body, by controlling how much calcium is taken from bones, absorbed in the intestines, and lost in urine. When too much parathyroid hormone is secreted, levels of calcium in the blood and urine rise, and bones may lose calcium, leading to osteoporosis.

13. Familial Isolated Hypoparathyroidism

The symptoms of hypoparathyroidism are predominantly due to low levels of calcium in the blood which leads to a variety of symptoms including fatigue, muscle weakness, twitching and cramping of the extremities, or spasms of the hands, feet, arms, or face (tetany). The onset of symptoms in cases of congenital hypoparathyroidism is usually during early childhood, but can occur any time from birth to adulthood. In some cases, seizures during infancy or childhood may be the first presenting sign. Chronic hypoparathyroidism in childhood may affect the teeth including the underdevelopment of the hard outer layer of the teeth (enamel hypoplasia). Sudden, muscular spasms affecting the larynx (laryngospasm) causes closure of the upper end of the trachea and prevents air from reaching the lungs. Affected individuals may develop calcium deposits (calcifications) in the brain or the kidneys (nephrocalcinosis). Fluctuations in serum calcium lead to neuromuscular irritability which may result in numbness, tingling, and cramping of the extremities or seizures. Chronic hypoparathyroidism may also lead stone formation in the kidney or collecting ducts (nephrolithiasis).

14. Primary pigmented nodular adrenocortical disease (PPNAD)

Patients with PPNAD have nodules on their adrenal glands (the adrenal glands sit above the kidneys) that result in excess hormone being released. Patients present with rapid weight gain, thin skin that bruises easily, muscle weakness, excessive sweating and slowed growth in children. Left untreated, these patients have an increased risk of heart attack and stroke due to high blood pressure. In some cases it can result in the death of the patient.

3b. Disorder/condition – if required please expand on the description of the disorder provided in answer to Q3a.

1. Congenital Hypothyroidism

CH is characterized by elevated levels of thyroid-stimulating hormone (TSH) resulting from reduced thyroid function. In 80 to 85% of cases, congenital hypothyroidism is associated with, and presumably is a consequence of, thyroid dysgenesis (Macchia et al., 1998). In these cases, the thyroid gland can be absent (agenesis), ectopically located, and/or severely reduced in size (hypoplasia). When thyroid hormone therapy is not initiated within the first 2 months of life, congenital hypothyroidism can cause severe neurologic, mental, and motor damage (cretinism). Inborn errors of thyroid hormone biosynthesis (dyshormonogeneses) account for 10-15% of cases

2. Familial Phaeochromocytoma and Paraganglioma

Phaeochromocytomas and extra-adrenal paragangliomas are rare tumors arising from neural crest tissue that develops into sympathetic and parasympathetic paraganglia throughout the body. Pheochromocytomas arise in adrenal gland tissue that cause an increase in the release of catecholamines (adrenaline and noradrenaline). Therefore phaeochromocytomas cause very high blood pressure, pounding headaches, heart palpitations, nausea and vomiting. Tumours can be unilateral or bilateral. Paragangliomas are identical to phaeochromocytomas but originate outside of the adrenal gland. Sympathetic paragangliomas are mainly located in the chest, abdomen and pelvis, and commonly secrete catecholamines whereas parasympathetic paragangliomas are typically located within the head and neck and usually do not secrete catecholamines but cause significant morbidity by pressure effects.

3. Hypophosphatemic Rickets

Hypophosphatemic rickets is a group of genetic diseases characterized by hypophosphatemia, rickets, and normal serum levels of calcium. Characteristic clinical features include slow growth, bone pain and bone deformities. These diseases comprise the FGF23-dependent forms (X-linked, autosomal dominant, and autosomal recessive hypophosphatemic rickets) that are caused by mutations in various genes involved in regulating renal phosphate reabsorption (PHEX, FGF23, DMP1, ENPP1) that induce an elevation in circulating levels of FGF23, and the FGF23-independent forms, such as hereditary hypophosphatemic rickets with hypercalciuria (HHRH), which is caused by mutations in a gene encoding a sodium-dependent phosphate transporter (SLC34A3). HHRH is characterised by hypophosphatemic rickets, growth retardation, osteomalacia, hypotonia and muscle weakness. Onset is within infancy or early childhood. Biochemically affected individuals have hypophosphatemia (low serum phosphate) due to decreased renal tubular reabsorption, hypercalciuria (high urine calcium), increased serum calcitriol (1,25-dihydroxyvitamin D) levels and decreased or low-normal serum parathyroid hormone (PTH).

4. Combined Pituitary Hormone Deficiency

Combined pituitary hormone deficiency (CPHD) is diagnosed when the production of two or more of these hormones is insufficient or absent. The etiology of CPHD is typically multifactorial and may be secondary to a neurological insult. However, a subset of patients, who present during infancy or childhood with CPHD, is classified as having idiopathic disease. These patients may be considered to have a genetic etiology for their deficiencies, associated with mutations in transcription factors responsible for pituitary development. Clinical presentation is variable, depending on the type and severity of deficiencies and on the age at diagnosis: PROP1 (somatolactotroph, thyrotroph,

gonadotroph and sometimes corticotroph deficiencies; pituitary hyper and hypoplasia), POU1F1 (somatolactotroph and thyrotroph deficiencies, pituitary hypoplasia), HESX1 (variable pituitary deficiencies, septo-optic dysplasia), and less frequently LHX3 (somatolactotroph, thyrotroph and gonadotroph deficiencies, limited head and neck rotation) and LHX4 (variable pituitary deficiencies, ectopic neurohypophysis, cerebral abnormalities). An appropriate replacement of hormone deficiencies is required. Strict follow-up is necessary because patients develop new deficiencies (for example late onset corticotroph deficiency in patients with PROP1 mutations). Type of transmission varies with the factor and the mutation involved (recessive transmission for PROP1 and LHX3, dominant for LHX4, autosomal or recessive for POU1F1 and HESX1).. If untreated, main symptoms include short stature, cognitive alterations or delayed puberty.

5. Congenital Generalised Lipodystrophy

Congenital generalized lipodystrophy, is an autosomal recessive disorder characterized by marked paucity of adipose tissue from birth, extreme insulin resistance, acanthosis nigricans, hypertriglyceridemia, hepatic steatosis and early onset of diabetes. Additional characteristics include accelerated growth during childhood, increased basal metabolic rate, voracious appetite, acromegaloid appearance (enlarged mandible, hands and feet), hepatosplenomegaly, umbilical hernia and in women, clitoromegaly and hirsutism.

6. Familial Glucocorticoid Deficiency

Familial Glucocorticoid deficiency (FGD) is a disease in which the cells of the zona fasciculata within the adrenal cortex fail to respond appropriately to stimulation by ACTH to produce cortisol. The disease is characterised by isolated glucocorticoid deficiency and patients therefore exhibit low or often undetectable serum cortisol with high plasma ACTH levels. Patients typically present with symptoms related to their low cortisol and high ACTH. Glucocorticoid deficiency may lead to hypoglycemic seizures and/or failure to thrive within the neonatal period or very early childhood, while older children may present with recurrent infections. The condition is potentially fatal if left untreated and in the long term recurrent hypoglycaemia can lead to learning difficulties in addition to other neurological sequelae. Excessive plasma ACTH often results in hyperpigmentation due to overstimulation of melanocortin 1 receptors (MC1R). This hyperpigmentation can be present from birth or may develop over time.

7. Familial Tumoral Calcinosis

Hyperphosphatemic familial tumoral calcinosis is characterised by ectopic calcifications in soft tissues around major joints. Vascular calcifications also occur in some patients. Other reported features of familial tumoral calcinosis include angioid streaks of the retina, dental abnormalities and testicular microlithiasis, however, it is not clear whether these findings are common in tumoral calcinosis. Biochemical features include hyperphosphatemia due to increased renal phosphate reabsorption, and elevated or inappropriately normal 1,25-dihyroxyvitamin D [1,25(OH)2D]. However, circulating calcium and parathyroid hormone (PTH) are usually normal. In contrast, biochemical investigations are normal in normophosphatemic familial tumoral calcinosis. The calcified tumor formation in normophosphatemic familial tumoral calcinosis (NFTC) is generally preceded by a vasculitis-like rash and is associated with inflammatory manifestations mostly evident in mucosal tissues.

8. Pseudohypoaldosteronism type 2

Pseudohypoaldosteronism type 2 is characterized by hypertension and hyperkalemia despite normal glomerular filtration rate (GFR). Other associated findings in both children and adults include hyperchloremia, metabolic acidosis, and suppressed plasma renin levels. Aldosterone levels are variable, but are relatively low given the degree of hyperkalemia (elevated serum potassium is a potent stimulus for aldosterone secretion). Hypercalciuria is well described.

9. Endocrine Neoplasia syndromes

- **Multiple Endocrine Neoplasia type 1:** characterised by the triad of parathyroid hyperplasia, pancreatic endocrine tumours and pituitary adenomas. A clinical diagnosis of MEN1 can be made in a patient with at least two of these three main MEN1-related endocrine tumours. Familial MEN1 is defined by the above criteria plus at least one first-degree relative with at least one of the three tumours. In addition, MEN1 patients may have carcinoid tumours, adrenal tumours, lipomas, angiofibromas, collagenomas and meningiomas.
- **Multiple Endocrine Neoplasia type 2A:** diagnosed clinically by the occurrence of two or more specific endocrine tumours: medullary thyroid carcinoma (MTC), phaeochromocytoma, or parathyroid adenoma/hyperplasia.
- **Multiple Endocrine Neoplasia type 2B:** characterised by the presence of mucosal neuromas of lips and tongue as well as distinctive facies with enlarged lips, a 'marfanoid' body habitus and medullary thyroid cancer.
- **Familial Medullary Thyroid Cancer:** characterised by the presence of medullary thyroid cancer (MTC) in the absence of phaeochromocytoma or parathyroid adenoma/hyperplasia.
- **Multiple Endocrine Neoplasia type 4:** characterised by the presence of parathyroid and pituitary tumours in addition to other malignancies. The full spectrum of features associated with MEN4 has not been clearly defined.
- **Familial Isolated Pituitary Adenoma:** characterised by early-onset disease, often aggressive tumour growth and a predominance of somatotroph and lactotroph adenomas.

10. General Aterial Calcification of Infancy (GACI)

Generalised Arterial Calcification of Infancy (GACI) is characterised by calcification of the internal elastic lamina of muscular arteries and stenosis due to myointimal proliferation. GACI is often fatal within the first 6 months of life because of myocardial ischemia resulting in refractory heart failure. Survival to adulthood has been reported.

11. Chondrodysplasia Punctata

Chondrodysplasia punctate is a name given to a heterogeneous collection of skeletal dysplasias characterised by punctate epiphyses.

12. Familial Isolated Primary Hyperparathyroidism

Hereditary primary hyperparathyroidism may develop as a solitary endocrinopathy or as part of the Multiple Endocrine Neoplasia or hereditary hyperparathyroidism-jaw tumour (HPT-JT) syndromes:

- Multiple Endocrine Neoplasia type 1due to mutations in the MEN1 gene
- Multiple Endocrine Neoplasia type 2A and 2B due to mutations in the RET gene
- Multiple Endocrine Neoplasia type 4 due to mutations in the CDKN1B gene
- Familial Isolated Primary Hyperparathyroidism and Hyperparathyroidism-jaw tumor (HPT-JT) syndrome due to mutations in the *CDC73* gene
- Familial Isolated Primary Hyperparathyroidism due to mutations in the CDKN2B, CDKN2C and CDKN1A genes
- Familial Isolated Primary Hyperparathyroidism and Familial Hypocalciuric Hypercalcaemia due to mutations in the CASR gene

13. Familial Isolated Hypoparathyroidism

Hypoparathyroidism is characterized by hypocalcemia and hyperphosphatemia due to inadequate supply or effectiveness of circulating parathyroid hormone (PTH). Isolated hypoparathyroidism is caused by mutations in the PTH gene (impaired synthesis of PTH), CASR gene (defects in PTH secretion or secretion) and GCM2 gene (defects that impair the embryological development of the

parathyroid glands). Mutations have also been reported in GNA11.

14. Primary Pigmented Nodular Adrenocortical Disease

Primary pigmented nodular adrenocortical disease (PPNAD) is a form of bilateral adrenocortical hyperplasia that is often associated with corticotrophin (ACTH)-independent Cushing's syndrome (CS) and is characterized by small to normal-sized adrenal glands containing multiple small cortical pigmented nodules. PPNAD may occur in an isolated form or associated with a multiple neoplasia syndrome, the complex of spotty skin pigmentation, myxomas, and endocrine overactivity, or Carney complex, in which Cushing's syndrome is the most common endocrine manifestation. Molecular studies have led to the identification of mutations in the PRKAR1A, PDE11A and PDE8B genes.

4. Disorder/condition – mode of inheritance

If this submission is for a panel test, please complete the mode of inheritance for each condition in the table in appendix 1.

N/A

5. Gene – approved name(s) and symbol as published on HGNC database (alternative names will be listed on the UKGTN website)

If this submission is for a panel test please complete appendix 1 listing all of the genes included using approved HGNC name, symbol, number and OMIM number.

N/A

6a. OMIM number(s) for gene(s)

If a panel test – see 5. Above

N/A

6b. HGNC number(s) for gene(s)

If a panel test – see 5. Above

N/A

7a. Gene – description(s)

If this submission is for a panel test, please provide total number of genes.

1. Congenital hypothyroidism – 8 genes
2. Familial Phaeochromocytoma and Paraganglioma – 10 genes
3. Hypophosphatemic Rickets – 5 genes
4. Combined Pituitary Hormone Deficiency – 5 genes
5. Congenital Generalised Lipodystrophy – 5 genes
6. Familial Glucocorticoid Deficiency – 5 genes
7. Hyperphosphatemic Familial Tumoral Calcinosis – 4 genes
8. Pseudohypoaldosteronism – 4 genes
9. Endocrine Neoplasias – 4 genes
10. Generalised Arterial Calcification of Infancy – 2 genes
11. Chondrodysplasia Punctata – 5 genes
12. Familial Isolated Primary Hyperparathyroidism – 8 genes
13. Familial Isolated Hypoparathyroidism – 4 genes
14. Primary Pigmented Nodular Adrenocortical Disease – 3 genes

7b. Number of amplicons to provide this test (molecular) or type of test (cytogenetic)

(n/a for panel tests)

N/A

7c. GenU band that this test is assigned to for index case testing.

G

8. Mutational spectrum for which you test including details of known common mutations

(n/a for panel tests)

If this application is for a panel test to be used for different clinical phenotypes and/or various sub panel tests – please contact the team for advice before completing a Gene Dossier

N/A

9a. Technical method(s) – please describe the test.

Mutation screening of the coding regions and intron/exon boundaries is performed using a targeted custom Agilent SureSelect exon-capture assay with sequencing on an Illumina HiSeq 2500. The regions analysed for each gene include coding exons, intronic sequences within 50bp upstream and 10bp downstream of each exon. Deletions/insertions/duplications >30 bp are identified by relative read depth coverage. All newly identified mutations are confirmed by Sanger sequencing or MLPA.

The regions analysed for each of the genes include coding exons, intronic sequences within 50bp upstream and 10bp downstream of each exon, and non-coding regions promoters and polyA sites) where pathogenic mutations have been reported. Deletions/insertions/duplications >30 bp are identified by relative read depth coverage. All newly identified mutations are confirmed by Sanger sequencing or MLPA.

9b. For panel tests, please specify the strategy for dealing with gaps in coverage.

On average, the assay achieves ≥30 reads for 99.7% of the region analysed. Sanger sequence analysis is performed for gaps in coverage (<20X per base) where the patient's phenotype is consistent with a mutation in that gene.

9c. Does the test include MLPA?

(For panel tests, please provide this information in appendix 1)

Partial/whole gene deletions/duplications in these genes are very rare but will be detected by the NGS assay (using relative read depth analysis).

9d. If NGS is used, does the lab adhere to the Association of Clinical Genetic Science Best Practice Guidelines for NGS?

Yes

10. Is the assay to be provided by the lab or is it to be outsourced to another provider?

If to be outsourced, please provide the name of the laboratory and a copy of their ISO certificate or their CPA number.

In-house

11. Validation process

Please explain how this test has been validated for use in your laboratory or submit your internal validation documentation. If this submission is for a panel test, please provide a summary of evidence of:

i) instrument and pipeline validation, and

ii) panel verification for the test

Please submit as appendices to the Gene Dossier (these will be included in the published Gene Dossier available on the website).

Please note that the preferred threshold for validation and verification is 95% sensitivity with 95% Confidence Intervals.

Details of the assay validation and panel verification are included in the attached document. We have tested a total of 126 unique variants previously identified by Sanger sequencing or MLPA (99 base substitutions, 22 indels and 5 partial/whole gene deletions). The NGS assay detected all variants with no false negative or positive results. Therefore we have 95% confidence that the sensitivity of the assay is >97.5% (error rate 0-2.3%).

12a. Are you providing this test already?

1. Congenital Hypothyroidism –TSHR sequencing by Sanger sequencing is available.
2. Familial Pheochromocytoma and Paraganglioma – yes, currently tested as a panel by Sanger sequencing.
3. Hypophosphatemic rickets – testing currently offered in a sequential manner by Sanger sequencing.
4. Combined Pituitary Hormone deficiency – No.
5. Congenital Generalised Lipodystrophy – testing currently offered in a sequential manner by Sanger sequencing.
6. Familial Glucocorticoid Syndrome – No.
7. Familial Tumoral Calcinosis - testing currently offered in a sequential manner by Sanger sequencing for Hyperphosphatemic Tumoral Calcinosis. We do not currently offer testing for Normophosphatemic Familial Tumoral Calcinosis.
8. Pseudohypoaldosteronism type 2 – testing currently offered for WNK4 only by Sanger sequencing.
9. Endocrine Neoplasias – testing currently offered on an individual gene basis by Sanger sequencing.
10. Generalised Arterial Calficiation of Infancy – testing currently offered in a sequential manner by Sanger sequencing.
11. Chondrodysplasia Punctata – testing currently offered on individual gene basis by Sanger sequencing.
12. Familial Isolated Primary Hyperparathyroidism - testing is available for MEN1, RET, CDC73, CDKN1B and CASR is available on individual gene basis using Sanger sequencing.
13. Familial Isolated Hypoparathyroidism – No.
14. Primary Pigmented Nodular Adrenocortical Disease – Yes.

12b(i). If yes, how many reports have you produced?

Data for those by Sanger sequencing:

Congenital Hypothyroidism (TSHR reports): 16 reports

Familial Phaeochromocytoma and Paraganglioma: 200

Hypophosphatemic rickets: 500

Congenital Generalised Lipodystrophy: 15

Familial Tumoral Calcinosis: 60

Pseudohypoaldosteronism type 2 – 9 reports

Endocrine Neoplasia syndromes

- Multiple Endocrine Neoplasia type 1: >1000
- Multiple Endocrine Neoplasia type 2A and 2B: ~800
- Familial Medullary Thyroid Cancer: 415
- Multiple Endocrine Neoplasia type 4: >100
- Familial Isolated Pituitary Adenoma: >600

Generalised Arterial Calcification of Infancy

Chondrodysplasia Punctata: ~200

Primary Pigmented Nodular Adrenocortical Disease - 1

12b(ii). Number of reports mutation positive?

Congenital Hypothyroidism: TSHR reports: 1

Familial Phaeochromocytoma and Paraganglioma: 14

Hypophosphatemic rickets: 250

Congenital Generalised Lipodystrophy: 3

Familial Tumoral Calcinosis: 12

Pseudohypoaldosteronism type 2: 1

Endocrine Neoplasia syndromes

- Multiple Endocrine Neoplasia type 1: ~200
- Multiple Endocrine Neoplasia type 2A and 2B: ~200
- Familial Medullary Thyroid Cancer: 33
- Multiple Endocrine Neoplasia type 4: 0
- Familial Isolated Pituitary Adenoma: ~100

Generalised Arterial Calcification of Infancy

Chondrodysplasia Punctata: ~70

Primary Pigmented Nodular Adrenocortical Disease - 0

12b(iii). Number of reports mutation negative?

Congenital Hypothyroidism: TSHR reports: 15

Familial Phaeochromocytoma and Paraganglioma: 186

Hypophosphatemic Rickets: 250

Congenital Generalised Lipodystrophy:12

Familial Tumoral Calcinosis: 48

Pseudohypoaldosteronism type 2: 8

Endocrine Neoplasia syndromes

- Multiple Endocrine Neoplasia type 1: ~800
- Multiple Endocrine Neoplasia type 2A and 2B: ~200
- Familial Medullary Thyroid Cancer: ~600
- Multiple Endocrine Neoplasia type 4: >100
- Familial Isolated Pituitary Adenoma: ~500

Generalised Arterial Calcification of Infancy

Chondrodysplasia Punctata: ~130

Primary Pigmented Nodular Adrenocortical Disease - 1

12b(iv). Please provide the time period in which these reports have been produced and whether in a research or a full clinical diagnostic setting.

All reports issued in a full diagnostic setting.

Congenital Hypothyroidism: since 2005 (Clinical)

Familial Phaeochromocytoma and Paraganglioma: panel test since 2011, RET testing since 1997

Hypophosphatemic rickets: PHEX since 2002, FGF23 since 2004, DMP1 since 2008, ENPP1 since 2010, SLC34A3 since 2009

Congenital Generalised Lipodystrophy: AGPAT2 since 2010; BSCL2, PTRF and CAV1 since 2012

Hyperphosphatemic Familial Tumoral Calcinosis: since 2007

Pseudohypoaldosteronism type 2: since 2008

Endocrine Neoplasia syndromes:

- Multiple Endocrine Neoplasia type 1: since 1998
- Multiple Endocrine Neoplasia type 2A and 2B: since 1996
- Familial Medullary Thyroid Cancer: since 1996
- Multiple Endocrine Neoplasia type 4: since 2008
- Familial Isolated Pituitary Adenoma: since 2008

Generalised Arterial Calcification of Infancy: since 2011 (ENPP1) and since 2014 (ABCC6)

Chondrodysplasia Punctata: since 2003 (EBP), 2004 (PEX7), 2006 (ARSE), 2012 (AGPS and GNPAT)

Primary Pigmented Nodular Adrenocortical Disease – since 2013

13a. Is there specialised local clinical/research expertise for this disorder?

Yes

13b. If yes, please provide details

Dr Bijay Vaidya, Consultant Endocrinologist

14. Based on experience what will be the national (UK wide) activity, per annum, for:

Index cases

1. **Congenital Hypothyroidism**:
   Estimate 50 per year, based on prevalence of new cases of CH per year in the UK and excluding Transient hypothyroidism caused by transplacental transfer of TSH binding inhibitory immunoglobulins from mothers with autoimmune thyroid disease (1 in 50,000 births) and exposure to iodine.

2. **Familial Phaeochromocytoma and Paraganglioma**:
   Approximately 60. Phaeochromocytoma/Paraganglioma has a prevalence in the population approximately 1/100,000 and the UK population is approximately 60 million. However not all patients will be identified or will consent to testing.

3. **Hypophosphatemic Rickets**
   Approximately 50 per year based on laboratory data

4. **Combined Pituitary Hormone Deficiency**
   Difficult to determine as we are not currently offering this test. The incidence of CPHD is 1/3000-1/4000 births but only a subset of these will be considered to be idiopathic. Therefore likely to be ~10 cases per year.

5. **Congenital Generalised Lipodystrophy**
   5 cases per year based on laboratory data.

6. **Familial Glucorticoid Deficiency**
   Difficult to determine as we are not currently offering this test. The prevalence of FGD is 1/200,000 (O'Riordan et al 2008 J Clin Endocrinol Metab. 93:2896-9). Therefore likely to be ~10 per year.

7. **Familial Tumoral Calcinosis**
   Approximately 10 cases per year based on laboratory data.

8. **Pseudohypoaldosteronism**
   Approximately 5 cases per year

9. **Endocrine Neoplasia syndromes:**

- Multiple Endocrine Neoplasia type 1: Approximately 150 cases per year
- Multiple Endocrine Neoplasia type 2A and 2B: Approximately 50 cases per year
- Familial Medullary Thyroid Cancer: Approximately 50 cases per year
- Multiple Endocrine Neoplasia type 4: Approximately 50 cases per year
- Familial Isolated Pituitary Adenoma: Approximately 150 cases per year

10. **Generalised Arterial Calcification of Infancy**
    Approximately 10 per year based on laboratory data

11. **Chondrodysplasia Punctata**
    Approximately 20 per year based on laboratory data

12. **Familial Isolated Primary Hyperparathyroidism**
    Approximately 100 cases per year based on laboratory data

13. Familial Isolated Hypoparathyroidism
Approximately 5

Family members where mutation is known

1. **Congenital Hypothyroidism:**
   Approximately 50.

2. **Familial Phaeochromocytoma and Paraganglioma:**
   Approximately 150 (on an estimated pick up rate of 10%). However this is highly dependent on individual family structure but generally in family cancer syndromes there is at least 2 or 3 at risk family member that comes forward for presymptomatic testing in a family.

3. **Hypophosphatemic Rickets**
   Approximately 30 cases per year, based on laboratory data.

4. **Combined Pituitary Hormone Deficiency**
   Difficult to predict but likely to be around 20.

5. **Congenital Generalised Lipodystrophy**
   5 per year based on laboratory data.

6. **Familial Glucocorticoid Deficiency**
   Difficult to predict but likely to be around 20.

7. **Familial Tumoral Calcinosis**
   Approximately 10 cases per year based on laboratory data.

8. **Pseudohypoadosteronism type 2**
   Approximately 10 per year

9. **Endocrine Neoplasia syndromes:**

- Multiple Endocrine Neoplasia type 1: Approximately 50 cases per year
- Multiple Endocrine Neoplasia type 2A and 2B: Approximately 30 cases per year
- Familial Medullary Thyroid Cancer: Approximately 30 cases per year
- Multiple Endocrine Neoplasia type 4: Approximately 10 cases per year
- Familial Isolated Pituitary Adenoma: Approximately 50 cases per year

10. **Generalised Arterial Calcification of Infancy**
    Approximately 10 per year

11. **Chondrodysplasia Punctata**
    Approximately 20 per year based on laboratory data

12. **Familial Isolated Primary Hyperparathyroidism**
    Approximately 50 cases per year

13. **Familial Isolated Hypoparathyroidism**
    Approximately 10 cases per year

15. If your laboratory does not have capacity to provide the full national need please suggest how the national requirement may be met. For example, are you aware of any other labs (UKGTN members or otherwise) offering this test to NHS patients on a local area basis only? This question has been included in order to gauge if there could be any issues in equity of access for NHS patients. If you are unable to answer this question please write "unknown".

N/A

16. If using this form as an Additional Provider application, please explain why you wish to provide this test as it is already available from another provider.

Congenital Hypothyroidism:

Our service will provide a more comprehensive test as it includes all genes in which mutations have been shown to cause Congenital Hypothyroidism.

Combined Pituitary Hormone Deficiency

Only two of the genes on this panel are available as individual tests. Although the clinical and biochemical evaluation can help direct to which gene to test, there is significant overlap between the clinical features seen with mutations in individual genes, therefore this test offers a larger set of genes to be tested simultaneously.

Familial Isolated Primary Hyerparathyroidism

Requested by users.

EPIDEMIOLOGY

17a. Estimated prevalence of conditions in the general UK population

Prevalence is total number of persons with the condition(s) in a defined population at a specific time.

Please identify the information on which this is based.

For panel tests, please provide estimates for the conditions grouped by phenotypes being tested.

1. **Congential Hypothyroidism**

   1 in 4000 births (Source: Grant DB, Smith I. Survey of neonatal screening for primary hypothyroidism in England, Wales, and Northern Ireland 1982-4. Br Med J 1988;296:1355-8).

2. **Familial Phaeochromocytoma and Paraganglioma:**

   Estimated to be 1 per 100,000 (data from Orphanet).

3. **Hypophosphatemic Rickets**

   Hypophosphatemia has a prevalence of 1 in 20,000 newborns in Europe (Lorenz-Depiereux et al, Am J Hum Genet 2010, 86:267-272). Accurate data is not available for the UK.

4. **Combined Pituitary Hormone Deficiency**

   1/8000 cases worldwide (Genetics Home Reference). UK-specific prevalence is not known.

5. **Congenital Generalised Lipodystrophy**

   More than 100 cases of CGL have been reported in the literature. Prevalence estimates: 1 in 10 000 000 in USA; 1 in 1 000 000 in Norway; 1 in 200 000 in Lebanon and 1 in 500 000 in Portugal (Agarwal and Garg, Annu Rev Genomics Hum Genet 2006, 7: 175-99). Accurate data is not available for the UK.

6. **Familial Glucocorticoid Deficiency**

   1/200,000 (O'Riordan et al 2008 J Clin Endocrinol Metab. 93:2896-9).

7. **Familial Tumoral Calcinosis**

   No epidemiological data are available. This is a rare syndrome and no accurate estimates of prevalence have been published.

8. **Pseudohypoaldosteronism type 2**

   No epidemiological data are available. This is a rare syndrome and no accurate estimates of prevalence have been published.

9. **Endocrine Neoplasia syndromes:**

- Multiple Endocrine Neoplasia type 1: 1-9/100,000 (Orphanet)
- Multiple Endocrine Neoplasia type 2A: 1-9/100,000 (Orphanet)
- Multiple Endocrine Neoplasia type 2B: Not known
- Familial Medullary Thyroid Cancer: 1-9/100,000 (Orphanet)
- Multiple Endocrine Neoplasia type 4: Not known
- Familial Isolated Pituitary Adenoma: Not known.

10. **Generalised Arterial Calcification of Infancy**

    No epidemiological data are available. This is a rare syndrome and no accurate estimates of prevalence have been published.

11. Chondrodysplasia Punctata

No epidemiological data are available. This is a rare syndrome and no accurate estimates of prevalence have been published.

12. Familial Isolated Primary Hyperparathyroidism

Primary Hyperparathyroidism has a prevalence of 3/1000. Approximately 5% is familial (Sharretts and Simonds 2010 Best Pract Res Clin Endocrinol Metab 24:491-502)

13. Familial Isolated Hypoparathyroidism

No epidemiological data are available. This is a rare syndrome and no accurate estimates of prevalence have been published.

14. Primary Pigmented Nodular Adrenocortical Disease

No epidemiological data are available. This is a rare syndrome and no accurate estimates of prevalence have been published.

17b. Estimated annual incidence of conditions in the general UK population

Incidence is total number of new cases in a year in a defined population.

Please identify the information on which this is based.

For panel tests, please provide for groups of conditions.

1. Congenital Hypothyroidism

150 in 600,000 births (source: UK Newborn Screening Programme Centre 'Screening for Congenital Hypothyroidism' Information sheet).

2. Familial Phaeochromocytoma and Paraganglioma:

1-8/1,000,000 cases

3. Hypophosphatemic Rickets

Not known, an average of 20 new cases diagnosed by the Exeter Laboratory each year from the years 2002 to 2013

4. Combined Pituitary Hormone Deficiency

The incidence of CPHD is 1/3000-1/4000 births (Orphanet). Approximately 10% have a genetic etiology (Phillips et al, 1995, The Metabolic Basis of Inherited Disease, 6 ed. New York, NY: McGraw-Hill;3023-44).

5. Congenital Generalised Lipodystrophy

Approximately 1 case per year based on estimates of incidence for US.

6. Familial Glucocorticoid Deficiency

Not known. Some patients may have episodes of recurring hypoglycemia or convulsions, but FGD may remain undiagnosed for many years.

7. Familial Tumoral Calcinosis

Not known.

8. Pseudohypoaldosteronism type 2

Not known.

9. Endocrine Neoplasia syndromes:

- Multiple Endocrine Neoplasia type 1: 1/30,000 (Orphanet)
- Multiple Endocrine Neoplasia type 2A: 1/40,000 (Orphanet)
- Multiple Endocrine Neoplasia type 2B: Not known, an average of 3 new cases diagnosed each year by our lab.
- Familial Medullary Thyroid Cancer: Not known
- Multiple Endocrine Neoplasia type 4: Not known
- Familial Isolated Pituitary Adenoma: 1/1000 (GeneReviews)

10. Generalised Arterial Calcification of Infancy

Not known

11. Chondrodysplasia Punctata

No epidemiological data are available. This is a rare syndrome and no accurate estimates of prevalence have been published.

12. Familial Isolated Primary Hyperparathyroidism

Not known. We have identified 5 cases with CDC73 mutations and 20 cases with CASR mutations for samples tested between 2013-2014. Cases with mutations identified in MEN1 and RET are classified as having MEN1 or MEN2.

13. Familial Isolated Hypoparathyroidism

Not known

14. Primary Pigmented Nodular Adrenocortical Disease

Not known

18. Estimated gene frequency (Carrier frequency or allele frequency)

Please identify the information on which this is based.

n/a for panel tests.

n/a

19. Estimated penetrance of the condition. Please identify the information on which this is based

n/a for panel tests

n/a

20. Estimated prevalence of conditions in the population of people that will be tested.

n/a for panel tests.

n/a

INTENDED USE (Please use the questions in Annex A to inform your answers)

21. Please tick either yes or no for each clinical purpose listed.

Panel Tests: a panel test would not be used for pre symptomatic testing, carrier testing and pre natal testing as the familial mutation would already be known in this case and the full panel would not be required.

Diagnosis	☒ **Yes**	☐ **No**
Treatment	☒ **Yes**	☐ **No**
Prognosis & management	☒ **Yes**	☐ **No**
Presymptomatic testing (n/a for Panel Tests)	☐ **Yes**	☐ **No**
Carrier testing for family members (n/a for Panel Tests)	☐ **Yes**	☐ **No**
Prenatal testing (n/a for Panel Tests)	☐ **Yes**	☐ **No**

TEST CHARACTERISTICS

22. Analytical sensitivity and specificity

This should be based on your own laboratory data for the specific test being applied for or the analytical sensitivity and specificity of the method/technique to be used in the case of a test yet to be set up.

Please note that the preferred threshold for validation and verification is 95% sensitivity with 95% Confidence Intervals.

Details of the assay validation and panel verification are included as appendices. We have tested a total of 126 unique variants previously identified by Sanger sequencing or MLPA (99 base substitutions, 22 indels and 5 partial/whole gene deletions). The NGS assay detected all variants with no false negative or positive results. Therefore we have 95% confidence that the sensitivity of the assay is >97.5% (error rate 0-2.3%).

There were two heterozygous variants in the HapMap sample identified by genome sequencing (Complete Genomics) that were not present in the v6 tNGS data. These SNVs are located in exons 3 and 4 of NOTCH2. Exons 1-4 of NOTCH2 (chr12) share high sequence homology with the pseudogene NOTCH2P1 located on chromosome 1. Sanger sequencing of these exons confirmed that the SNVs are not present in the HapMap sample and represent a false positive in the Complete Genomics data. Please see Verification document (Appendix 3).

23. Clinical sensitivity and specificity of test in target population

The *clinical sensitivity* of a test is the probability of a positive test result when condition is known to be present; the *clinical specificity* is the probability of a negative test result when disorder is known to be absent. The denominator in this case is the number with the disorder (for sensitivity) or the number without condition (for specificity).

Please provide the best estimate. UKGTN will request actual data after one year service.

The clinical specificity for all disorders is likely to be ≥99%.

Clinical Sensitivity:

1. **Congenital Hypothyroidism**

   This panel tests all of the genes known to cause congenital hypothyroidism, accounting for 5% of all congenital hypothyroidism cases. For the remaining cases, the molecular aetiology has not been elucidated. However by targeting the known genes we have maximised the probability of detecting the causative mutation.

2. **Familial Phaeochromocytoma and Paraganglioma**

   Probability of identifying a SDH mutation in individuals with familial Phaeochromocytoma/ Paraganglioma (PHEO/PGL) is estimated to be 70% (Baysal et al 2002 J Med Genet 39:178). This is higher than for sporadic forms of the disease. TMEM127 mutations have been detected in 30%

of familial PHEO/PGL's and in ~3% of sporadic cases with unknown genetic cause (Qin et al 2010 Nat Genet 42:229). MAX mutations have been detected in 13% of familial PHEO/PGL's with unknown genetic cause (Comino-Mendez et al 2011 Nature Genet. 43: 663-667) and VHL and RET mutations account for up to 10% of familial cases (OMIM). FH mutations were identified in 5/598 (1%) patients with Pheo/PGL but not mutation in the other known genes (Castro-Vega et al, 2014, Hum Mol Genet 23:2440).

3. Hypophosphatemic Rickets

PHEX mutations in account for approximately 70% of hypophosphatemic rickets (Gaucher et al 2009 Hum Genet 125, 401-411). The severity of the disease and specific clinical manifestations are variable even among members of the same family. It is currently unknown in what proportion of individuals the clinical diagnosis of hypophosphatemic rickets is a result of a mutation in FGF23, DMP1 or ENPP1 genes. However by targeting the known genes we have maximised the probability of detecting the causative mutation.

4. Combined Pituitary Hormone Deficiency

PROP1 mutations account for up to 35% of familial CPHD cases (GeneReviews Table 2, PROP1 mutation detection frequency in cohorts with CPHD). It is currently unknown in what proportion of individuals the clinical diagnosis of CPHD is a result of a mutation in POUF1, HESX1, LHX3 or LHX4 genes. However by targeting the five genes listed we have maximised the probability of detecting the causative mutation.

5. Congenital Generalised Lipodystrophy

Mutations in AGPAT2 and BSCL2 genes are found in approximately 80% of CGL families (Agarwal et al, Nat Genet 2002, 31:21-23; Agarwal et al, J Clin Endocrinol Metab 2003, 88:4840-4847). The proportion of patients with mutations in CAV1, PTRF and PPARG is not known due to low numbers tested to date.

6. Familial Glucocorticoid Deficiency

Mutations in all of the genes tested account for approximately 60% of all cases of FGD (Eirini et al 2013, Molecular and Cellular Endocrinology 371:195-200).

7. Familial Tumoral Calcinosis

Most cases of Hyperphosphatemic Familial Tumoral Calcinosis are due to mutations in the GALNT3 gene. Reports in the literature relate to individual case studies. Hyperphosphatemic Familial Tumoral Calcinosis is also biochemically similar to hyperostosis-hyperphosphatemia syndrome (HHS) which is characterised by hyperphosphatemia and recurrent episodes of localised bone lesions with hyperostosis. GALNT3 mutations have also been identified in HHS patients (Frishberg et al. J Mol Med 2005.83:33-38). Ichikawa et al reported a case of a 13-year-old girl with hyperphosphatemic tumoral calcinosis and hyperparathyroidism who had a homozygous mutation in the KLOTHO gene (Ichikawa et al. J Clin Invest 2007. 117: 2684-2691). Topaz et al, 2006, identified SAMD9 mutations in 5 Jewish Yemenite families with Normophosphatemic Familial Tumoral Calcinosis (Am. J. Hum. Genet. 79: 759-764, 2006).

8. Pseudohypoaldosteronism type 2

Mutations in WNK1 or WNK4 are present in approximately 13% of cases (Boyden et al 2012 Nature. 482: 98–102). The majority of families reported have mutations in CUL3 or KLH3. Glover et al identified disease-causing variants in CUL3 and KLHL3 in 63% of pedigrees with PHA2 in whom no WNK1 or WNK4 mutations were identified (Clin Sci [Lond]. 2014; 126: 721–726).

9. Endocrine Neoplasia syndromes:

- **Multiple Endocrine Neoplasia type 1**: Germline mutations in the MEN1 gene are found in approximately 87% of patients with familial MEN1 and 45% of sporadic cases (Ellard et al 2005 Clin Endocrinol 62:169-175).
- **Multiple Endocrine Neoplasia type 2A**: Mutations in exons 5, 8, 10, 11, 13, 14, 15 and 16 of the RET gene have been identified in >98% MEN2A cases (Eng et al 1996 JAMA 276:1575-1579).
- **Multiple Endocrine Neoplasia type 2B**: Approximately 95% of individuals with the MEN 2B phenotype have a single point mutation, p.Met918Thr (p.M918T) in exon 16 of the RET gene (Eng et al 1996 JAMA 276:1575-1579).
- **Familial Medullary Thyroid Cancer**: Mutations in exons 5, 8, 10, 11, 13, 14, 15 and 16 of the RET gene have been identified in >95% FMTC cases (Hansford and Mulligan 2000 J Med Genet 37:817-827).
- **Multiple Endocrine Neoplasia type 4**: 12 index cases have been reported in the literature with mutations in the CDKN1B gene and no mutation in the MEN1 gene.
- **Familial Isolated Pituitary Adenoma:** Mutations in the AIP gene have been reported in approximately 20% of FIPA families (Beckers and Daly 2007 Eur J Endocrinol. 157:371-82.

10. Generalised Arterial Calcification of Infancy (GACI)

Mutations in ENPP1 are identified in ~73% of GACI cases and ABCC6 mutations in 8% of cases (Nitschke et al 2012 Am J Hum Genet 90, 25-39).

11. Chondrodysplasia Punctata

- Autosomal recessive Rhizomelic Chondrodysplasia Punctata: mutations in PEX7 have been identified in 94% cases (Braverman et al 2002 Hum Mutat., 20:284–97), with mutations in AGPS and GNPAT possibly accounting for remaining 6%.
- X-linked recessive Chondrodysplasia Punctata: mutations have been identified in 60-75% males (Nino et al Am J Med Genet A. 2008;146A:997–1008).
- X-linked dominant Chondrodysplasia Punctata: mutations in EBP gene have been identified in 85-95% females with a clinical diagnosis (Has et al 2000 Hum Mol Genet.;9:1951–5).

12. Familial Isolated Primary Hyperparathyroidism

MEN1 mutations have been reported in between 20% (Miedlich et al, 2001, Eur J Endocrinol, 145:155-160, Villablanca et al, 2002, Eur J Endocrinol, 147:313-322) and 57% (Pannett et al, 2003, Clin Endocrinol, 58:639-646) of families with familial isolated hyperparathyroidism. Inactivating mutations of the tumour suppressor gene, CDC73 (formerly known as HRPT2), account for approximately 14% of familial isolated hyperparathyroidism cases and 50% of HPT-JT cases (Cascon et al 2011 Genes Chromosomes and Cancer 50:922-929). Mutations in CASR account for approximately 15-18% of Familial Isolated Hyperparathyroidism cases (Warner et al 2004 J Med Genet 41:155-160). Therefore, the clinical sensitivity in patients presenting with isolated primary hyperparathyroidism is likely to be in excess of 60%.

13. Familial Isolated Hypoparathyroidism

To date, no studies have been performed on a cohort of isolated Hypoparathyroidism cases to determine the clinical sensitivity when tested for all four genes. However, mutations in CASR are found in approximately 42% of isolated Hypoparathyroidism cases (Orphanet). Individual case reports have demonstrated mutations in PTH and GCM2 as causes of Familial Isolated Hypoparathyroidism. Nesbit et al identified GNA11 mutations in 2/8 patients with hypocalcemia and low or normal serum parathyroid hormone concentrations (Nesbit et al, 2013, New Eng. J. Med. 368: 2476-2486).

14. Primary Pigmented Nodular Adrenocortical Disease

In a cohort of 6 paediatric patients with PPNAD, a PRKAR1A mutation was identified in one patient (16%) (Storr et al, 2004, Clin Endocrinol, 61:553-559) and in a cohort of 5 patients, a PRKAR1A mutation in 5/5 (100%) (Groussin et al, 2002, J Clin Endocrinol Metab, 87:4324–4329). A PDE11A mutation was identified in 5/16 (31%) patients with isolated PPNAD (Horvath et al, Nature Genetics, 2006, 38:794-800). A PDE8B mutation was identified in 1/84 (1.2%) of patients with PPNAD (Rothenbuhler et al, 2012, Clin Endocrinol, 77, 195–199) and in a cohort of 20 patients, 1 mutation was identified (5%) (Horvath et al 2008, N Engl J Med, 358:751-752). To date, no studies have been performed on a cohort of isolated PPNAD cases to determine the clinical sensitivity when tested for all three genes.

24. Clinical validity (positive and negative predictive value in the target population)

The *clinical validity* of a genetic test is a measure of how well the test predicts the presence or absence of the phenotype, clinical condition or predisposition. It is measured by its *positive predictive value* (the probability of getting the condition given a positive test) and *negative predictive value* (the probability of not getting the condition given a negative test).

Not currently requested for panel tests

n/a

25. Testing pathway for tests where more than one gene is to be tested sequentially

Please include your testing strategy if more than one gene will be tested and data on the expected proportions of positive results for each part of the process. Please illustrate this with a flow diagram. This will be added to the published Testing Criteria.

n/a for panel tests

n/a

CLINICAL UTILITY

26. How will the test change the management of the patient and/or alter clinical outcome? Please describe associated benefits for patients and family members. If there are any cost savings AFTER the diagnosis, please detail them here.

1. Congenital Hypothyroidism

Mutations in the genes listed in Appendix 1 would enable confirmation of the biochemical diagnosis of Congenital Hypothyroidism, and would enable appropriate counselling for the parents, with risks to future pregnancies determined. Subsequent pregnancies could then be monitored for the presence of a fetal goitre (for genes causing thyroid dyshormonogenesis) and thyroid investigations and genetic analysis performed at birth. This would allow earlier treatment intervention, leading to a better outcome. The identification of a mutation would also prevent the distress for the patient and their parents caused during the thyroxine challenge at 3 years).

2. Familial Phaeochromocytoma and Paraganglioma

Analysing a panel of genes that could be responsible for familial Phaeochromocytoma/Paraganglioma means the test offered has a high sensitivity. When a mutation is identified reporting time and hence patient waiting time is reduced, therefore reducing patient anxiety. In addition identification of specific mutations in specific genes may lead to more investigations to detect associated conditions; for example, detection of a mutation in the RET gene will lead to investigation for a medullary thyroid carcinoma (as a part of multiple endocrine neoplasia type 2) which may result in early detection and treatment of the condition. The type of mutation also affects the prognosis. For example, hereditary phaeochromocytomas are usually bilateral or multifocal and detection of a mutation will allow more careful monitoring for the development of phaeochromocytoma from the second adrenal gland after surgery of the original phaeochromocytoma. SDHB and VHL mutations are more likely to be associated with malignant phaeochromocytoma therefore needing more careful monitoring for recurrence and metastasis; in comparison RET gene mutations almost always cause benign phaeochromocytomas. Mutations in the FH gene have been found in patients with Renal Cell Carcinoma and these patients will therefore need appropriate screening. For family members – presymptomatic testing will allow early diagnosis and hence surveillance of at risk family members.

3. Hypophosphatemic Rickets

Determining the genetic cause in individuals with a clinical diagnosis of hypophosphatemic rickets will allow a definitive diagnosis. A molecular diagnosis determines mode of inheritance and provides a means by which carrier testing can be offered to relatives and offspring at risk. Testing will enable appropriate treatment to be given early for those affected. The different forms of hypophosphatemic rickets are indistinguishable from each other biochemically and a definitive diagnosis can only be made by molecular genetic testing.

4. Combined Pituitary Hormone Deficiency

The main principle of treatment in CPHD is replacement therapy with the appropriate hormones. The identification of the genetic cause would enable appropriate hormones to be given and, in the case of patients with PROP1 mutations without known ACTH deficiency, cortisol levels should be monitored because ACTH deficiency may develop at a later time. A molecular diagnosis determines mode of inheritance and provides a means by which carrier testing can be offered to relatives and offspring at risk and appropriate treatment to be administered.

5. Congenital Generalised Lipodystrophy

A definitive diagnosis of CGL will allow patients to commence treatment and identify those requiring regular follow up. Treatment includes restriction of total fat intake (20-30% total dietary energy) to maintain normal serum triglyceride levels, management of diabetes (which does not differ from that of childhood onset diabetes) and special education for individuals with mental retardation. The following is recommended for follow up: regular screening for glycosuria to identify those affected with diabetes and

then six monthly review to monitor possible retinal, peripheral nerve and renal complications. A yearly liver ultrasound is also recommended to detect fatty infiltration which precedes liver cirrhosis and a yearly echocardiogram is also recommended to monitor cardiomyopathy. Definitive diagnosis allows appropriate treatment of the affected individuals and will allow regular monitoring, potentially avoiding diabetic, cardiac or hepatic complications developing later in life. It is estimated that treating diabetic complications costs up to 10% of the NHS budget.

6. Familial Glucocorticoid Deficiency

Familial Glucocorticoid Deficiency (FGD) syndrome is an adrenal insufficiency without mineralocorticoid deficiency. Therefore establishing a genetic diagnosis of FGD is important both in providing reassurance that mineralocorticoid replacement is not necessary and ensuring appropriate treatment with a replacement therapy of oral hydrocortisone/dexamethasone is undertaken.

7. Familial Tumoral Calcinosis

A genetic diagnosis will allow a definitive diagnosis and the appropriate treatment to be given. Treatment is with phosphate-binding antacids together with dietary phosphate and calcium deprivation, and therapies to increase renal phosphate excretion. This treatment has been shown to reduce ectopic calcification masses and reducing phosphate levels in this way can also assist in preventing their recurrence (Martinez et al. Sem Musculoskeletal Radiol. 2002. 6: 331-339). Surgery may be performed to excise large calcification masses which can be both painful and disabling. Identification of individuals at risk in early childhood will allow treatment to be started sooner potentially avoiding large calcification masses forming and requiring surgical removal.

8. Pseudohypoaldosteronism type 2

The importance of making a genetic diagnosis of PHA2 is that thiazide diuretics effectively reverse the hypertension and hyperkalaemia. Hypertension in patients with PHA2 is estimated to be six times more sensitive to thiazide treatment than in individuals with essential hypertension. Angiotensin converting enzyme inhibitors and angiotensin II receptor blockers should not be used to treat hypertension in PHA2 as they may worsen the hyperkalaemia.

9. Endocrine Neoplasia syndromes

The identification of the genetic cause of the endocrine neoplasias will provide a definitive diagnosis and guide clinical management, specific to the disorder. It also has important implications for family members as it will provide a molecular tool for the identification and management of predisposed individuals, thereby reducing morbidity.

10. Generalised Arterial Calcification of Infancy

GACI can be treated with Bisphosphonates and this has been shown to increase chance of survival. In a study by Rutsch et al 17 patients, who survived their first day of life, were treated with bisphosphonates (as etidronate, pamidronate, clodronate or risedronate). The treatment was associated with survival beyond infancy in 11 cases (65%), whereas 18/26 (69%) patients not treated with bisphosphonates died in infancy (Rutsch et al 2008 Circ Cardiovasc Genet 1, 133-140). Confirmation of the diagnosis enables life-saving treatment, but also means that further investigations attempting to identify the underlying cause are no longer required, thereby saving resources and sparing the patient any associated inconvenience and risk.

11. Chondrodysplasia Punctata

The identification of the genetic cause will allow a definitive diagnosis and resolve the mode of inheritance and enable appropriate carrier testing . A molecular diagnosis will then provide a means by which carrier testing and prenatal testing can be offered.

12. Familial Isolated Primary Hyperparathyroidism

The identification of the genetic cause of primary hyperparathyroidism has a significant impact on clinical management. For example, the identification of a mutation in the *MEN1* gene would direct surgery and prompt surveillance for other MEN1-associted tumours. The identification of mutations causing Familial Hypocalciuric Hypercalcaemia would indicate that no surgical intervention was required. The detection of a mutation in one of the genes tested would enable presymptomatic genetics testing for family members.

13. Familial Isolated Hypoparathyroidism

Treatment is aimed at raising calcium levels high enough to provide symptom relief without causing abnormally high levels of calcium (hypercalciuria). Vitamin D analogs and calcium supplements are the conventional therapy for all forms of hypoparathyroidism.

14. Primary Pigmented Nodular Adrenocortical Disease

Establishing the diagnosis of PPNAD can be challenging, in particular when PPNAD is the only manifestation of the disease and when there are no other members affected in the family. Hypercortisolism usually progresses only slowly and in some cases periodically, and the clinical and laboratory features can be normal during nonhypersecretory periods. The typical growth failure observed in children with Cushing's syndrome is therefore not constant. Plasma ACTH levels are not fully suppressed in all cases in which hypercortisolism is mild or periodic. Furthermore, adrenal imaging can be normal or show only minor alterations with minimal adrenal nodularity that cannot always be distinguished from Cushing's disease or normal glands. In some instances, the diagnosis may be confused with factitious exogenous glucocorticoid excess. (Groussin *et al,* 2006, J Clin Endocrinol Metab 91: 1943–1949). Genetic testing therefore can provide a definitive diagnosis of PPNAD and enable genetic counselling for family members and implementation of appropriate management therefore requires genetic testing. For example, the identification of a *PRKAR1A* mutation enables screening for other Carney complex neoplasias.

27. If this test was not available, what would be the consequences for patients and family members?

1. Congenital Hypothyroidism

If this test was not available, in order to determine if the CH is permanent, or to confirm the diagnosis, patients would have to undergo a thyroxine challenge, where thyroxine treatment is withheld for 6 weeks and the child is monitored biochemically. LaFranchi SH, Austin J. How should we be treating children with congenital hypothyroidism? J Pediatr Endocrinol Metab 2007; 20:559-78). Patients with permanent CH would therefore develop symptoms of CH. If a definitive diagnosis is made by identifying the genetic cause, then this challenge would not need to be undertaken. The clinical management of subsequent pregnancies would need to be based on biochemical testing in the newborn and could result in a delay to commencement of treatment with a less favourable outcome.

2. Familial Phaeochromocytoma and Paraganglioma

For patients, not having a definitive diagnosis may prevent screening for other associated conditions (e.g. Medullary thyroid cancer in the case of a RET mutation or renal cell carcinoma in the case of a VHL or FH mutation), which if left untreated could be life-threatening. Presymptomatic testing would not be available for other family members. Presymptomatic testing also provides useful information for genetic counselling. Also by identifying those individuals who are not at risk of the disease, anxiety is reduced in those individuals.

3. Hypophosphatemic Rickets

Without a definitive diagnosis, offspring of affected individuals would require routine monitoring and are at risk of requiring surgical correction of skeletal malformations due to rickets. Distinguishing hypophosphatemic rickets is particularly important for those who have ENPP1 mutations - current treatment for hypophosphatemia is phosphoris and 1,25-dihydroxyvitamin D supplementation.

However this regime may trigger arterial calcification in these patients and treatment with bisphosphonates is therefore recommended. Identifying patients with ENPP1 mutations will also highlight those at risk of arterial calcifications.

4. Combined Pituitary Hormone Deficiency

Without a genetic diagnosis the long term management of these patients is affected. Subsequent pregnancies would need to be based on biochemical testing in the newborn and could result in a delay to commencement of treatment with a less favourable outcome.

5. Congenital Generalised Lipodystrophy

Not identifying the genetic cause will mean that those at risk of developing diabetic, cardiac or hepatic complications developing later in life will not be identified.

6. Familial glucocorticoid Deficiency

Not identifying those at risk could potentially lead to patients having an Addisonian crisis. Delays in administration of replacement therapy could lead to periods of severe hypoglycaemia at time of infections, and could lead to hypoglycaemic coma. The identification of the genetic cause would enable appropriate counselling and management of family members at risk.

7. Hyperphosphatemic Familial Tumoral Calcinosis

A definitive diagnosis would not be made and family members at risk of morbidity associated with ectopic calcifications would not be identified.

8. Pseudohypoaldosteronism type 2

Without a definitive diagnosis in the proband diagnosis and treatment of other family members with the disorder may be delayed.

9. Endocrine Neoplasia syndromes

For patients, not having a definitive diagnosis may prevent screening for other tumours, which if left untreated could be life-threatening. Presymptomatic testing would not be available for other family members.

10. Generalised Arterial Calcification in Infancy

Without a molecular genetic test a definitive diagnosis will not be achieved and appropriate life-saving treatment may be denied to affected infants. Identifying the genetic also means that further investigations attempting to identify the underlying life-saving cause are no longer required, thereby saving resources and sparing the patient any associated inconvenience and risk.

11. Chondrodysplasia Punctata

Future affected pregnancies would need to rely on USS findings but this may only be possible for the more severely affected fetuses and not until late in the 2nd and 3rd trimesters.

12. Familial Isolated Primary Hyperparathyroidism

Familial Isolated Hyperparathyroidism can occur as a single endocrinopathy but without genetic testing it would not be possible to determine whether the patient is likely to develop other endocrinopathies. This would result in potentially unnecessary screening and repeated surgical interventions. Families of patients would also need to undergo routine biochemical screening if the genetic cause was not identified. The introduction of a panel test would reduce the overall time for a diagnosis to be made than if the genes were analysed in a stepwise manner.

13. Familial Isolated Hypoparathyroidism

Without the identification of the genetic aetiology appropriate clinical management cannot be implemented and could result in a delay in at-risk family members, leading to increased morbidity.

14. Primary Pigmented Nodular Adrenocortical Disease

The lack of a genetic test could result in a delay in diagnosis. The diagnosis of hypercortisolism itself in PPNAD patients can be difficult. Groussin *et al* showed that the cyclic variations of cortisol oversecretion in their patients resulted in a delay of 2–4 years before a diagnosis of Cushing's syndrome was established (Groussin *et al* J Clin Endocrinol Metab, 87:4324–4329). Patients may be misdiagnosed clinically as having Carney complex and undergo unnecessary screening for Carney complex-related neoplasias. Family members may need to undergo unnecessary screening.

28. Is there an alternative means of diagnosis or prediction that does not involve molecular diagnosis? If so (and in particular if there is a biochemical test), please state the added advantage of the molecular test.

1. **Congenital Hypothyroidism:**

   In most cases, the diagnosis of CH is made through the newborn screening program. Patients considered to have CH based on newborn screening test are followed up with measurements of T4 and TSH levels. A thyroid scan is performed to help determine whether the thyroid gland is structurally normal. However these tests will not determine the genetic cause of the thyroid dysgenesis and cannot determine whether the patient is affected by CH due to dyshormonogenesis, as the results are similar to those cases due to severe iodine deficiency in the mother. The advantage of having a molecular test is that it would provide a definitive diagnosis.

2. **Familial Phaeochromocytoma and Paraganglioma**

   No definitive test is available.

3. **Hypophosphatemic Rickets**

   Diagnosis of hypophosphatemic rickets can be made biochemically by identifying hypophosphatemia, hyperphosphaturia, elevated plasma alkaline phosphatase, inappropriately normal levels of vitamin D metabolites. However a biochemical test will not distinguish between the X-linked, autosomal dominant and autosomal recessive forms of hypophosphatemic rickets, important for genetic counselling and to identify those at risk of arterial calcification.

4. **Combined Pituitary Hormone Deficiency**

   Without the availability of a genetic test diagnosis is made using a combination of biochemical and MRI. However, many patients may initially be diagnosed with one pituitary hormone deficiency, but over time develop additional deficiencies.

5. **Congenital Generalised Lipodystrophy**

   Diagnosis of CGL can be made biochemically (e.g. elevated serum triglycerides, elevated serum insulin), by physical examination and/or MRI scans for whole body fat distribution. However these tests will not identify the causative gene and hence the subtype which can be useful in predicting prognosis.

6. **Familial Glucocorticoid Deficiency**

   Diagnosis can be made biochemically. However it will not identify the genetic cause, important for genetic counselling and early identification of the disease.

7. **Familial Tumoral Calcinosis**

   The hyperphosphatemia associated with Hyperphosphatemic Familial Tumoral Calcinosis can be detected biochemically along with serum levels of FGF23, Vitamin D and calcium. The ectopic

calcifications can be identified by a skeletal survey. However each test in isolation will not allow a definitive clinical diagnosis and all these tests together will not identify which gene is likely to carry a mutation. Therefore, a definitive diagnosis relies on molecular genetic testing. Biochemical investigations are normal for Normophosphatemic tumoral calcinosis and diagnosis therefore relies on X-ray findings.

8. Pseudohypoaldosteronism type 2

Diagnosis is based on biochemical examinations that show hyperkalaemia, hyperchloremic acidosis, low or suppressed plasma renin activity and normal to high levels of aldosterone. Glomerular filtration rate is normal. Hypervolemia is frequently observed. However it will not identify the genetic cause, important for genetic counselling and early identification of the disease.

9. Endocrine Neoplasia syndromes

- **Multiple Endocrine Neoplasia type 1:** No definitive test is available.
- **Multiple Endocrine Neoplasia type 2A and 2B:** No definitive test is available. Genetic testing is required to guide surgical management.
- **Familial Medullary Thyroid Cancer:** No definitive test is available. Genetic testing is required to guide surgical management.
- **Multiple Endocrine Neoplasia type 4:** No definitive test available.
- **Familial Isolated Pituitary Adenoma:** No definitive test is available. Genetic testing is required since pituitary adenomas are part of the MEN1 and MEN4 syndromes. By confirming a diagnosis of FIPA, patients do not need to undergo monitoring for other MEN-related tumours.
- **Pure Mucosal Neuroma syndrome:** No definitive test is available

10. Generalised Arterial Calcification of Infancy

Although a diagnosis of GACI can be made on X-ray or ultrasound this does not identify the underlying cause. It is not possible to diagnose this disorder by biochemical testing; therefore a definitive diagnosis relies on molecular genetic testing.

11. Chondrodysplasia Punctata

No definitive test is available.

12. Familial Hyperparathyroidism

Biochemical screening will identify hypercalcaemia but will not provide a definitive diagnosis.

13. Familial Isolated Hypoparathyroidism (FIH)

Biochemical diagnosis is made when hypocalcaemia, hyperphosphoremia, and low or undetectable PTH levels are observed but this does not determine the genetic cause and therefore the availability of genetic testing for at risk family members. It is also important to differentiate FIH from other conditions where the symptoms can be similar to those of FIH, e.g. Barakat syndrome (hypoparathyroidism - sensorineural deafness - renal disease), Kenney-Caffey disease, Sanjad-Sakati syndrome (hypoparathyroidism - retardation - dysmorphism), autoimmune polyendocrine syndrome type I or lymphedema-hypoparathyroidism syndrome.

14. Primary Pigmented Nodular Adrenocortical Disease

Confirmation of hypercortisolism (24hr urinary free cortisol, late night salivary cortisol, overnight and low-dose dexamethasone-suppression test and assessment of midnight plasma cortisol) and plasma ACTH detection to distinguish ACTH-independent CS (values lower than 5-10 pg/ml) from ACTH-dependent Cushing syndrome. In some cases, nodules are visible on adrenal gland computed tomography (CT) or magnetic resonance imaging (MRI). Biochemical confirmation is complicated by the cyclic variations of cortisol oversecretion sometimes seen in patients with PPNAD and is therefore not sensitive. Collection of 24hr urine samples in children may be difficult. Radiologic imaging can be normal or show only subtle nodularity. Molecular Genetic testing allows definitive diagnosis. If the test is not available, there may be repeated MRI scans which in children

could require a general anaesthetic as well as repeated biochemical testing (measuring plasma cortisol requires venepuncture). Multiple paediatric follow up appointments to identify a diagnosis as well as onward referral to tertiary specialist paediatric appointments would be needed. Costs not possible to estimate but likely to be substantial.

29a. What unexpected findings could this test show? For example, lung cancer susceptibility when testing for congenital cataract because ERCC6 gene (primarily associated with lung cancer) is included in a panel test for congenital cataract.

Congenital Hypothyroidism (CH)

NKX2-1: mutations in this gene have been reported in patients with Hereditary Benign Chorea (OMIM 118700) and Brain-Lung-Thyroid syndrome (OMIM 610978). All CH patients reported in the literature also had neurological symptoms (Nettore et al 2013 J Endocrinol Invest 36:654-664).

Familial Glucocorticoid Deficiency

STAR: Mutations in this gene have been reported in patients with Congenital lipoid adrenal hyperplasia (lipoid CAH), a rare autosomal recessive disorder of adrenal and gonadal steroidogenesis. Patients with lipoid CAH typically present with adrenal crisis in early infancy, and those with a 46,XY karyotype have female genitalia. However, it has been recently recognized that the phenotype can be quite variable, in that adrenal insufficiency is detected later in life and patients may have partially masculinized or even normal male genitalia.

Primary Pigmented Nodular Adrenocortical Disease

A mutation in PDE8B has also been reported in a German family with autosomal dominant striatal degeneration (ADSD). These patients did not respond to L-Dopa therapy and no other effective treatment has been reported. The pathomechanism of ADSD has not been elucidated. There have been no further reports of mutations in PDE8B causing ADSD.

29b. Please list any genes where the main phenotype associated with that gene is unrelated to the phenotype being tested by the panel.

Generalised Arterial Calcification of Infancy

ABCC6: mutations in ABCC6 also cause Pseudoxanthoma Elasticum (PXE) is an autosomal recessive multisystem disorder characterised histologically by ectopic mineralisation and fragmentation of elastic fibres of soft connective tissues such as skin, retina and cardiovascular system. Patients have yellowish skin papules on neck and flexural areas, angioid streaks on retina, retinal haemorrhage, diffuse arteriosclerosis and narrowing of small and medium sized arteries. Skin and eye manifestations occur in adolescence but may appear earlier in childhood. Cardiovascular complications usually develop later in mid-adulthood.

30. If testing highlights a condition that is very different from that being tested for, please outline your strategy for dealing with this situation.

ABCC6: findings would be reported. There have been reports in the literature of patients having both disorders.

31. If a panel test, is this replacing an existing panel/multi gene test and/or other tests currently carried out through UKGTN using Sanger sequencing? If so, please provide details below.

- TPO and TG genes (Thyroid dyshormonogenesis dossier submitted by Glasgow RGC)
- TSHR gene (Congenital Hypothyroidism dossier submitted by Cardiff SAS Porphyria and Cambridge RGC
- Phaeochromocytoma and Paraganglioma genes (gene dossier submitted by our laboratory and approved in 2012: PROP1 and POU1F1 genes (testing listed on UKGTN as being offered by the Birmingham laboratory)
- AGPAT2 and BSCL2 genes (gene dossier submitted by our laboratory and approved in 2010:
- GALNT3 and KL (gene dossiers submitted by our laboratory and approved in 2009 and 2014, respectively;

- MEN1, RET, AIP (no evaluation numbers available as pre-2009)
- ABCC6 gene (gene dossier submitted by our laboratory and approved in 2014;
- ARSE, AGPS, GNPAT, PEX7 and EBP (gene dossiers submitted by our laboratory;
- Familial Hyerparathyroidism dossier submitted by Oxford RGC. CDC73 gene dossier submitted by our laboratory and approved in 2012)
- Primary Pigmented Nodular Adrenocortical Disease dossier submitted by our laboratory and approved in 2014

32. Please describe any specific ethical, legal or social issues with this particular test.

This panel has been designed such that it is divided into specific subpanels. This enables the clinician to obtain consent for the specific sub-panel of genes rather than the whole panel. Disease-specific request forms will be available listing the genes tested under specific subpanels.

To ensure that data from non-consented genes are not analysed, Variant calling initially occurs across the genomic intervals (based around the coordinates of the start and end of the genetic feature in question) of all of the genes included in the relevant panel. A filtering stage then selects variants that fall within the genomic intervals for the genes specific to the phenotype in question. This process is fully automated, and the initial full variant call file is kept in case reanalysis against a different set of genes is required.

IS IT A REASONABLE COST TO THE PUBLIC?

33. In order to establish the potential costs/savings that could be realised in the diagnostic care pathway, please list the tests/procedures that would be required in the index case to make a diagnosis if this genetic test was not available.

1. Congenital Hypothyroidism

This test replaces testing offered by another laboratory in a sequential manner by Sanger sequencing and is therefore cost neutral.

2. Familial Phaeochromocytoma and Paraganglioma

This test replaces testing currently offered by our laboratory by Sanger sequencing and is therefore cost neutral.

3. Hypophosphatemic Rickets

This test replaces testing currently offered by our laboratory by Sanger sequencing and is therefore cost neutral.

4. Combined Pituitary Hormone Deficiency

	Type of test	Cost (£)
Costs and type of imaging procedures		
Costs and types of laboratory pathology tests (other than molecular/cyto genetic test proposed in this Gene Dossier)	Biochemical tests	>50
Costs and types of physiological tests (e.g. ECG)		
Cost and types of other investigations/procedures (e.g. biopsy)		
Total cost of tests/procedures no longer required (please write n/a if the genetic test does not replace any other tests procedures in the diagnostic care pathway)		**N/A**

5. Congenital Generalised Lipodystrophy

This test replaces testing currently offered by our laboratory by Sanger sequencing and is therefore cost neutral.

6. Familial Glucocorticoid Deficiency

	Type of test	Cost (£)
Costs and type of imaging procedures		
Costs and types of laboratory pathology tests (other than molecular/cyto genetic test proposed in this Gene Dossier)	Biochemical tests	>50
Costs and types of physiological tests (e.g. ECG)		
Cost and types of other investigations/procedures (e.g. biopsy)		
Total cost of tests/procedures no longer required (please write n/a if the genetic test does not replace any other tests procedures in the diagnostic care pathway)		**N/A**

7. Familial Tumoral Calcinosis

This test replaces testing currently offered by our laboratory by Sanger sequencing and the addition of SAMD7 as part of the panel test costs less than the current charge for sequence analysis of the 3 genes by Sanger sequencing.

8. Pseudohypoaldosteronism type 2

	Type of test	Cost (£)
Costs and type of imaging procedures		
Costs and types of laboratory pathology tests (other than molecular/cyto genetic test proposed in this Gene Dossier)	Biochemical tests	>50
Costs and types of physiological tests (e.g. ECG)		
Cost and types of other investigations/procedures (e.g. biopsy)		
Total cost of tests/procedures no longer required (please write n/a if the genetic test does not replace any other tests procedures in the diagnostic care pathway)		**N/A**

9. Endocrine Neoplasias
This test replaces testing currently offered by our laboratory by Sanger sequencing and is therefore cost neutral.

10. Generalised Arterial Calcification of Infancy
This test replaces testing currently offered by our laboratory by Sanger sequencing and is therefore cost neutral.

11. Chondrodysplasia Punctata
This test replaces testing currently offered by our laboratory by Sanger sequencing and is therefore cost neutral. Each subtype will continue to be available as a single gene test based on phenotype.

12. Familial Isolated Primary Hyperparathyroidism
This test replaces testing currently offered by our laboratory by Sanger sequencing and is therefore cost neutral.

13. Familial Isolated Hypoparathyroidism

	Type of test	**Cost (£)**
Costs and type of imaging procedures		
Costs and types of laboratory pathology tests (other than molecular/cyto genetic test proposed in this Gene Dossier)	Biochemical tests	>50
Costs and types of physiological tests (e.g. ECG)		
Cost and types of other investigations/procedures (e.g. biopsy)		
Total cost of tests/procedures no longer required (please write n/a if the genetic test does not replace any other tests procedures in the diagnostic care pathway)		**N/A**

14. Primary Pigmented Nodular Adrenocortical Disease
This test replaces testing currently offered by our laboratory by Sanger sequencing and is therefore cost neutral.

34. Based on the expected annual activity of index cases (Q14), please calculate the estimated annual savings/investments based on information provided in Q33.

Combined Pituitary Hormone Deficiency

Number of index cases expected annually	(a) 10
Cost to provide tests for index cases if the genetic test in this Gene Dossier was not available (see Q32)	(b)
Total annual costs pre genetic test	(a) x (b) = (c)
Total annual costs to provide genetic test	£7500- cost for two genes already covered by approved dossiers therefore marginal costs = £360
Additional investment for 100% positive rate for index cases	(d) – (c) = (e) £360
Percentage of index cases estimated to be negative	(f)
Number of index cases estimated to be negative	(f) x number of index cases = (g)
Costs to provide additional tests for index cases testing negative	(g) x (b) = (h)
Total costs for tests for index patient activity	(e) + (h) = (i) £360
Total costs for family members	Costs for family member test x number of family members expected to test in a year (j) £2000
If there is a genetic test already available and some of the family testing is already being provided, please advise the cost of the family testing already available	Cost for family member testing already available x estimated number of tests for family members already provided (k) £2000
Total costs for family members minus any family member testing costs already provided	(j) – (k) = (l) £0
Additional costs for all activity expected in a year	(i) + (j) or (i) + (l) £360

Familial Glucocorticoid Deficiency

Number of index cases expected annually	(a) 10
Cost to provide tests for index cases if the genetic test in this Gene Dossier was not available (see Q32)	(b)
Total annual costs pre genetic test	(a) x (b) = (c)
Total annual costs to provide genetic test	(a) x cost of genetic testing for index case = (d) £7500
Additional investment for 100% positive rate for index cases	£7500
Percentage of index cases estimated to be negative	(f) 40
Number of index cases estimated to be negative	(f) x number of index cases = (g) 4
Costs to provide additional tests for index cases testing negative	(g) x (b) = (h) n/a
Total costs for tests for index patient activity	(e) + (h) = (i) £7500
Total costs for family members	Costs for family member test x number of family members expected to test in a year (j) £2000
If there is a genetic test already available and some of the family testing is already being provided, please advise the cost of the family testing already available	Cost for family member testing already available x estimated number of tests for family members already provided (k)
Total costs for family members minus any family member testing costs already provided	(j) – (k) = (l) £2000
Additional costs for all activity expected in a year	(i) + (j) or (i) + (l) £9500

Pseudohypoaldosteronism type 2

Number of index cases expected annually	(a) 5
Cost to provide tests for index cases if the genetic test in this Gene Dossier was not available (see Q32)	
Total annual costs pre genetic test	(a) x (b) = (c)
Total annual costs to provide genetic test	(a) x cost of genetic testing for index case = (d) £3750
Additional investment for 100% positive rate for index cases	(d) – (c) = (e) £3750
Percentage of index cases estimated to be negative	(f) 33
Number of index cases estimated to be negative	(f) x number of index cases = (g) 1
Costs to provide additional tests for index cases testing negative	(g) x (b) = (h) n/a
Total costs for tests for index patient activity	(e) + (h) = (i) £3750
Total costs for family members	Costs for family member test x number of family members expected to test in a year (j) £1000
If there is a genetic test already available and some of the family testing is already being provided, please advise the cost of the family testing already available	Cost for family member testing already available x estimated number of tests for family members already provided (k)
Total costs for family members minus any family member testing costs already provided	(j) – (k) = (l) £1000
Additional costs for all activity expected in a year	(i) + (j) or (i) + (l) £4750

Familial Isolated Hypoparathyroidism

Number of index cases expected annually	(a)
Cost to provide tests for index cases if the genetic test in this Gene Dossier was not available (see Q32)	(b)
Total annual costs pre genetic test	(a) x (b) = (c)
Total annual costs to provide genetic test	(a) x cost of genetic testing for index case = (d)
Additional ~~savings~~/investment for 100% positive rate for index cases	(d) – (c) = (e)
Percentage of index cases estimated to be negative	(f) not known
Number of index cases estimated to be negative	(f) x number of index cases = (g)
Costs to provide additional tests for index cases testing negative	(g) x (b) = (h)
Total costs for tests for index patient activity	(e) + (h) = (i)
Total costs for family members	Costs for family member test x number of family members expected to test in a year (j)
If there is a genetic test already available and some of the family testing is already being provided, please advise the cost of the family testing already available	Cost for family member testing already available x estimated number of tests for family members already provided (k)
Total costs for family members minus any family member testing costs already provided	(j) – (k) = (l)
Additional cost -for all activity expected in a year	(i) + (j) or (i) + (l) **cost neutral**

35. REAL LIFE CASE STUDY
Please provide a case study that illustrates the benefits of this test

Familial Phaeochromocytoma and Paraganglioma

A 39 year old woman was referred to the headache clinic with a 10 year history of episodic headaches, sweating, and palpitations. Investigation showed bilateral phaeochromocytoma. Genetic testing showed RET gene (p.C634G) mutation. She was also found to have a small nodule in her thyroid, which further investigation with fine needle aspiration showed features of medullary thyroid carcinoma. She underwent total thyroidectomy and the histology confirmed the diagnosis of medullary thyroid carcinoma. She had two children, who underwent predictive testing. Her son did not have the mutation; however her teenaged daughter carried the mutation. In view of this, she underwent prophylactic total thyroidectomy. There was no evidence of full blown medullary thyroid carcinoma; however, the histology showed that she had already developed c-cell hyperplasia (precursor medullary thyroid carcinoma). On biochemical testing, she has not developed phaeochromocytoma yet but she is at risk and is under close surveillance.

Hypophosphataemic Rickets

Patient S was a 39 year old female who was affected with Hypophosphatemic Rickets. The parents were not known to be consanguineous. She was initially referred in March 2007 for mutation analysis of the PHEX gene. No mutation was identified and FGF23 testing was offered. This was requested by the clinician and reported in May 2007 (no mutation was identified). In 2008 our laboratory was asked to carry out mutation analysis of the DMP1 gene but no mutations were identified. In 2009 we were then asked to test for mutations in the SLC34A3 gene but no mutations were identified. In 2010 we were asked to carry out mutation analysis of the ENPP1 gene. The patient was shown to be compound heterozygous for a novel missense mutation and a nonsense mutation. In silico evidence was consistent with the novel missense mutation being pathogenic. Parental samples were tested that showed that these mutations were in trans. A diagnosis of autosomal recessive Hypophosphatemic Rickets was confirmed. This case demonstrates the utility of testing these genes as a panel. By testing each of the genes in a sequential manner it took 3 years to elucidate the genetic cause of the hypophosphatemic rickets observed in this patient and cost £1,620 (panel test cost: £750).

Hyperphosphatemic Familial Tumoral Calcinosis

Patient N was a 3 year old girl with tumoral calcinosis referred to our laboratory for testing. Her parents were consanguineous. Mutation analysis of the GALNT3 gene showed that she was homozygous for a novel GALNT3 missense mutation, p.C401R, located in Catalytic B domain of GALNT3. This cysteine is conserved across GALNT3, GALNT8, GALNT12 and GALNT13 (Hussain and Nasir 2014 J Cell Biochem 115:313-327) and is predicted by UniPROT to form a disulphide bond with the cysteine at position 482 in GALNT3. Therefore this finding was consistent with a diagnosis of HFTC. Her parents, who were clinically unaffected, were heterozygous for the variant.

Pseudohypoaldosteronism type 2

A 41 year old man was found to have high blood pressure of 220/100mmHg during a routine health check-up for medical insurance. Initial investigations showed serum sodium 144mmo/L (reference range 132-144), potassium 6mmol/L (3.5-5.5) and creatinine 100µmol/L (45-120). Renal ultrasound, renal perfusion scan and chest radiograph were also normal. The patient was given lifestyle advice, and was commenced on a beta blocker. Over the next seven years, further antihypertensive agents were gradually added. During this period, following an episode of renal colic, he was found to have a renal stone, and was treated with lithotripsy. Despite multiple antihypertensive agents, his blood pressure was still consistently above 150/90mmHg. In addition, his serum potassium levels, measured on several occasions, remained high between 5.8-6.6mmol/L. Therefore, the patient was referred to the Endocrine outpatient clinic for further review. In the Endocrine clinic, it was noted that his mother and his only sister also suffered from hypertension and hyperkalaemia. As well as a high serum potassium level, he also had hyperchloraemic metabolic acidosis and marked hypercalciuria. His renal function, renin and aldosterone levels were normal. These results, taken together with the history of familial hypertension and hyperkalaemia, were considered to be consistent with a diagnosis of pseudohypoaldosteronism type 2 (PHA2). Sequence analysis identified a novel heterozygous missense mutation in WNK4. Therefore, his antihypertensive agents were switched to bendrofluazide 2.5mg once a day. On this drug, his blood pressure improved and has remained well controlled below 140/80mmHg. In addition, his biochemical abnormalities, including hyperkalaemia, hyperchloraemic metabolic acidosis and hypercalciuria, all reverted to normal.

Endocrine Neoplasia syndromes

Patient L was a 19 year old male with a growth hormone secreting pituitary tumour. His brother had a non-secreting pituitary tumour diagnosed at the age of 18 years and his mother had a spinal ependymona. Mutation analysis of the MEN1 gene in 2003 did not identify a mutation. In 2008 mutation analysis of the AIP gene was carried out which showed that Patient L was heterozygous for a deletion of exon 2 of the AIP gene, thus confirming a diagnosis of Familial Isolated Pituitary Adenoma. This family no longer needed monitoring for other MEN1-related tumours.

Generalised Arterial Calcification of Infancy

Patient S was a neonate who had persistent pulmonary hypertension and calcification of the great vessels. Mutation analysis of the ENPP1 gene identified a homozygous frameshift mutation. Unfortunately the patient died before the diagnosis was confirmed.

36. Please only complete this question if there is previously approved Testing Criteria.
Please contact the UKGTN office if you are unsure whether testing criteria is available.

36a. Do you agree with the previously approved Testing Criteria? Yes/No

- Congenital Hypothyroidism – agree with previously approved testing criteria
- Familial Phaeochromocytoma and Paraganglioma – agree with previous criteria
- Hypophosphataemic Rickets – suggested additions to previously approved criteria (see below)
- Congenital Generalised Lipodystrophy – agree with previously approved testing criteria
- Familial Tumoral Calcinosis – suggested additions to previously approved criteria (see below)
- Endocrine Neoplasias
  - Multiple Endocrine Neoplasia types 2A and 2B – agree with previous criteria
  - Familial Isolated Pituitary Adenoma – agree with previous criteria
- Generalised Arterial Calcification of Infancy – agree with previous criteria
- Chondrodysplasia Punctata – agree with previous criteria
- Familial Isolated Primary Hyperparathyroidism – agree with previous criteria
- Primary Pigmented Nodular Adrenocortical Disease – agree with previous criteria

Sub panel: Congenital hypothyroidism
Currently there are TC for the TSHR gene and hypothyroidism type 1 only. There is also TC for types 2a and 3 of thyroid dyshormonogenesis (genes TPO and TG) but this differs to the TC for TSHR. The sub panel also has five genes for other types of disorders and therefore a new TC is provided to cover the clinical entry point for this sub panel.

Sub panel: Congenital generalised lipodystrophy
There is one TC for types 1 and 2 and a separate and different TC for type 3 – there are no TC for type 4. Therefore a new TC for this sub pane has been provided.

Sub panel: generalised arterial calcification of infancy
It is noted that there are only two genes on this sub panel and they are for types 1 and 2 of arterial calcification of infancy. The TC currently available is for type 1 only, therefore new TC is provided below to cover entry point for types 1 and 2.

Sub panel: chondrodysplasia punctate
There are five genes on this sub panel for three types of rhizomelic chondrodysplasia punctate and two types of chondrodysplasia punctate x linked. There are three separate TC already available that differ for three of the conditions and no TC for the other two conditions. Therefore a new TC for this sub panel has been created.

Sub panel: familial isolated hypoparathyroidism
There are no TC for any of these conditions on this panel test even though Oxford already offers sanger testing for three of the genes. Therefore a TC for this sub panel has been provided.

36b. If you do not agree, please provide revised Testing Criteria on the Testing Criteria form and explain below the reasons for the changes.

UKGTN Testing Criteria

Test name: **Congenital Hypothyroidism 8 gene panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Paediatric Endocrinologists	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Raised serum thyroid stimulating hormone level **AND** • Nongoitrous thyroid gland in a normal position **AND** absence of thyroid autoantibodies **OR** • Presence of an enlarged thyroid gland in situ **OR** • Muscular hypotonia **AND** thyroid hypoplasia agenesis	
OR Cleft palate **AND** thyroid hypoplasia agenesis	
OR Congenital Hypothyroidism, nongoitorous	
OR Choreoathetosis and congenital hypothyroidism with or without pulmonary dysfunction	
OR Thyroid dyshormonogenesis	
OR Hypothyroidism with spiky hair and cleft palate	

Additional Information:
For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name: **Hypophosphataemic Rickets 5 gene panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Clinical diagnosis of Hypophosphatemic Rickets with evidence of decreased renal phosphate reabsorption **AND**	
Family history suggestive of X-linked dominant, autosomal dominant or autosomal recessive inheritance **OR**	
Early arterial onset calcification	

Additional Information:
For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name: **Combined Pituitary Hormone Deficiency 5 gene panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Endocrinologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Neonatal/childhood onset **AND**	
Biochemical evidence of CPHD (deficiency of at least two pituitary hormones)	

Additional Information:
For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name: **Congenital Generalised Lipodystrophy 5 gene panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Endocrinologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Patient meets 3 major criteria **OR** 2 of the major criteria **AND** 2 or more minor criteria: **Major criteria:** • Lipoatrophy affecting the trunk, limbs, and face. • Acromegaloid features • Hepatomegaly • Elevated serum concentration of triglycerides • Insulin resistance **Minor criteria:** • Hypertrophic cardiomyopathy • Psychomotor retardation or mild to moderate cognitive impairment • Hirsuitism • Precocious puberty in females • Bone cysts • Phlebomegaly • Congenital myopathy	

For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

See over the page for additional information

Additional Information:

Major criteria

•Lipoatrophy affecting the trunk, limbs, and face. Generalized lipodystrophy is apparent at birth. In some individuals, the face may be normal at birth with lipoatrophy becoming apparent during the first months of life. Lipoatrophy gives an athletic appearance, especially because skeletal muscle hypertrophy is also present.

•Acromegaloid features include gigantism, muscular hypertrophy, advanced bone age, prognathism, prominent orbital ridges, enlarged hands and feet, clitoromegaly, and enlarged external genitalia in the male.

•Hepatomegaly. Liver enlargement is secondary to fatty liver early on and to cirrhosis late in the disease course.

•Elevated serum concentration of triglycerides. Serum concentration of triglycerides can be elevated up to 80 g/L, and is sometimes associated with hypercholesterolemia.

•Insulin resistance. Elevated serum concentrations of insulin and C-peptide may occur starting in the first years of life. Overt clinical diabetes mellitus usually develops during the second decade. Its early clinical expression is acanthosis nigricans of the groin, neck, and axillae, which may have, in some cases, a verrucous appearance.

Minor criteria

•Hypertrophic cardiomyopathy may be present in infancy or develop later in life.

•Psychomotor retardation or mild (IQ 50-70) to moderate (IQ 35-50) intellectual impairment. Approximately 80% of individuals with mutations in BSCL2 have mild-to-moderate intellectual impairment, whereas only 10% of individuals with mutations in AGPAT2 have intellectual impairment.

•Hirsutism manifests with low frontal and posterior hairline; hypertrichosis is apparently independent of hormonal stimulation.

•Precocious puberty in females. In a series of 75 individuals with BSCL, three females underwent puberty before age seven years [Van Maldergem et al 2002].

•Bone cysts occur in 8%-20% of affected individuals and have a polycystic appearance on x-ray. Located in the epiphyseal and metaphyseal regions of the long bones, bone cysts are often diagnosed during the second decade and are mostly observed in individuals with mutations in AGPAT2.

•Phlebomegaly. Prominence of the veins of the lower and upper limbs is observed, in part because of the lack of subcutaneous fat

UKGTN Testing Criteria

Test name: **Familial Glucocorticoid Deficiency 5 gene panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Endocrinologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Biochemical findings consistent with FGD (low serum cortisol and high plasma ACTH levels) **AND**	
Suggestive family history **OR** no other obvious cause	

Additional Information:
For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name: **Pseudohypoaldosteronism type II 4 gene panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Endocrinologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Hypertension that is unresponsive to anti-hypertensives **AND**	
Elevated serum potassium and chloride levels, hypercalciuria **AND**	
Normal renal function (renin and aldosterone levels within the normal range)	

Additional Information:
For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name: **Endocrine Neoplasia syndromes 4 gene panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Endocrinologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Two MEN related tumours **OR**	
Parathyroid tumour in a person < 30 years where the result will affect immediate clinical management **OR**	
1 MEN tumour with family history **OR**	
Medullary thyroid cancer in a person < 40 years	

Additional Information:
Where an individual presents and it is clear that they have either MEN1 or MEN2, then these individual sanger tests should be carried out first.

For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name: **Generalised Arterial calcification of Infancy 2 gene panel test**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Paediatrician/Neonatologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Early onset arterial calcification in neonatal period	

Additional Information:
For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name **Chondrodysplasia Punctata 5 gene panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Stippling involving the epiphyses of the long bones and vertebrae, the trachea and distal ends of the ribs seen on x-ray **OR**	
Rhizomelia with stippling involving the epiphyses knee, hip, elbow, and shoulder **OR**	
Biochemical evidence of Chondrodysplasia punctata	

Additional Information:
For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name **Familial Isolated Hypoparathyroidism 4 gene panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Endocrinologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Biochemical evidence of hypoparathyroidism: low serum PTH and calcium levels	

Additional Information:
For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name: **Hyperphosphatemic & Normophosphatemic Familial Tumoral Calcinosis 4 gene panel test**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Endocrinologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Individuals with a clinical diagnosis of Hyperphosphatemic Familial Tumoral Calcinosis (HFTC) with all of the following: • Ectopic calcification • Hyperphosphatemia • Normal or elevated levels of vitamin D	
Individuals with a clinical diagnosis of Normophosphatemic Familial Tumoral Calcinosis (NFTC) with all of the following: • Ectopic calcification • Normal phosphate and vitamin D levels	

Additional Information:
For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name: **Familial Pheochromocytoma and Paraganglioma 10 Gene Panel**	
Approved name and symbol of disorder/condition(s): See appendix 1 **Approved name and symbol of gene(s):** See appendix 1	
Patient name:	
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Endocrinologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
One of the following:	
PGL in neck or elsewhere	
PHEO with family history of PGL	
PHEO <45 yrs of age	
PHEO over 45yrs of age*	
PHEO with other syndromic features[#] e.g. MEN2, VHL – **specify**	
Malignant PHEO over 45yrs of age	
Bilateral PHEO / Multiple tumours	
Confirmation of affected status in a family with known mutation	

- indicates testing for genes specific to stated syndrome
*****Indicates testing for only *TMEM127* and *SDHB* as patients with a later age of diagnosis are more likely to have mutations in these genes than mutations in the other genes (Yao *et al* 2010, Jafri *et al*, 2012)

Additional Information:
For panel tests: At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation.

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name: **Familial Isolated Primary Hyperparathyroidism 8 Gene Panel**
Approved name and symbol of disorder/condition(s): See appendix 1 **Approved name and symbol of gene(s):** See appendix 1
Patient name: **Patient postcode:** **NHS number:** **Name of referrer:** **Title/Position:** **Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Endocrinologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Primary hyperparathyroidism in the index case **AND** affected first degree relative	
OR Clinical, biochemical or radiological features suggestive of either the MEN1, MEN2A or HPT-JT syndromes	
OR History of parathyroid carcinoma	
OR Features suggestive of FHH e.g. benign hypercalcaemia and suppressed urine calcium excretion	

Additional Information:
For panel tests: At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation.

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name: **Primary Pigmented Nodular Adrenocortical Disease 3 Gene Panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Endocrinologist	
Consultant Clinical Geneticist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Primary Pigmented Nodular Adrenocortical Disease **OR**	
Clinical diagnosis of ACTH independent Cushing syndrome of unknown etiology	

Additional Information:
For panel tests: At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation.

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

Appendix 1

Genes in panel test and associated conditions

Rows that are highlighted yellow indicate where the gene was currently being fully analysed in the context of a single separate UKGTN test at time of submission.

HGNC standard name and symbol of the gene	HGNC number	OMIM number	OMIM standard name of condition and symbol	Mode of inheritance	OMIM number	Evidence of association between gene(s) and condition	% of horizontal coverage of gene	MLPA	Comments
Congenital Hypothyroidism									
TSHR	12373	603372	Hypothyroidism, congenital nongoitrous, 1; CHNG1	AR	275200	PubMed: 7528344	100	No	
PAX8	8622	167415	Hypothyroidism, congenital nongoitrous, 2; CHNG2	AD	218700	PubMed: 23698639	100	No	
NKX2-1	11825	600635	Choreoathetosis and congenital hypothyroidism with or without pulmonary dysfunction; CAHTP	AD	610978	PubMed: 24714694	100	No	
FOXE1	3806	602617	Hypothyroidism, athyroidal, with spiky hair and cleft palate	AR	241850	PubMed: 23698639	100	No	
TPO	12015	606765	Thyroid dyshormonogenesis 2a; TDH2A	AR	274500	PubMed: 8964831	100	No	
TG	11764	188450	Thyroid dyshormonogenesis 3; TDH3	AR	274700	PubMed: 1752952	100	No	
DUOX2	13273	606759	Thryoid dyshormonogenesis 6; TDH6	AR	607200	PubMed: 12110737	100	No	

THRA	11796	190120	Hypothyroidism, congenital nongoitrous, 6; CHNG6	AD	614450	PubMed: 22494134 and 22168587	100	No	
Familial Phaeochromocytoma and Paraganglioma									
RET	9967	164761	Pheochromocytoma	AD	171300	PubMed: 8825918	Exons and flanking introns of exons 5, 8, 10, 11, 13, 14, 15 and 16	No	
VHL	12687	608537	Pheochromocytoma	AD	171300	PubMed: 8825918	100	No	
SDHA	10680	600857	Paragangliomas 5; PGL5	AD	614165	PubMed: 21752896	100	No	
SDHB	10681	185470	Pheochromocytoma Paragangliomas 4; PGL4	AD	171300 115310	PubMed: 11404820	100	No	
SDHC	10682	602413	Paragangliomas 3; PGL3	AD	605373	PubMed: 11062460	100	No	
SDHD	10683	602690	Pheochromocytoma Paragangliomas 1; PGL1	AD	171300 16800	PubMed: 10657297	100	No	
SDHAF2	26034	613019	Paragangliomas 2; PGL2	AD	601650	PubMed: 19628817	100	No	
TMEM127	26038	613403	Pheochromocytoma	AD	171300	Pubmed: 20154675	100	No	
MAX	6913	154950	Pheochromocytoma	AD	171300	PubMed: 21685915	100	No	
FH	3700	136850	No OMIM name	AD		PubMed: 24334767	100	No	
Hypophosphatemic Rickets									
PHEX	8918	307800	Hypophosphatemic rickets, X-linked dominant; XLHR	XLD	300550	PubMed: 7550339	100	No	
FGF23	3680	605380	Hypophosphatemic rickets, autosomal dominant; ADHR	AD	193100	PubMed: 11062477	100	No	
DMP1	2932	600980	Hypophosphatemic	AR	241520	PubMed: 17033625	100	No	

			Rickets, Autosomal Recessive, 1; ARHR1						
ENPP1	3356	173335	Hypophosphatemic Rickets, Autosomal Recessive, 2; ARHR2	AR	613312	PubMed: 20137773	98.61	No	
SLC34A3	20305	609826	Hypophosphatemic Rickets With Hypercalciuria, Hereditary; HHRH	AR	241530	PubMed: 16358215	100	No	
Combined Pituitary Hormone Deficiency									
PROP1	9455	262600	Pituitary Hormone Deficiency, Combined, 2; CPHD2	AR	604538	PubMed: 9462743	100	No	
POU1F1	9210	173110	Pituitary Hormone Deficiency, Combined, 1; CPHD1	AD (dominant negative) and AR	613038	PubMed: 1472057 PubMed: 1302000	100	No	
HESX1	4877	601802	Pituitary Hormone Deficiency, Combined, 5; CPHD5	AR (mild form shows AD inheritance)	182230	PubMed: 9620767 PubMed: 11136712	100	No	
LHX3	6595	600577	Pituitary Hormone Deficiency, Combined, 3; CPHD3	AR	221750	PubMed: 10835633	100	No	
LHX4	21734	602146	Pituitary Hormone Deficiency, Combined, 4; CPHD4	AD	262700	PubMed: 11567216	100	No	
Congenital Generalised Lipodystrophy									
AGPAT2	325	603100	Lipodystrophy, Congenital Generalized, Type 1; CGL1	AR	608594	PubMed: 11967537	100	No	
BSCL2	15832	606158	Lipodystrophy, Congenital Generalized, Type 2; CGL2	AR	269700	PubMed: 11479539	100	No	

CAV1	1527	601047	Lipodystrophy, Congenital Generalized, Type 3; CGL3	AR	612526	PubMed: 18211975	100	No	
PTRF	9688	603198	Lipodystrophy, Congenital Generalized, Type 4; CGL4	AR	613327	PubMed: 19726876	100	No	
PPARG	9236	601487	Lipodystrophy, familial partial, type 3; FPLD3	AR	604367	PubMed: 24980513	100	No	
Familial Glucocorticoid Deficiency									
MC2R	6930	607397	Glucocorticoid deficiency 1; GCCD1	AR	202200	PubMed: 8094489 PubMed: 8227361	100	No	
MRAP	1304	609196	Glucocorticoid deficiency 2; GCCD2	AR	607398	PubMed: 15654338	100	No	
STAR	11359	600617	Lipoid congenital adrenal hyperplasia; LCAH	AR	201710	PubMed: 7892608	100	No	
MCM4	6947	602638	Natural killer cell and glucocorticoid deficiency with DNA repair defect; NKGCD	AR	609981	PubMed: 18430777	100	No	
NNT	7863	607878	Glucocorticoid deficiency 3; GCCD3	AR	614736	PubMed: 22634753	100	No	
Familial Tumoral Calcinosis									
GALNT3	4125	601756	Tumoral Calcinosis, Hyperphosphatemic, Familial; HFTC	AR	211900	PubMed: 15133511	100	No	
FGF23	3680	605380	Tumoral Calcinosis, Hyperphosphatemic, Familial; HFTC	AR	211900	PubMed: 15590700	100	No	
KL	6344	604824	Tumoral Calcinosis, Hyperphosphatemic, Familial; HFTC	AR	211900	PubMed: 17710231	99.10	No	

SAMD9	1348	610456	Tumoral Calcinosis, Normophosphatemic, Familial; NFTC	AR	610455	PubMed: 15133511	100	No	
Pseudohypoaldosteronism type 2									
WNK1	14540	605232	Pseudo-hypoaldosteronism, Type IIC; PHA2C	AD	614492	PubMed: 11498583 PubMed: 22266938	Coverage of intron 1	No	Both mutations reported to date are located in this intron
WNK4	14544	601844	Pseudo-hypoaldosteronism, Type IIB; PHA2B	AD	614491	PubMed: 11498583	Sequencing of exons 7 and 17	No	
CUL3	2553	603136	Pseudo-hypoaldosteronism, Type IIE; PHA2E	AD	614496	PubMed: 22266938	100	No	
KLHL3	6354	605775	Pseudo-hypoaldosteronism, Type IID; PHA2D	AR and AD	614495	PubMed: 22266938	100	No	
Endocrine Neoplasia syndromes									
MEN1	7010	613733	MULTIPLE ENDOCRINE NEOPLASIA, TYPE I; MEN1	AD	131100	Ellard et al 2005 Clin Endocrinol 62:169-175	100	No	
RET	9967	164761	Multiple Endocrine Neoplasia type IIA; MEN2A	AD	171400	PubMed: 8099202	Exons and flanking introns of exons 5, 8, 10, 11, 13, 14, 15 and 16	No	
RET	9967	164761	Multiple Endocrine Neoplasia type IIB; MEN2B	AD	162300	PubMed: 7906866	Exons 15 and 16	No	
RET	9967	164761	Thyroid Carcinoma, Familial Medullary; MTC	AD	155240	PubMed: 11073534	Exons and flanking introns of	No	

							exons 5, 8, 10, 11, 13, 14, 15 and 16		
AIP	358	605555	PITUITARY ADENOMA, GROWTH HORMONE-SECRETING	AD	102200	PubMed: 20506337	100	No	
CDKN1B	1785	600779	Multiple endocrine neoplasia IV; MEN4	AD	610755	PubMed: 20980721	100	No	
Generalised Arterial Calcification of Infancy									
ENPP1	3356	173335	Arterial calcification, generalized, of infancy, 1; GACI1	AR	208000	PubMed: 20016754	100	No	
ABCC6	57	603234	Arterial calcification, generalized, of infancy, 2; GACI2	AR	614473	PubMed: 22209248	100	No	
Chondrodysplasia Punctata									
PEX7	8860	601757	Rhizomelic chondrodysplasia punctata, type 1; RCDP1	AR	215100	PubMed: 9090381	100	No	
GNPAT	4416	602744	Rhizomelic chondrodysplasia punctata, type 2; RCDP2	AR	222765	PubMed: 9536089	100	No	
AGPS	327	603051	Rhizomelic chondrodysplasia punctata, type 3; RCDP3	AR	600121	PubMed: 21990100	100	No	
ARSE	719	300180	Chondrodysplasia punctata, X-linked recessive; CDPX1	XLR	302950	PubMed: 12567415	100	No	
EBP	3133	300205	Chondrodysplasia punctata, X-linked dominant; CDPX2	XLD	302960	PubMed: 10391218	100	No	

Familial Isolated Primary Hyperparathyroidism									
MEN1	7010	613733	MULTIPLE ENDOCRINE NEOPLASIA, TYPE I; MEN1	AD	131100	PubMed: 15670192	100	No	
RET	9967	164761	Multiple endocrine neoplasia IIA; MEN2A	AD	171400	PubMed: 8918855	100	No	
CDKN1B	1785	600779	Multiple endocrine neoplasia IV; MEN4	AD	610755	PubMed: 20980721	100	No	
CDC73	16783	607393	Hyperparathyroidism 1; HRPT1 Hyperparathyroidism 2 HRPT2	AD	145000 145001	PubMed: 21837707	100	No	
CASR	1514	601199	Hypocalciuric hypercalcemia, familial, type I; HHC1	AD	145980	PubMed: 14985373	100	No	
CDKN1A	1784	116899	No disease name stated	AD		PubMed: 19141585	100	No	
CDKN2C	1789	603369	No disease name stated	AD		PubMed: 19141585	100	No	
CDKN2B	1788	600431	No disease name stated	AD		PubMed: 19141585	100	No	
Familial Isolated Hypoparathyroidism									
PTH	9606	168450	Hypoparathyroidism, Familial Isolated; FIH	AR	146200	PubMed: 10523031	100	No	
CASR	1514	601199	Hypocalcemia, autosomal dominant; HYPOC1	AD	601198	PubMed: 7874174	100	No	
GCM2	4198	603716	Hypoparathyroidism, Familial Isolated; FIH	AR	146200	PubMed: 15863676	100	No	
GNA11	4379	139313	Hypocalcemia, autosomal dominant 2; HYPOC2	AD	615361	PubMed: 23802516	100	No	

Primary Pigmented Nodular Adrenocortical Disease									
PRKAR1A	9388	188830	Pigmented nodular adrenocortical disease, primary, 1; PPNAD1	AD	610489	PubMed: 12213893	100	No	
PDE11A	8773	604961	Pigmented nodular adrenocortical disease, primary, 2; PPNAD2	AD	610475	PubMed: 16767104	100	No	
PDE8B	8794	603390	Pigmented nodular adrenocortical disease, primary, 3; PPNAD3	AD	614190	PubMed: 18272904	100	No	