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

Submitting laboratory: Oxford 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.

CRANIOSYNOSTOSIS AND DENTAL ANOMALIES; CRSDA

2. OMIM number for disorder/condition

If a panel test – see 1. above

#614188

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

This disorder is characterized by craniosynostosis (premature fusion of the growth plates of the skull), maxillary hypoplasia (under-development of the upper jaw), and dental anomalies, including malocclusion (misalignment of teeth), delayed and ectopic tooth eruption (teeth erupting late or in the wrong place), and/or supernumerary teeth (additional teeth). Some patients also display minor digit anomalies, such as syndactyly (fusion of two or more fingers and/or toes) and/or clinodactyly (bent fingers).

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

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.

autosomal recessive

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.

interleukin 11 receptor, alpha; IL11RA

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

If a panel test – see 5. above

***600939

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

If a panel test – see 5. above

HGNC:5967

7a. Gene – description(s)

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

IL11RA is located on 9p13.3 and consists of 13 exons, 12 of which are coding. It encodes IL11RA, the receptor for the cytokine interleukin 11. IL11RA plays a role in bone formation and remodelling and is expressed in both osteoblasts and mature osteoclasts.

7b. Number of amplicons to provide this test (molecular) or type of test (cytogenetic) (n/a for panel tests)
10
7c. GenU band that this test is assigned to for index case testing.
Band E
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
Nonsense, missense, splicing and in-frame duplications have been reported so far.
9a. Technical method(s) – please describe the test.
Bidirectional fluorescent sequencing
9b. For panel tests, please specify the strategy for dealing with gaps in coverage.
N/A
9c. Does the test include MLPA? **(For panel tests, please provide this information in appendix 1)**
No
9d. If NGS is used, does the lab adhere to the Association of Clinical Genetic Science Best Practice Guidelines for NGS?
N/A
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.**
To be provided by the lab
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.
Bidirectional fluorescent sequencing is used by our laboratory for mutation scanning of several genes including the craniofacial genes *FGFR1, FGFR2, FGFR3, TWIST1, EFNB1, RUNX2, TCF12* and *ERF.* Prior to use all primers were checked for SNPs and 2 normal controls sequenced to confirm specific amplification. Confirmation of known mutations using controls from Professor Wilkie was also carried out.
12a. Are you providing this test already?
No. This service was offered on a research basis by Professor Andrew Wilkie's laboratory and will transfer to our laboratory in February 2014.

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

Professor Wilkie analysed 240 nonsyndromic and syndromic craniofacial samples (previously screened for other craniofacial genes) in a research setting, which revealed two positive cases (Nieminen et al 2011, Am J Hum Genet 89:67-81)

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

Professor Wilkie found 2 positive samples (~1%) in the initial cohort. Subsequent to this his lab has found mutations in a further 4 families based on targeted analysis of individuals from a consanguineous union.

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

Professor Wilkie found 238 negative samples in the initial cohort.

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.

Professor Wilkie has been offering this service for 3 years in a research setting.

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

Yes

13b. If yes, please provide details

Professor Andrew Wilkie's research group at the Weatherall Institute of Molecular Medicine was amongst the first to identify *IL11RA* mutations in patients with autosomal recessive craniosynostosis and dental anomalies (Nieminen et al 2011, Am J Hum Genet 89:67-81). In addition his laboratory has worked on many of the craniofacial genes. A HSS-funded referral centre for craniofacial disorders is based at the John Radcliffe, Oxford, with weekly clinics for patients. The lead clinician is Mr Steve Wall.

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

Index cases
Approx 20 per year, of which approx 1-2 (5-10%) will be positive based on estimated prevalence.

Figures are an estimate of cases which would meet our diagnostic criteria of multisuture synostosis, together with either a known or suspected consanguineous parental relationship, or dental anomalies (supernumerary teeth or delated eruption), and that have screened negative on our initial level 1 screen.

Family members where mutation is known At least 2

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".

Our laboratory would be able to provide the full national need.

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.

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.

We know of 14 cases in the UK at present, giving a minimum prevalence of 1 in 5 million. The majority of these 14 cases are of Pakistani ethnic origin.

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.

Assuming a prevalence of 1 in 1 million, the birth incidence is predicted to be approximately one new case per year. This is based on a UK population of 63 million, and a birth rate of 12.3 per 1000 population.

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

Please identify the information on which this is based.

n/a for panel tests.

The mutation prevalence is approximately 1 in 1000 based on the population prevalence and penetrance of 100%.

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

n/a for panel tests

Non-penetrance has not been reported in the few reported cases to date, but as clinical expression is variable it cannot be ruled out.

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

n/a for panel tests.

IL11RA mutations are expected to comprise approximately 5-10% of our target population which consists of individuals with multisuture synostosis, either the result of a consanguineous union or of Pakistani ethnic background, or who also have delayed tooth eruption or supernumerary teeth; and in whom no mutation was identified following a level 1 screen.

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.

Bidirectional sequencing is expected to have an analytical sensitivity >90%, as no whole exon deletions or duplications have been reported to date. Sequencing will not detect whole exon duplications, however whole exon deletions may be suspected if non-amplification occurs in an individual with known or suspected consanguinity. The specificity is also expected to be high, as mutations appear to be clearly pathogenic causing null alleles, or missense variants and in-frame duplications within functional domains of the protein that are highly conserved between family members.

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 two years service.

Clinical specificity approaches 100% as the disorder is very rare in the general population. Clinical sensitivity in cases with multi-suture synostosis, consanguinity or tooth anomalies, and in whom no mutation has been detected on a level 1 screen is ~10-20%. In subjects with multisuture synostosis only that have no mutation detected on a level 1 screen the sensitivity is ~1%.

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

Positive predictive value approaches 100% in probands and affected relatives. Due to genetic heterogeneity *IL11RA* mutations are expected to be identified in only 10-20% of individuals in the target population.

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

Cases which fully meet the diagnostic criteria will be tested for the most frequent craniofacial mutations initially; this 'level 1' screen encompasses *FGFR1* exon 7, *FGFR2* exons 8 and 10, *FGFR3* exons 7 and 10 and *TWIST1* exon 1. Clearly consanguineous or Pakistani cases, or isolated cases with dental anomalies, that test negative for this screen will then go on to be screened *IL11RA*. All referrals will be assessed for appropriateness by the Oxford craniofacial service. Testing will not be carried out until appropriate clinical details have been received.

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.

A positive diagnosis will alert clinicians to the possibilities of dental complications (delayed tooth eruption, supernumerary teeth) and insidious onset of craniosynostosis associated with raised intracranial pressure, enabling more personalised prognostic information to be given to the family, and specific monitoring of the patient to be planned. In addition it will enable appropriate genetic counselling and prenatal testing to be offered; further molecular studies including further analysis of the FGFR2 gene (level 2 screen), ERF analysis, array CGH and whole exome analysis would not be required (cost saving approx £2039).

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

It will be impossible to give a correct diagnosis for the cause of craniosynostosis. Therefore accurate genetic counselling will not be possible. The sibling and offspring recurrence risks for craniosynostosis may range from 0-50%; giving the correct figure within this wide range requires a molecular diagnosis. In addition, no follow on pre-implantation or prenatal diagnosis would be possible.

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.

The clinical features are too non-specific to enable accurate clinical diagnosis in the absence of molecular genetic testing.

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.

N/A

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

N/A

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

N/A

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.

N/A

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

Due to the variation of clinical presentation, prediction of precise phenotype severity in homozygote/compound heterozygote fetuses following prenatal testing may be difficult. Detailed ultrasound scanning would be indicated, although it does not always reliably detect craniosynostosis prenatally. Diagnostic testing in minors may inadvertently reveal carrier status.

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.

	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)	Level 2 screen aCGH ERF screen	490 (HSS) 500 490 (HSS)
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)		**1480**

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

Number of index cases expected annually	(a) 20
Cost to provide tests for index cases if the genetic test in this Gene Dossier was not available (see Q32)	(b) £1,480
Total annual costs pre genetic test	(a) x (b) = (c) £29,600
Total annual costs to provide genetic test	(a) x cost of genetic testing for index case = (d) 20 x £510 = £10,200
Additional savings for 100% positive rate for index cases	(d) – (c) = (e) £10,200 – £29,600 = -£19,400
Percentage of index cases estimated to be negative	(f) 95%
Number of index cases estimated to be negative	(f) x number of index cases = (g) 95% x 20 = 19
Costs to provide additional tests for index cases testing negative	(g) x (b) = (h) £28,120
Total costs for tests for index patient activity	(e) + (h) = (i) -£19,400 + £28,120 = £8,720
Total costs for family members	Costs for family member test x number of family members expected to test in a year (j) £153 x 2 = £306
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) 0
Total costs for family members minus any family member testing costs already provided	(j) – (k) = (l) £306 –£0 = £306
Additional costs for all activity expected in a year	(i) + (j) or (i) + (l) £8,720 +£306 = £9,026

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

A 7 year old male presented to the craniofacial unit with pansynostosis and a suggested diagnosis of Crouzon syndrome (autosomal dominant). Parents were 1st cousins and of Pakistani origin; in another branch of the family (still in Pakistan), 3 siblings were reported to be similarly affected, but no further details were available. The proband was also noted to have eczema/atopy and mild learning disability. Genetic testing of FGFR2 and FGFR3 (Crouzon syndrome genes) and array CGH was normal. The family was identified by the consultant clinical geneticist as a high priority for exome sequencing because the multisuture involvement made a genetic cause more likely and the mother was-anxious for a diagnosis.

Exome sequencing in a research lab revealed a previously described homozygous mutation in IL11RA, confirmed on Sanger sequencing in the same research lab. This changed the diagnosis from Crouzon syndrome to CRSDA and confirmed an autosomal recessive, rather than dominant mechanism of inheritance.

TESTING CRITERIA

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

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: **Craniosynostosis And Dental Anomalies**	
Approved name and symbol of disorder/condition(s): Craniosynostosis and Dental Anomalies; CRSDA	**OMIM number(s):** #614188
Approved name and symbol of gene(s): interleukin 11 receptor, alpha; IL11RA	**OMIM number(s):** ***600939**

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**
Craniosynostosis affecting multiple sutures **AND** no mutation identified in *FGFR1*, *FGFR2*, *FGFR3* or *TWIST1*	
AND inheritance pattern compatible with autosomal recessive inheritance **OR** of Pakistani ethnic origin	
AND supernumerary teeth or delayed dental eruption	
OR At risk family members where familial mutation is known	

Additional Information:
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.