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

Test – Disease – Population Triad

Disease – name	Primary congenital glaucoma (PCG)
OMIM number for disease	231300
Disease – alternative names Please provide any alternative names you wish listed	Glaucoma, congenital; GLC3 Buphthalmos Primary infantile glaucoma; PIG Infantile congenital glaucoma; ICG
Disease – please provide a brief description of the disease characteristics	Primary congenital glaucoma (PCG) is a congenital or infantile onset condition, characterised by raised intraocular pressure. Signs of this may include enlargement of the globe (buphthalmos), cloudy/hazy corneas, lacrimation and optic disc changes. The condition causes irreversible optic nerve damage and blindness if untreated.
Disease - mode of inheritance	Autosomal recessive
Gene – name(s)	CYP1B1
OMIM number for gene(s)	601771
Gene – alternative names Please provide any alternative names you wish listed	Cytochrome P450 Subfamily 1, Polypeptide 1 (P4501B1)
Gene – description(s) (including number of amplicons).	Located at 2p22.2 (38294746-38303323) (hg19) 3 exons, 2 of which are coding Longest transcript is 5347bp and 543 amino acids (NM_000104.3) Amplified in 6 amplicons to span the coding region Coding sequence 1632 bp
Mutational spectrum for which you test including details of known common mutations.	Analysis for point mutations and small insertions / deletions. A wide variety of mutations have been reported, occurring throughout the coding region, no common mutations have been described. A single case has been identified with a whole gene deletion (Kelberman et al., 2011. Ophthalmology, in press).
Technical Method (s)	Bi-directional sequence analysis of coding exons (2 and 3)
Validation Process Note: please explain how this test has been validated for use in your laboratory	Sequencing primers have been designed and optimised for the two coding amplicons in six overlapping fragments. Validation has been carried out on a panel of mutation positive patients (n=9) identified in a research laboratory and also on normal controls (n=60). The laboratory participates in all relevant technical EQA schemes that are available through UK NEQAS and EMQN: 2007 -Genotypes correctly assigned in 3/3 sequencing EQA samples 2006 - Genotypes correctly assigned in 3/3 sequencing EQA samples 2005 - Genotypes correctly assigned in 3/3 sequencing EQA

	samples 2004 - Genotypes correctly assigned in 4/4 sequencing EQA samples.
Are you providing this test already? **If yes, how many reports have you produced?** **Please give the number of mutation positive/negative samples you have reported**	Currently offered by research laboratory at the Institute of Child Health and being validated for a diagnostic service. If Yes: Number of reports issued: 24 (Research) Number of reports mutation positive: 9 (Research) Number of reports mutation negative: 15 (Research)
For how long have you been providing this service?	
Is there specialised local clinical/research expertise for this disease?	Yes [ ] Please provide details Clinical: Paediatric glaucoma clinics at Great Ormond Street Hospital and Moorfields Eye Hospital (Mr Ken Nischal, Professor Peng Khaw, Miss Maria Papadopoulos). Research: Dr Jane Sowden, Dr Lily Islam (UCL Institute of Child Health), studying eye development and the genetics of congenital eye defects.
Are you testing for other genes/diseases closely allied to this one? Please give details	No
Your Current Activity If applicable - How many tests do you currently provide annually in your laboratory?	Index cases: 20 Family members where mutation is known: 40
Your Capacity if Gene Dossier approved How many tests will you be able to provide annually in your laboratory if this gene dossier is approved and recommended for NHS funding?	Index cases: 45 Family members where mutation is known: 80
Based on experience how many tests will be required nationally (UK wide)? Please identify the information on which this is based	Index cases: 45 (Papadopoulos et al., 2007. IOVS 48:4100-4106) Family members where mutation is known: 50 (estimated, confirmations of heterozygote status in parents of probands with mutations, and a small number of sibling tests)
National Activity (England, Scotland, Wales & Northern Ireland) **If your laboratory is unable to provide the full national need please could you provide information on how the national requirement may be met.**	Our laboratory can meet the national requirement

Epidemiology

Estimated prevalence of disease in the general UK population Please identify the information on which this is based	1:10,000 (Francois, 1980. Ophthalmologica 181:61–73; in Vasiliou et al., 2008. Annu. Rev. Pharmacol. Toxicol. 48:333–58).
Estimated gene frequency (Carrier frequency or allele frequency) Please identify the information on which this is based	Approximately 1/50 carrier frequency based on incidence quoted above.
Estimated penetrance Please identify the information on which this is based	>90% (Suri et al., 2009. Ophthalmology 116:2101-2109).
Target Population Description of the population to which this test will apply (i.e. description of the population as defined by the minimum criteria listed in the testing criteria)	Patients with primary congenital glaucoma (PCG), defined by elevated intraocular pressure (IOP) >21 mm Hg and/or signs consistent with elevated IOP, including • disc cupping >0.3 or disc asymmetry ≥0.2 • progressive disc cupping • buphthalmos (prominent, enlarged eye) • enlarged corneal diameter (>11 mm in newborn, >12 mm in a child <1 year, or >13 mm in a child >1 year) • corneal edema • Descemet's membrane splits (Haab's striae) • visual field defects • progressive myopia in a child < 2 years of age.
Estimated prevalence of disease in the target population	50% of patients with a clinical diagnosis of PCG would be expected to have mutations in CYP1B1, in our experience (Kelberman et al., 2011. Ophthalmology, in press).

Intended Use (Please use the questions in Annex A to inform your answers)

Please tick the relevant clinical purpose of testing	YES	NO
Diagnosis	X	
Treatment		X
Prognosis & Management	X	
Presymptomatic testing	X	
Risk Assessment for family members	X	
Risk Assessment – prenatal testing	X	

Test Characteristics

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.	Direct sequence analysis has a high sensitivity in this laboratory. We use Big Dye chemistry, ABI analysers (3130XL, 3730XL) and analyse using Mutation Surveyor software. We participate and perform successfully in EQA programmes for sequence analysis (see above). Bidirectional sequence analysis has specificity approaching 100% although large insertions/deletions and deep intronic mutations will not be detected.
Clinical sensitivity and specificity of test in target population The *clinical sensitivity* of a test is the probability of a positive test result when disease is known to be present; the *clinical specificity* is the probability of a negative test result when disease is known to be absent. The denominator in this case is the number with the disease (for sensitivity) or the number without disease (for specificity)	Clinical sensitivity: 50% of patients with the symptoms described above will be expected to have a mutation in CYP1B1. No other genes have yet been described. Clinical specificity: High. Later-onset disease (e.g. in middle childhood/adolescence) caused by CYP1B1 mutations is rarely reported.
Clinical validity (positive and negative predictive value in the target The *clinical validity* of a genetic test is a measure of how well the test predicts the presence or absence of the phenotype, clinical disease or predisposition. It is measured by its *positive predictive value* (the probability of getting the disease given a positive test) and *negative predictive value* (the probability of not getting the disease given a negative test).	Positive predictive value: High. A patient with 2 mutations is expected to exhibit PCG symptoms. Negative predictive value: High. Biallelic mutations have not been detected in patients with other ocular phenotypes or in normal controls.
Testing pathway 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 can be added to the document as a separate sheet if necessary.	Direct sequence analysis of CYP1B1 is the only test offered.
Clinical utility of test in target population (Please refer to Appendix A) Please provide a description of the clinical care pathway.	Clinical care pathway: Children present with the signs of raised intraocular pressure (as detailed above). Clinical care includes surgical management and medical therapy as appropriate. This aims to lower intraocular pressure and thus preserve sight. Probands for testing will be identified from clinical examination and imaging by ophthalmologists, and at-risk family members will be identified by

	ophthalmologists or clinical geneticists. 89% of children in the UK who have PCG are treated at one of four centres (Papadopoulos et al., 2007. IOVS 48:4100-4106), so we anticipate that most referrals will come from these, with some additional referrals coming from ophthalmologists at other centres and the UK network of clinical genetics departments. Testing will allow accurate genetic counselling of offspring risk and recurrence risk for the parents of an affected child, together with some prognosticating information: while corneal clouding is expected to clear with glaucoma control, it has been shown that some CYP1B1 mutations are associated with endotheliopathy (Kelberman et al., 2011. Ophthalmology, in press). Waiting for corneal clearing in these circumstances leads to stimulus deprivation amblyopia which could be treated by timely corneal transplant.
How will the test add to the management of the patient or alter clinical outcome?	Early diagnosis and management of raised intraocular pressure is crucial to prevent or minimise permanent visual deficit. While surgical management is the treatment of choice for PCG, the finding of CYP1B1 mutations may indicate prognosis, depending on already published genotype/phenotype correlations, and over time will allow correlation of outcome and genotype data. A 25% recurrence risk is confirmed if recessive CYP1B1 mutations are identified, so accurate genetic counselling can then be given to families.
What impact will this test have on the NHS i.e. by removing the need for alternative management and/or investigations for this clinical population? Please provide evidence from your own service.	Family members (e.g. newborn siblings of an affected child with confirmed CYP1B1 mutations) can be tested pre- or post-natally, and discharged from subsequent follow-up if no CYP1B1 mutation is found. All children identified as having CYP1B1 mutations will need lifelong surveillance and management of their glaucoma. In infancy, accurate ascertainment of intraocular pressure can be technically difficult and may require examination under anaesthesia which presents risks to the child. These risks, and considerable expense to the NHS for the years of screening, can be obviated if the child does not carry biallelic mutations. If he/she does have biallelic mutations, this information can be used to inform decisions about their clinical care (as above).
What are the consequences of not doing this genetic test? Commissioners have asked for specific information to support introduction of tests.	Clinicians would have reduced information available to assist them with decision-making regarding clinical management. At-risk siblings will be subjected to continued, potentially invasive investigations throughout childhood if mutation status cannot be determined.
Utility of test in the NHS In a couple of sentences explain the utility of this test for the disease(s)	Molecular testing provides early and accurate diagnosis and identifies children at high risk of sight loss who require immediate surgical and medical management to reduce intraocular pressure. It also allows accurate genetic counselling to be given to families, and removes unnecessary and potentially invasive treatments for at-risk

	family members.
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 alternative test is available. Clinical diagnosis relies upon signs of high intraocular pressure and therefore potential permanent damage already being present. The earlier this diagnosis can be made – e.g. by molecular testing – the earlier intervention can be made to reduce pressure and prevent loss of sight.
Please describe any specific ethical, legal or social issues with this particular test?	None.

UKGTN Testing Criteria

Name of Disease(s): GLAUCOMA 3, PRIMARY CONGENITAL, A; GLC3A (231300)

Name of gene(s): cytochrome P450, family 1, subfamily B, polypeptide 1; CYP1B1 (601771)

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 Paediatric Ophthalmologist	
Consultant Clinical Geneticist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:

Criteria	Tick if this patient meets criteria
Birth to two years of age **AND**	
Primary congenital glaucoma with elevated intraocular pressure (IOP) >21 mm Hg **OR**	
>1 ocular sign consistent with elevated IOP: • disc cupping >0.3 or disc asymmetry ≥0.2 • progressive disc cupping • buphthalmos (prominent, enlarged eye) • enlarged corneal diameter (>11 mm in newborn, >12 mm in a child <1 year, or >13 mm in a child >1 year) • corneal edema • Descemet's membrane splits (Haab's striae) **OR**	
Testing for known mutation in relative of affected child.	

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.