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

Submitting laboratory: Leeds RGC

1. Disorder/condition – approved name (please provide UK spelling if different from US) and symbol as published on the OMIM database (alternative names will be listed on the UKGTN website).
If NGS panel test, please provide a name.

If this submission is for a panel test please complete the Excel spread sheet, Appendix 1, available for download from the UKGTN website, and list all of the conditions grouped by sub panels if applicable.

Amelogenesis Imperfecta 21 Gene Panel

2. OMIM number for disorder/condition

If a panel test – see 1 above. If a number of subpanels exist with different clinical entry points e.g. cancer panel test but different subpanels for different types of cancer (breast cancer, colon, phaeochromocytoma), then please list the sub panels here – providing name of each sub panel.

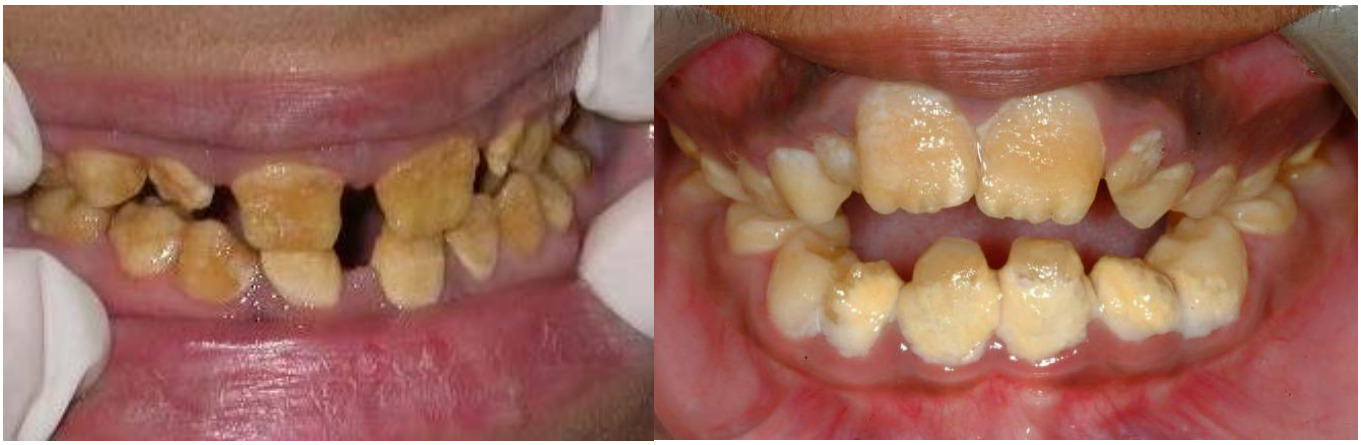
See Appendix 1.

3a. Disorder/condition – to help commissioners to understand the impact of this condition please provide, in laymen's terms (e.g tubes in the kidney (renal tubule) or low sugar in the blood (hypoglycaemia), a brief (2-5 sentences/no more than 50 words) description of how the disorder(s) affect individuals and prognosis.

Amelogenesis Imperfecta (AI) is a collective term for a group of inherited conditions with abnormal dental (tooth) enamel quality and/or quantity. This group of conditions can be inherited in a number of different ways. AI affects the primary ('baby') and secondary ('adult') teeth. It can be associated with a significant negative impact on day-to-day living. Individuals with AI require specialist care through childhood and into adulthood. This group of disorders may be associated with other serious medical complications such as Immunodeficiency which require specialist monitoring.

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

Dental enamel is the hardest substance in the body. Secondary ('adult') teeth formed in childhood can be expected to last a lifetime with an increasing proportion of older adults retaining their natural dentition. Teeth affected by AI have abnormalities of shape, colour, texture and how the upper and lower teeth come together (occlude).



Examples of AI illustrating the poor aesthetics, dental plaque accumulation due to dental sensitivity and the abnormal relationship between the upper and lower teeth.

AI teeth can be sensitive to temperature and other stimuli that are tolerated by those with normal teeth (including toothbrushing). Where enamel has formed in AI this can fail very early compounding the

negative impact on aesthetics and function. Self-conscious children become very aware that they are different from their peers (including unaffected siblings). The importance of the smile should not be underestimated and dental sensitivity to foods/drinks enjoyed by their peers sets them apart. A negative impact on self-esteem is recognised from an early stage. Some adults with AI have chosen not to have children of their own as they would not want their child to go through what they have.

Care of patients with AI is extremely challenging with a range of clinical presentations and it may not always be recognised. Primary care dental practitioners will encounter AI infrequently and for those without a dentist there can be mis-diagnoses such as dental caries. Specialist dental services (children's and adult) can be difficult to access with only some specialists having a particular interest and experience of AI.

Some individuals with AI require multiple, regular visits to the dental clinic over extended periods of time. Other specialist services may also be indicated, such as Clinical Psychology or Clinical Genetics. A subset of patients with AI have other health problems (e.g. of vision or hearing or immunodeficiency linked to the same underlying genetic problem).

Whilst the impact on the child with AI is typically obvious, there may also be a negative impact on other immediate family members. There can be unexpected challenges such as others interpreting AI being a consequence of parental neglect.

The transition from child to adult services can be suboptimal for many reasons including service availability and the attitudes and priorities of the young adult after living with AI as a child.

In summary, the impact of AI can be considerable on the affected individual, other family members and NHS clinical services. A molecular diagnosis for AI has the potential to act as a focus for a range of issues that will enhance patient care.

We know that amelogenesis can be a feature of a number of other conditions which may have other serious medical complications. These include:

- Epidermolysis Bullosa
- FAM20C – Raine Syndrome which can cause bone problems
- FAM20C – nephrocalcinosis
- Zellweger-spectrum disorders which are serious metabolic disorders and
- Immunodeficiency

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.

See Appendix 1.

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 the Excel spread sheet, Appendix 1, available for download from the UKGTN website, and list all of the genes grouped by sub panels if applicable.

See Appendix 1.

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

If a panel test – see 5. above

See Appendix 1.

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

If a panel test – see 5. above

See Appendix 1.

7a. Gene – description(s)

If this submission is for a panel test, please provide total number of genes and if there are subpanels, please also list the number genes per sub panel.

21 genes (see Appendix 1 for details)

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.
For NGS panel tests if there are sub panels, please provide GenU per subpanel.

Band G (1-50 genes analysed by NGS)

8. Mutational spectrum for which you test including details of known common mutations
(n/a for panel tests)

n/a

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

Genomic regions comprising the consensus coding regions of 6000+ disease genes are captured using the Agilent SureSelectXT Focused Exome. Libraries are prepared using standard reagents as per the maufacturer's protocol. Sequencing is performed on an Illumina Next-Generation Sequencing (NGS) platform, typically the HiSeq 2500 utilising the rapid run settings, multiplexing 6 samples per lane. Sufficient sequencing is performed to cover >95% of the targeted genes to a minimum of 15X read-depth, generally producing average exome and target coverage of approximately 100X.

An in-house bioinformatics pipeline is used to process the data. In summary, genomic alignment of raw Illumina data is performed using the Burrows-Wheeler Aligner (BWA-MEM; Li H. and Durbin R. (2010) Fast and accurate long-read alignment with Burrows-Wheeler Transform. Bioinformatics, Epub. [PMID: 20080505]), post-processing using Picard and the GATK-lite suite of applications (Broad Institute), variant calling using GATK-lite (Broad Institute), and variant annotation using Alamut Batch (Interactive Biosoftware).

Assessment of the panel of relevant genes (21 genes - see Appendix 1) takes place using Microsoft Visual Basic (VBA)-driven analysis templates, which filter the raw variant list and determine coverage of the targeted regions. Standard "moderate" filtering criteria involve the removal of any variants outside the selected gene list, any variants with a dbSNP minor allele frequency (rsMAF) exceeding 0.5%, any synonymous variants in coding regions, any variants in the 3' untranslated region, and any variants further than 10 nucleotides outside a coding exon; internal data indicates a sensitivity exceeding 98.5% for likely-pathogenic variation (n = 1,250 known pathogenic and likely-pathogenic variants detected historically by this laboratory). A "relaxed" filtering criteria (to include coding variants (including synonymous variants), 5' and 3' UTR variants, and "splice site" (+/-10bp from splice junction) variants, all with rsMAF (dbSNP minor allele frequency) <2%) is available for assessment, but not routinely used. Any variants passing the filtering stage are assessed manually by two scientists using the standard 5-class system, as per ACGS Best-Practice Guidelines. Variants considered of clinical utility (class 4 and 5) are confirmed by dideoxy sequencing, with primers designed in-house, and published in a fully interpretive report. Coverage figures are reported at 15X and 30X read-depths, and may be assessed for specific single genes if required for particular referrals.

Exonic dosage is calculated by comparative assessment of intra-lane read-depths; this method is not diagnostically validated, therefore negative results are not reported. Any clinically-relevant copy number imbalances that are identified are confirmed and reported using a second validated method.

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

The overall panel coverage is stated on the reports. Gaps in coverage are not routinely filled, unless there is a specific clinical need to do so (i.e. a gap in coverage in a particular gene with a very high prior probability of being causative of the patient's phenotype and there no pathogenic/likely pathogenic variant(s) identified).

9c. Does the test include MLPA?
(For panel tests, please provide this information in appendix 1)

No. Exonic dosage is calculated from NGS data, however, this method is not diagnostically validated and negative results are not reported. Any clinically-relevant copy number imbalances are confirmed and

reported using a second validated method.

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.

Provided by the laboratory.

11. Validation process

Please explain how this test has been validated for use in your laboratory, including calculations of the sensitivity and specificity for the types of mutations reported to cause the clinical phenotype. Note that the preferred threshold for validation and verification is ≥95% sensitivity (with 95% Confidence Intervals). Your internal validation documentation can be submitted as an appendix (and will be included in the published Gene Dossier available on the website). The validation information should include data on establishing minimum read depth and horizontal coverage for the regions of interest, reproducibility of the pipeline, accuracy of variant calling, filtering of common variants and artefacts.

If this submission is for a panel test, please provide a summary of evidence of instrument and pipeline validation and complete the tables below.

Instrument (HiSeq2000) and bioinformatics pipeline was validated on equivalent panel chemistry (hereditary cancer genes panel), enabling a comparison to be made against sensitivity of existing pipelines (Sanger sequence analysis and NGS analysis based on enrichment by long range PCR). 100% concordance was recorded for 480 variants (101 unique) between the panel test and gold-standard PCR-based analysis.

Specific validation of the reagent used for this panel (Agilent SureSelectXT Focused Exome) involved a variant detection assessment (unfiltered) against an established reagent ("SelGen" selected genes NGS reagent analysis), an assessment of the variant filtering criteria used, and an assessment of the sensitivity of the specific panel(s) used in this service as a function of read-depth, based on internal and published NGS data.

Variant detection sensitivity of reagent:

A panel of 590 known unique genomic variants from a panel of 125 diagnostically relevant genes ("SelGen") across 4 validation samples (two male, two female) was assayed on the Focused Exome reagent, using the standard laboratory protocol and bioinformatics pipeline. No additional filtering was applied. 561 were detected.

Of the 29 discordant variants, 24 were in genes which have no/low coverage on the exome reagent. These genes were not part of this diagnostic panel, and would be omitted from any putative diagnostic panel during our laboratory's defined validation/feasibility studies; they were therefore outside the scope of the assay. Of the remaining 5 variants (out of a total of 555) in valid genes, 4 were found in isolated regions of zero NGS coverage. The remaining variant was a call with a very low vcf quality score and a skewed allele balance (17 of 89 reads) in a gene with a known pseudogene (HYDIN NM_001270974.1:c.3840G>A); this was deemed likely to be a pseudogene-derived artefact, although this was not formally proven as the measured sensitivity was already >98%.

550 of 555 variants within valid genes were therefore detected by the clinical exome technical process, a measured sensitivity of 99%, minimum sensitivity of 98% at 95% confidence. The only undetected variants were in low coverage and/or pseudogenic regions; sensitivity is therefore a function of coverage (mostly) and uniqueness of target sequence.

For panel tests:

Sensitivity 98% (95% CI)

Read depth minimum cut off:15X

	Previously tested	NGS test concordant results	NGS False negative
Number of patient samples	**4**	**-**	**-**
Unique variants (total)	**555**	**550**	**5**
SNV	**536**	**531**	**5**
Indel (1bp to 18 bp)	**19**	**19**	**0**
CNV	**0**	**0**	**0**

Note: The analysis of the 4 local control samples was undertaken in place of analysis of a reference sample (eg HapMap/CEPH DNA).

Specificity 92.3% (95% CI)

(Calculated using 'Jeffreys Interval')

	Variant confirmed by other method	NGS False positive
29 patient samples with a variant detected by NGS		
Unique variants (total)	**31**	**0**
SNV	**23**	**0**
Indel (1bp to 25 bp)	**7**	**0**
CNV	**1**	**0**

Panel-specific sensitivity figures

A set of known variants within the targeted genes is not available, due to a paucity of positive controls and the expense of sequencing large numbers of samples using this technical pipeline. The sensitivity figures at two measured read-depths (15X and 30X), derived from local data and published data using a comparable informatics pipeline (Meynert et al, BMC Bioinformatics. 2013 Jun 18;14:195), were used to estimate a minimum sensitivity for each representative sub-panel.

Meynert et al. 2013 (supplementary data) derived a single-nucleotide variant (SNV) sensitivity figure (lower 95% confidence interval) of 98.8% at 30X and 96.2% at 15X for exome-based testing (largely agnostic of reagent and platform, using a consistent bioinformatics pipeline comparable to ours).

Local data using the Focused Exome reagent and our standard pipeline supports these figures:

38 of 38 variants with coverage between 15X and 30X were detected (100% sensitivity; 93.6% minimum sensitivity at 95% CI).

472 of 473 variants with coverage >30X were detected (99.8% sensitivity, 99% minimum sensitivity at 95% CI).

Local data were not subject to a downsampling analysis due to the potential for introducing bias when imposing such methods using public domain tools (personal observation).

The SNV sensitivity of the entire set of 21 AI genes was assessed (using the 15X and 30X thresholds, and the Meynert 2013 sensitivity figures, which use a larger sample-size than was possible in our hands).

It is assumed that these figures represent an under-estimate of SNV sensitivity, as they make the assumption that all nucleotides with fewer than 15 reads have zero sensitivity; in our hands, 17 of 45 (38%) of variants <15X were detected in a validation experiment, and the vast majority of false-negatives (27 of 28) were in regions with 0X or 1X coverage. Sensitivity estimates are also based on heterozygous SNPs, and would be higher for homozygotes (Meynert et al. estimate a minimum read-depth of 3X is required to detect homozygous variants at 95% sensitivity in typical genomic assays).

Panel	# Genes	15X Coverage	30X Coverage	Estimated SNV sensitivity:
Amelogenesis Imperfecta	21	99.71%	98.45%	96.67%

Variant filtering validation:

Variant filtering is applied as a standard part of the analysis. The standard criteria ("moderate") are as follows:

- By gene
- By location (within coding region +/-10bp)
- By minor allele frequency (dbSNP minor allele frequency (rsMAF) <0.5%)
- By coding consequence (synonymous coding variation excluded)

Internal validation (comparing those criteria to 1249 unique "likely pathogenic" (class 4) and "pathogenic" (class 5) variants derived from the laboratory's service history) indicated a measured sensitivity of 98.9% (1235/1249 detected, 98.2% sensitivity 95% CI); of the 14 "missed" variants, 4 were relatively high-frequency variants in very common recessive conditions, 5 were synonymous exonic variants with a proven effect on splicing, and 5 were variants beyond the +/-10bp region with a proven effect on splicing. None of the genes included on this panel are common recessive conditions with any known mutations exceeding 0.5% rsMAF (therefore making the first criteria irrelevant). We believe that, for the majority of the genes on this panel, there is little published evidence of characterisation of non-coding splicing variants outside of the consensus donor/acceptor dinucleotides, and therefore the impact of the second two criteria is likely to be minimal.

In addition, a "relaxed" filtering criteria is available (this is not reported as standard, but may be informally applied by analysts when the "moderate" criteria return no variants of clinical significance, or a single heterozygous variant in a recessive gene) which will include synonymous variation and common recessive mutations (up to 2% rsMAF), at the expense of specificity.

All variants which pass the filtering criteria are assessed manually by an analyst and checked by a second.

12a. Are you providing this test already?

Yes

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

Sanger Based Tests	NGS Based Tests
0	10

12c. Number of reports with a pathogenic (or likely pathogenic) mutation identified?

Sanger Based Tests	NGS Based Tests
0	4 (+2 with suspicious VUS)

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

Initial research cohort tested in diagnostic laboratory between March 2015 and January 2016.

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

Yes

13b. If yes, please provide details

Clinical expertise: There is clinical expertise in centres in Leeds with close liaison with other clinical specialists providing care for patients with AI across Yorkshire and the Humber. There are excellent links with specialists providing care for AI centres elsewhere in the UK. Our current genetics research study is recruiting through specialist children's dentistry clinics in Leeds, Bradford, Sheffield, Birmingham and Newcastle. There is a UK network of children's specialist dentists with an interest in AI clinical care.

Research expertise: There is a well-established multi-disciplinary AI research team in Leeds that

includes geneticists, biochemists and clinicians who collaborate closely with other centres [Kirkham J, Brookes S, Inglehearn CF, Mighell AJ]. Achievements include: 1) internationally recognised leader for advancing the genetic understanding of AI and the associated genotype/phenotype correlations, including through nano-CT and other ultrastructural analyses of enamel that are informing disease mechanisms; 2) description of the only AI disease mechanism with an effective intervention in an animal model of AI where the intervention is already in use in humans for other conditions); 3) development from the laboratory to the clinic of an effective intervention for porous enamel.
14. 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.
n/a

EPIDEMIOLOGY

15. Estimated prevalence and/or incidence of conditions in the general UK population
For panel tests, please provide estimates for the conditions grouped by phenotypes being tested.
Prevalence is total number of persons with the condition(s) in a defined population at a specific time (i.e. new and existing cases).
e.g. CF prevalence approx. 12 per 100,000 with UK population of approx. 63 million the prevalence of affected individuals in the UK is 7560
Incidence is total number of newly identified cases in a year in a defined population. e.g. CF incidence 1/2650 live births in a UK population with 724,000 live births in a year = 273 new cases a year
Please identify the information on which this is based.

There is currently no accurate prevalence or incidence data for AI in the UK. This reflects wide-ranging factors including: 1) absence of systems/processes for data collection; 2) classification limitations; 3) absence of coordinated patient pathways within NHS services.

It is likely that AI prevalence in the UK varies between different ethnic groups with geographic variation possible too. Data from outside the UK is indicative of a variable prevalence within study limitations – e.g. 43:10,000 (Turkey) 14:10,000 (Sweden) 10:10,000 (Argentina) and 1.25:10,000 (Israel).

Initiatives to address the limitations of the prevalence and incidence data for AI locally include: 1) developing registration of individuals with AI with NorCAS (Northern Region Congenital Abnormality Survey) run by PHE as part of the national database. 2) Using the increasing knowledge of AI genetics generated by ourselves and others to promote a genetics-based classification of AI with an associated move away from the historical classification of clinical features. 3) Actively engaging with a network of clinicians caring for AI patients to translate genetic and associated phenotype advances to patient care on an individual basis, as well as engaging with those developing the new NHS commissioning guides and patient pathways.

16. Estimated gene frequency (Carrier frequency or allele frequency)
Please identify the information on which this is based.
n/a for panel tests.

n/a

17. Estimated penetrance of the condition. Please identify the information on which this is based
n/a for panel tests

n/a

18. 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)

19. 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

20. Analytical sensitivity and specificity

The *analytical sensitivity* of a test is the proportion of positive results correctly identified by the test (true positive/true positive + false negative). The *analytical specificity* of a test is the proportion of negative results correctly identified by the test (true negative/true negative + false positive).

This should be based on your own laboratory data for (a) the specific test being applied for or (b) the analytical sensitivity and specificity of the method/technique to be used in the case of a test yet to be set up. Please specify any types of mutations reported to cause the clinical phenotype that cannot be detected by the test.

Note that the preferred threshold is ≥95% sensitivity (with 95% Confidence Intervals).

Sensitivity 98% (95% CI)

Specificity 92.3% (95% CI)

This testing strategy will not detect intronic or regulatory variants that are outside of the coding regions +/-10bp and may not detect all copy number variants (single exon/partial exon deletions).

21. 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.

For a panel test, the expected percentage diagnostic yield for the test in the target population can be presented as an alternative to clinical sensitivity and specificity?

From local research studies the underlying genetic defect has been identified in around a third of AI patients recruited. Therefore the diagnostic yield is likely to be at least 33%.

Analysis of the initial cohort tested using this 21 gene panel approach has identified pathogenic/likely pathogenic variants that are consistent with the clinical diagnosis in 40% of cases, increasing to 60% of cases if two patients with suspicious VUS (insufficient evidence currently for higher classification) are included.

22. 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

(Not currently requested for panel tests)

23. 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

24. How will the test change the management of the patient and/or alter clinical outcome? Please summarise in 2-3 sentences – no more than 50 words.

Genetic testing has the potential to act as a focus for the transformation to coordinated clinical care across multiple centres with development of systematic approaches to care informed by better understanding of genotype-phenotype relationships.

Genetic testing will confirm the diagnosis and inform the mode of inheritance allowing for appropriate advice and targeted specialist treatment. This may include anticipation of potential complications such as Nephrocalcinosis with FAM20A mutations.

25. Please provide full description on likely impact on management of patient and describe associated benefits for family members. If there are any cost savings AFTER the diagnosis, please detail them here.

Provision of care for patients with AI is extremely challenging with many barriers to patients accessing the required specialist care.

Specialist dental care is typically required throughout childhood and into adulthood. This reflects the extended sequence of tooth eruption. The primary teeth start to erupt in to the mouth by around 6 months of age with the first adult tooth erupting around 6 years of age and the last by 14 years of age (excluding the 'wisdom' teeth, which erupt in late teen years or early adulthood). Extraction of all the teeth is a poor option that is not consistent with contemporary standards of clinical care. Accordingly, there is a need for regular specialist dental care to maintain the compromised dentition as it erupts that far exceeds that of someone without AI.

AI can have a significant adverse impact on affected children (e.g. appearance when smiling, pain on eating/drinking and related self-esteem issues) with an associated adverse impact on parents and unaffected siblings (e.g. psychological, financial, time off work, decisions about having children). Multiple visits are often required from a young age, which can lead to negative attitudes to dental care that complicates future care.

Molecular diagnosis can provide clarity to the family on reason for occurrence, implications for extended at risk family members and the chances of recurrence in a future

26a. If this test was not available, what would be the consequences for patients and family members? Please describe in not more than 50 of words.

Patients and families will be disadvantaged. Care would continue on an *ad hoc* basis but is likely to be sub optimal and not within a systematic approach. Other medical complications may not be anticipated and addressed in a timely manner.

26b. The consequences for patients and family members if this test was not available – if required please expand on the response provided in question 26a.

Access to appropriate care for patients with AI and their families is currently inequitable with many barriers in place. The introduction of AI genetics testing has the potential to be the pivot point around which care pathways can be reorganised and quality managed to deliver better care, make best use of the available NHS resources and underpin advances in care that matter to patients and their families. Failure to introduce genetic testing for AI as part of NHS clinical care will lead to those who have AI and those who provide the care for them, being disadvantaged. It will not be possible to provide accurate risk of recurrence to the parents of an affected child or clarify status of at risk family members.

27. 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.

No. The current clinic classification of AI has many limitations including that features can be lost due to changes to the enamel after the teeth have erupted. Value judgements are made that vary between clinicians and these do not necessarily inform care choices in the way that they could do either on an individual patient basis or within a more systematic approach to AI patients as a group.

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

LAMA3/LAMB3/LAMC2/ITGB4/COL17A1: Epidermolysis Bullosa (Panel to test AI patients with no skin features of EB only)

FAM20C: Raine Syndrome (Attenuated presentation with AI as the predominant clinical phenotype with other poorly defined features).

PEX1/PEX6: Zellweger-spectrum disorders (Attenuated presentation of AI with hearing problems).

STIM1/ORAI1: Immunodeficiency (Attenuated presentation with AI as the predominant clinical phenotype)

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

In the event that this occurs the family will be referred to the appropriate specialists including clinical genetics.

30. If a panel test, is this replacing an existing panel/multi gene test and/or other tests currently carried out by your lab e.g. Noonan Spectrum Disorders 12 Gene Panel replaced multigene Sanger test for KRAS, RAF1, PTPN11 and SOS1? If so, please provide details below.

No

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

The proposed test for AI does not raise any specific ethical, legal or social issues.

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

The parents of AB recognised that his teeth were not normal in infancy. On eruption of the secondary dentition from around the age of 6 years old, it became clear that he had Amelogenesis Imperfecta (AI). Over time, dental care was undertaken by different providers before starting in the specialist child dental health clinic in Leeds. There was no other history of AI in either the immediate family or the more distant relatives. The parents of AB were of different ethnicity with nothing to suggest consanguinity in the family. Other than the AI, AB was well with a clear medical history.

Genetic testing of AB identified compound heterozygote variants in *FAM20A* that were predicted to be damaging. The variants segregated in other family members. *FAM20A* variants are associated with nephrocalcinosis, which typically will not present until extensive and can lead to renal impairment and failure. The natural history of development of nephrocalcinosis in this situation is poorly understood with a variable age of clinical presentation. AB was evaluated in paediatric nephrology with no features of nephrocalcinosis detected. He is being kept under periodic review.

This case illustrates how genetic testing can provide a molecular diagnosis that will be the focus for improving the delivery of AI care, but also can prompt targeted, timely investigation by other services ahead of a late clinical presentation. Furthermore, this approach will give new insight in to the natural history of nephrocalcinosis due to *FAM20A* mutations and allow future development of a stratified risk approach.

UKGTN Testing Criteria

Test name: Amelogenesis Imperfecta 21 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	
Specialist or Consultant in Paediatric Dentistry	
Specialist or Consultant in Restorative Dentistry	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
• Significant developmental abnormalities of enamel quality and/or quantity affecting all or nearly all teeth of both dentitions (primary and secondary) **AND** • environmental factors excluded (Note: Enamel abnormalities affecting unerupted permanent teeth can be detected on dental radiographs meaning that information about both dentitions is available well before eruption of the first permanent tooth)	

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.

IS IT A REASONABLE COST TO THE PUBLIC?

36. Based on experience what will be the national (UK wide) expected activity for requesting this test, per annum, for:

Index cases :

The projected numbers of index cases for diagnostic testing are:

- Year 1: 25 cases
- Year 2: 50 cases
- Year 3 onwards: 50 cases per annum

Family members where mutation is known

If a NGS panel test, it is recognised that the full panel will not be used to test family members where the familial mutation is known. Please provide expected number of tests to inform completion of Q40

Assuming a referral rate of 50 index cases per annum, and a diagnostic yield of 33%, the estimate of the number of family member tests where mutation is known is approximately 33 per annum.

37. 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".

Laboratory has capacity to provide service for the full national need.

38. In order to establish the potential costs/savings that could be realised in the diagnostic care pathway, please list the tests/procedures that are no longer required to make a diagnosis for index cases where index cases would have the molecular genetic test proposed in this gene dossier at an earlier stage in the pathway. It is the tests/procedures that would be stopped for patients that are eligible for the gene test.
This information will be used to calculate the overall investment / savings required in Q39
Example:
The introduction of a 95 gene panel for syndromic and non-syndromic hearing loss would allow those patients who are recognised early enough in their pathway to diagnosis to be offered the genetic test instead of having sequential gene tests for individual genes already available and repeated ECGs, ERGs & renal ultrasounds as part of the diagnostic pathway although these may still be required as part of management after diagnosis.

At this stage it is difficult to accurately forecast the savings to be made through establishment of defined care pathways with key clinical decisions informed by NHS genetics testing.

	Type of test	Cost (£)
Imaging procedures	No significant impact anticipated	
Laboratory pathology tests (other than molecular/cyto genetic test proposed in this Gene Dossier)	Hard tissue histopathology of extracted or exfoliated teeth*	**£130**
Physiological tests (e.g. ECG)	None	
Other investigations/procedures (e.g. biopsy)	See *FAM20A* above.	See above
Associated inpatient stays in the diagnostic pathway	Limited impact on inpatient stays but could reduce the number of procedures under general anaesthesia that are required (estimated 1 fewer on average per patient) and the number of outpatient clinic appointments (estimated 3-5 fewer per patient).	1x£700 (day case for patient requiring care under GA) + 4x£115 (Outpatient appointment) = **£1160**
Total cost of tests/procedures to be stopped (please write n/a if the genetic test does not replace any other tests procedures in the diagnostic care pathway)		**£1290**
If any of the tests/procedures listed above would be carried out on individuals after having the genetic test because the genetic test did not pick up a pathogenic mutation (i.e. negatives), please indicate the costs for these tests to continue to diagnosis. ***For example a panel test replaces single gene tests that have been included above, but after the panel test an individual that tests negative would not need to have these single gene tests, because the genes were on the NGS panel.***		**£1290**

***** This is not a mainstream investigation, but is requested by some clinicians to support diagnosis (i.e. difficult to know what proportion of individuals with AI are investigated in this way (especially across the UK)

39. Please complete the Excel spread sheet available to download from the UKGTN website to calculate the estimated investment or savings, based on the expected annual activity of index & family cases (Q36 above) and using the information provided in Q38. Please submit this separately.

Number of index cases expected annually	50
Number of family member tests expected annually	33
Cost to provide index case test	£860
Cost to provide family member test	£195
Costs associated with tests/procedures for index cases if the genetic test in this Gene Dossier was not available	£1,290
Costs associated with tests/procedures for index cases that test negative for the genetic test in this Gene Dossier	£1,290
Total annual costs for diagnostic tests prior to introduction of the genetic test submitted for evaluation in this Gene Dossier	£64,500
Total annual costs to provide genetic test	£43,000
Additional savings or investment for 100% pick up rate for index cases	-£21,500
Percentage of index cases expected not to find a pathogenic mutation (negatives)	67%
Number of index cases estimated to not find a pathogenic mutation (negatives)	33.5
Costs or savings to provide additional tests for index cases that test negative	£43,215
Total savings / investment prior to application of marginal reduction if applicable	£21,715
If a panel test and there are genes on the panel test that are already available on either other panel tests or single gene tests please estimate/suggest a marginal percentage reduction of the investment/savings. If you feel this is NOT applicable please leave this as 0%.	0%
Marginal percentage reduction if applicable applied to the savings/investment	£0
TOTAL SAVINGS / INVESTMENT for tests for INDEX CASES	**£21,715**
Total costs for family members	£6,435
If family testing is already available for any of the genes on this panel across the Network, please estimate the associated funding for these tests.	£0
TOTAL SAVINGS / INVESTMENT for tests for FAMILY MEMBERS	£6,435
ADDITIONAL INVESTMENT / SAVINGS FOR ALL ACTIVITY EXPECTED PER ANNUM	£28,150

40. Please indicate the healthcare outcomes that apply to this test after diagnosis. It is recognised that all tests recommended by the UKGTN for NHS service improve clinical management and, if a familial mutation is found, allows for prenatal testing and therefore these are not included in the list below.

Healthcare outcomes	Does this apply to this test?
1. Alerts significant clinical co-morbidities	**Yes** This will not be relevant to the majority of cases. However, it will be important with respect to *FAM20A* mutations (see section 32) and where there is AI as part of an attenuated clinical presentation that is milder than the full syndrome presentation.
2. Reduces mortality/saves lives	**No** Any impact on mortality reduction will be minimal and relate to a small number of selected examples where AI is part of a wider syndrome that will typically present with attenuated clinical features

	(see section 25).
3. Avoids irreversible harm	**Yes** The introduction of a molecular diagnosis for AI will provide the consistency needed to plan, deliver and quality assure improved multi-disciplinary care informed by an expanding evidence base for decision-making. Harm reduction may include that related to the consequences of tooth restoration and also related to psychological impact of how dental care is delivered.
4. Avoids diagnostic procedures/tests (some of which may be invasive) and/or multiple hospital appointments	**Yes** Establishment of AI molecular diagnostic testing will lead to more stream-lined care. Within a specialist dental team the timing of care and who this care is delivered by will be critical with an expectation that clinic appointments will be more effectively used, thus reducing the impact on the patient and the team providing care.
5. Avoids incorrect management (e.g. medication or treatment) that could be harmful	**Yes** Care will become more focused with involvement of other healthcare professionals in a targeted way. The likelihood of inappropriate management should reduce.
6. Confirms targeted therapy/management	**Yes** For the reasons outlined elsewhere in this section.
7. Earlier diagnosis allowing commencement of treatment earlier with associated improved prognosis	**Yes** AI molecular testing as part of better-defined patient pathways will help to promote effective patient care pathways. This will include more rapid referral to those best-placed to deliver specialist care for AI and appropriate shared-care. There will be associated benefits to patient care.
8. Enables access to educational and social support	**Yes** AI molecular testing will help promote the education of healthcare professionals and lay-people including those with

	AI. There will be professional development opportunities arising for those healthcare workers involved in specialist care as well as for those colleagues who encounter this condition infrequently. A molecular diagnosis has the potential to act as a focus for a much-needed patient support group. All too often AI patients and their families feel very isolated.
9. At risk family members that test negative for a familial mutation can be discharged from follow up	**Yes** This will affect a small, but important number of individuals where environmental factors have resulted in abnormal dental enamel that does not fit within the definitions of AI. Such individuals may belong to families with (inherited) AI.
10. At risk family members that test positive for a familial mutation have appropriate follow up	**Yes** Care will be stream-lined to reduce the likelihood of inappropriate care. This can be a particular issue where family members live at different places in the UK.

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Appendix 1

Genes in panel test and associated conditions

OMIM standard name of condition (please provide the conditions that the test is for which may NOT necssarily be the condition that is linked to the gene on OMIM)	OMIM symbol of condition	OMIM number of condition	Mode of inheritance	HGNC standard name of gene	HGNC symbol of the gene	HGNC number of the gene	OMIM number of the gene	Evidence of association between gene and condition	% of horizontal coverage of gene	MLPA
AMELOGENESIS IMPERFECTA, TYPE IE	AI1E	301200	X-linked	amelogenin	AMELX	461	300391	Kim JW, Simmer JP, Hu YY, et al. Amelogenin p.M1T and p.W4S mutations underlying hypoplastic X-linked amelogenesis imperfecta. J Dent Res. 2004 May;83(5):378-83.	100%	No
AMELOGENESIS IMPERFECTA, TYPE III	AI3	130900	Autosomal Dominant	family with sequence similarity 83, member H	FAM83H	24797	611927	Wright JT, Frazier-Bowers S, Simmons D, et al. Phenotypic Variation inFAM83H-associated Amelogenesis Imperfecta. Journal of Dental Research. 2009;88(4):356-360.	99.69%	No
AMELOGENESIS IMPERFECTA, TYPE IB	AI1B	104500	Autosomal Dominant	enamelin	ENAM	3344	606585	Rajpar MH, Harley K, Laing C, Davies RM and Dixon MJ. Mutation of the gene encoding the enamel-specific protein, enamelin, causes autosomal-dominant amelogenesis imperfecta. Hum Mol Genet. 2001 Aug 1;10(16):1673-7.	100%	No

AMELOGENESIS IMPERFECTA, TYPE IC	AI1C	204650	Autosomal Recessive	enamelin	ENAM	3344	606585	Hart T, Hart P, Gorry M, et al. Novel ENAM mutation responsible for autosomal recessive amelogenesis imperfecta and localised enamel defects. Journal of Medical Genetics. 2003;40(12):900-906.	100%	No
AMELOGENESIS IMPERFECTA, HYPOMATURATION TYPE, IIA4	AI2A4	614832	Autosomal Recessive	chromosome 4 open reading frame 26	C4orf26	26300	614829	Parry DA, Brookes SJ, Logan CV, et al. Mutations in C4orf26, Encoding a Peptide with In Vitro Hydroxyapatite Crystal Nucleation and Growth Activity, Cause Amelogenesis Imperfecta. American Journal of Human Genetics. 2012;91(3):565-571.	100%	No
AMELOGENESIS IMPERFECTA, HYPOMATURATION TYPE, IIA1	AI2A1	204700	Autosomal Recessive	kallikrein-related peptidase 4	KLK4	6365	603767	Hart P, Hart T, Michalec M, et al. Mutation in kallikrein 4 causes autosomal recessive hypomaturation amelogenesis imperfecta. Journal of Medical Genetics. 2004;41(7):545-549.	100%	No
AMELOGENESIS IMPERFECTA, HYPOMATURATION TYPE, IIA2	AI2A2	612529	Autosomal Recessive	matrix metallopeptidase 20	MMP20	7167	604629	Kim J, Simmer J, Hart T, et al. MMP-20 mutation in autosomal recessive pigmented hypomaturation amelogenesis imperfecta. Journal of Medical Genetics. 2005;42(3):271-275.	99..35%	No

AMELOGENESIS IMPERFECTA, HYPOMATURATION TYPE, IIA3	AI2A3	613211	Autosomal Recessive	WD repeat domain 72	WDR72	26790	613214	El-Sayed W, Parry DA, Shore RC, et al. Mutations in the Beta PropellerWDR72 Cause Autosomal-Recessive Hypomaturation Amelogenesis Imperfecta. American Journal of Human Genetics. 2009;85(5):699-705.	96.54%	No
AMELOGENESIS IMPERFECTA	Not currently associated with any conditions on OMIM	--	Autosomal Recessive	G protein-coupled receptor 68	GPR68	4519	601404	Almost certainly a cause of AI in isolation – Research group have a family with an exonic deletion, but have yet to find a 2nd family.	100%	No
AMELOGENESIS IMPERFECTA, TYPE IA	AI1A	104530	Autosomal Dominant	laminin, beta 3	LAMB3	6490	150310	Poulter JA, El-Sayed W, Shore RC, Kirkham J, Inglehearn CF, Mighell AJ. Whole-exome sequencing, without prior linkage, identifies a mutation in LAMB3as a cause of dominant hypoplastic amelogenesis imperfecta. European Journal of Human Genetics. 2014;22(1):132-135.	98.66%	No
AMELOGENESIS IMPERFECTA	Not currently associated with AI on OMIM	--	Autosomal Dominant	laminin, alpha 3	LAMA3	6483	600805	Research group aware of at least 1 case of AI in isolation – unclear if this is being missed or whether it is a rare cause of AI	98.31%	No

AMELOGENESIS IMPERFECTA	Not currently associated with AI on OMIM	--	Autosomal Dominant	integrin, beta 4	ITGB4	6158	147557	Good candidate as ITGB4 mutations cause junctional epidermolysis bullosa (JEB) with pyloric atresia, which includes enamel defects as a feature. Isolated AI has been observed in heterozygous relatives of JEB patients with mutations in COL17A1, LAMA3 and LAMB3, therefore it is possible that heterozygous ITGB4 mutations could cause AI in isolation also.	97.16%	No
AMELOGENESIS IMPERFECTA	Not currently associated with AI on OMIM	--	Autosomal Dominant	collagen, type XVII, alpha 1	COL17A1	2194	113811	Nakamura, H., Sawamura, D., Goto, M., Nakamura, H., Kida, M., Ariga, T. ... Shimizu, H. (2006). Analysis of the COL17A1 in non-Herlitz junctional epidermolysis bullosa and amelogenesis imperfecta. International Journal of Molecular Medicine, 18, 333-337.	98.20%	No
AMELOGENESIS IMPERFECTA	Not currently associated with AI on OMIM	--	Autosomal Recessive	latent transforming growth factor beta binding protein 3	LTBP3	6716	602090	Huckert M, Stoetzel C, Morkmued S, et al. Mutations in the latent TGF-beta binding protein 3 (LTBP3) gene cause brachyolmia with amelogenesis imperfecta. Human Molecular Genetics. 2015;24(11):3038-3049.	98.04%	No
AMELOGENESIS IMPERFECTA, TYPE IG	AI1G	204690	Autosomal Recessive	family with sequence similarity 20, member A	FAM20A	23015	611062	Wang S-K, Aref P, Hu Y, et al. FAM20A Mutations Can Cause Enamel-Renal Syndrome (ERS). Spinner NB, ed. PLoS Genetics. 2013;9(2):e1003302.	99.82%	No

AMELOGENESIS IMPERFECTA, TYPE IV	AI4	104510	Autosomal Dominant	distal-less homeobox 3	DLX3	2916	600525	Dong, J., Amor, D., Aldred, M. J., Gu, T., Escamilla, M., MacDougall, M. DLX3 mutation associated with autosomal dominant amelogenesis imperfecta with taurodontism. Am. J. Med. Genet. 133A: 138-141, 2005.	99.41%	No
IMMUNODEFICIENCY 10	IMD10	612783	Autosomal Recessive	stromal interaction molecule 1	STIM1	11386	605921	Wang S, Choi M, Richardson AS, et al. STIM1 and SLC24A4 Are Critical for Enamel Maturation. Journal of Dental Research. 2014;93(7 Suppl):94S-100S. Parry D.A., Holmes T.D., Gamper N., et al. A homozygous STIM1 mutation impairs store-operated calcium entry and natural killer cell effector function without clinical immunodeficiency. J Allergy Clin Immunol. (2015). [Epub ahead of print]	99.25%	No
IMMUNODEFICIENCY 9	IMD9	612782	Autosomal Recessive	ORAI calcium release-activated calcium modulator 1	ORAI1	25896	610277	Lisa J Robinson, Salvatore Mancarella, Duangrat Songsawad, et al. Gene disruption of the calcium channel Orai1 results in inhibition of osteoclast and osteoblast differentiation and impairs skeletal development. Laboratory Investigation (2012) 92, 1071–1083.	94.86%	No

AMELOGENESIS IMPERFECTA	Not currently associated with AI on OMIM	--	(Autosomal Dominant)	laminin, gamma 2	LAMC2	6493	150292	Not yet identified as a cause of AI in isolation, but an excellent candidate and possibly only a matter of time before it is described as cause of AI with parallels to LAMB3 and other EB genes.	98.98%	No
RAINE SYNDROME	RNS	259775	Autosomal Recessive	family with sequence similarity 20, member C	FAM20C	22140	611061	Ana Carolina Acevedo, James A Poulter, Priscila Gomes Alves, et al. Variability of systemic and oro-dental phenotype in two families with non-lethal Raine syndrome with FAM20C mutations. BMC Medical Genetics (2015) 16:8.	100%	No
HEIMLER SYNDROME 1	HMLR1	234580	Autosomal Recessive	peroxisomal biogenesis factor 1	PEX1	8850	602136	Ratbi, I., Falkenberg, K. D., Sommen, M., et al. Heimler syndrome is caused by hypomorphic mutations in the peroxisome-biogenesis genes PEX1 and PEX6. Am. J. Hum. Genet. (2015) 97: 535-545.	96.07%	No
HEIMLER SYNDROME 2	HMLR2	616617	Autosomal Recessive	peroxisomal biogenesis factor 6	PEX6	8859	601498	Ratbi, I., Falkenberg, K. D., Sommen, M., et al. Heimler syndrome is caused by hypomorphic mutations in the peroxisome-biogenesis genes PEX1 and PEX6. Am. J. Hum. Genet. (2015) 97: 535-545.	94.98%	No