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

TEST – DISEASE/CONDITION – POPULATION TRIAD			
Submitting laboratory:	**Leeds RGC**	**Approved:**	**September 2012**
1. Disease/condition – approved name and symbol as published on the OMIM database (alternative names will be listed on the UKGTN website)	Various diseases of brain malformation, primarily within the **lissencephaly/polymicrogyria** spectrum. Complete list of linked OMIM diseases: Epileptic encephalopathy, early infantile, 1 (West syndrome, Ohtahara syndrome) (308350) Hydranencephaly with abnormal genitalia (300215) Lissencephaly, X-linked 1 (300067) & 2 (XLAG, 300215) Subcortical laminar heterotopia, X-linked (300067) Mental retardation, X-linked, with or without seizures, ARX-related (MRXARX/MRX29 300419) Partington X-linked mental retardation syndrome (309510) Corpus callosum, agenesis of, with abnormal genitalia (Proud syndrome) (300004) Lissencephaly 1 (300004), 3 (611603) & 4 (with microcephaly) (614019) Subcortical laminar heterotopia (607432) Polymicrogyria, bilateral frontoparietal (606854) Polymicrogyria, asymmetric (610031) Polymicrogyria with optic nerve hypoplasia (613180)		
2. OMIM number for disease/condition	See above.		
3. Disease/condition – please provide a brief description of the characteristics of the disease/condition and prognosis for affected individuals. Please provide this information in laymen's terms.	**Lissencephaly** (LIS), literally meaning smooth brain, is characterized by smooth or nearly smooth cerebral surface and a paucity of gyral and sulcal development, encompassing a spectrum of brain surface malformations ranging from complete agyria to subcortical band/laminar heterotopia (SBH), and characterised by an abnormally thick and underdeveloped cerebral cortex. Agyria is a brain lacking any convolutions or gyri (the most severe end of the spectrum). Pachygyria involves unusually thick and malformed convolutions/gyri in the cerebral cortex. SBH involves displaced (ectopic) bands of neuronal tissue just beneath the cortex, separated by a band of white matter. Lissencephaly syndromes are frequently diagnosed by MRI or ultrasound before or shortly after birth, and are characterised by a heterogeneous range of neurological symptoms of varying severity, including mental retardation, seizures, muscle		

	spasticity/hypotonia, microcephaly and dysmorphism. The most severe presentations may cause death in infancy or early childhood, although patients with milder presentations (e.g. SBH) may survive until adulthood and have only mild developmental delay and seizures. **Polymicrogyria** is similar to lissencephaly both in the nature of brain malformation and (to some extent) clinical presentation. It is characterised by excessive numbers of very small gyri on the brain surface (which may be misdiagnosed as lissencephaly on brain scans). Again, clinical presentation may be varied and complex, with developmental delay and mental retardation common, in concert with varying degrees of motor dysfunction and seizures. In addition to X-linked lissencephaly, mutations in the **ARX gene** have been associated with a nearly continuous series of developmental disorders ranging from lissencephaly (described above, but also including hydranencephaly with abnormal genitalia in some reported cases), to Proud syndrome (agenesis of corpus callosum with abnormal genitalia, severe mental retardation, seizures and spasticity, with milder and in some cases non-penetrant effects on female carriers), to early infantile epileptic encephalopathy (severe infantile spasms without obvious brain malformation), to Partington syndrome (syndromic mental retardation with variable movement and muscle disturbances including dystonia and dysarthia, and mild dysmorphism), to non-syndromic mental retardation. These disorders are inherited in an X-linked recessive fashion, although in some cases (specifically Proud syndrome) females may be mildly affected. There is significant genotype-phenotype correlation within this spectrum, with common mutations associated with certain phenotypes (most notably two polyalanine expansions in ARX exon 2 associated with infantile encephalopathy and mental retardation), see **Kato et al 2004, Human Mutation, 23(2):147-159** and **Shoubridge et al 2010, Human Mutation, 31(8):889-900**.
4. Disease/condition – mode of inheritance	**LIS1 & Subcortical laminar heterotopia (PAFAH1B1 gene)** – autosomal dominant. **LISX1 (DCX gene)** – X-linked recessive (i.e. affects males with DCX mutations) **X-linked subcortical laminar heterotopia (DCX gene)** – X-linked dominant (i.e. affects females with DCX mutations) **Polymicrogyria, bilateral frontoparietal (GPR56 gene)** – autosomal recessive. **LIS3 (TUBA1A gene)** – autosomal dominant.

	Polymicrogyria, asymmetric (TUBB2B gene) – autosomal dominant. **Polymicrogyria with optic nerve hypoplasia (TUBA8 gene)** – autosomal recessive. **LIS4 (with microcephaly; NDE1 gene)** – autosomal recessive. ***ARX-related disorders:*** **Early infantile epileptic encephalopathy, X-linked mental retardation with or without seizures, ARX-related, & Partington syndrome** – X-linked recessive (caused principally by polyalanine expansions in exon 2 and missense changes; no reported cases of clearly linked clinical phenotype in carrier females). **LISX2 (XLAG), Hydranencephaly with abnormal genitalia, Proud syndrome** – X-linked semi-dominant (affects males & females; males with classical disease, females with reduced severity and penetrance, in some cases mirroring the male phenotypes in the X-linked recessive forms **Wallerstein et al. 2008, Clinical neurology and neurosurgery, 110(6): 631-634**).
5. Gene – approved name(s) and symbol as published on HUGO database (alternative names will be listed on the UKGTN website)	This service proposes to test for mutations in a panel of cerebral malformation genes currently comprising: • ARISTALESS-RELATED HOMEOBOX, X-LINKED; **ARX** (***300382**) • DOUBLECORTIN; **DCX** (***300121**) • PLATELET-ACTIVATING FACTOR ACETYLHYDROLASE, ISOFORM 1B, ALPHA SUBUNIT 1 (45kDa); **PAFAH1B1** (***601545**) • G PROTEIN-COUPLED RECEPTOR 56; **GPR56** (***604110**) • TUBULIN, ALPHA-1A; **TUBA1A** (***602529**) • TUBULIN, BETA-2B; **TUBB2B** (***612850**) • TUBULIN, ALPHA-8; **TUBA8** (***605742**) nudE nuclear distribution gene E homolog 1 (A. nidulans) Other genes may be added to the service as evidence is gained; some of the listed conditions are very rare recessive disorders, and other genetic causes of cerebral malformation may be determined in future.
6. OMIM number for gene(s)	See above.
7. Gene – description(s)	**ARX:** Encodes the aristaless-related homeobox protein, which is a homeodomain transcription factor protein. These play important roles in cerebral development. Xp21.3, 1686bp, 5 exons, 562 amino acids. **DCX:** Encodes the brain-specific doublecortin protein, which interacts with LIS1 (PAFAH1B1). Influences neurite outgrowth during neuronal differentiation, and is involved in microtubule stabilisation. Xq23, 1080bp,

	9 exons (6 coding), 360 amino acids. **PAFAH1B1:** Encodes the alpha subunit of the platelet-activating factor acetylhydrolase (PAFAH) enzyme (aka LIS1), which catalyses the removal of an acetyl group from the platelet activating factor (PAF), rendering it inactive. It has been shown to interact with the protein products of DCX and NDE1, and is involved in microtubule function (including interactions with the protein product of TUBA1A) and neuronal migration, differentiation and growth. 17p13.3, 1233bp, 11 exons, 411 amino acids. **GPR56:** Encodes the G protein-coupled receptor 56, a membrane-bound "class B" (secretin-like) receptor (with 7 transmembrane domains) which undergoes post-translational cleavage into two active subunits. It is widely expressed (especially in brain regions engaged in neurogenesis), and thought have a regulatory role in cortical patterning. 16q21, 2061bp, 14 exons, 687 amino acids. **TUBA1A:** Encodes the tubulin-alpha 1A chain protein, which is a constituent of the microtubule cytoskeleton most prominently expressed in differentiated neuronal cells. It is expressed particularly heavily during fetal brain development. 12q13.12, 1356bp, 4 exons, 451 amino acids. **TUBB2B:** Encodes the tubulin-beta 1B chain protein, which is a constituent of the microtubule cytoskeleton expressed in migrating, differentiating and mature neuronal cells. It is expressed particularly heavily during fetal brain development, and remains in some regions of the adult brain. It is thought to be involved in neuronal migration and has been shown to form heterodimers with some tubulin-alpha subunits. 6p25.2, 1338bp, 4 exons, 445 amino acids. **TUBA8:** Encodes the tubulin-alpha 8 protein, which is involved in microtubule assembly and function and is heavily expressed in developing neuronal structures. 22q11.21, 1350bp, 5 exons, 449 amino acids. **NDE1:** Encodes the protein homolog of the *A. nidulans*/rat Nude protein, which is involved in nuclear distribution in *A. nidulans*, and has been shown to interact with PAFAH1B1, and have some involvement in microtubule function and neuronal tissues. It is also known to be phosphorylated by CDK1, which occurs during mitotic progression. 16p.13.11, 1008bp, 11 exons (8 coding), 335 amino acids.
7b. Number of amplicons to provide this test	22 long-range PCR fragments (analysed by next generation sequencing) 4 regular PCR fragments (analysed by dideoxy sequencing) Sizing PCRs for ARX polyalanine expansions if necessary (see 8. below)

7c. MolU/Cyto band that this test is assigned to	Band F (25 MolU) 2012/13 GenU Band G 2013/14
8. Mutational spectrum for which you test including details of known common mutations	Testing covers entire coding region for all genes involved (exons in untranslated regions are omitted in some cases; no mutations have been described in these regions as far as we know), and should cover all described missense, nonsense and small deleletion/duplication/insertion mutations described. Analysis also includes intron/exon boundaries; generally a minimum of 20 bases into 5' and 3' introns. Two polyalanine expansions in exon 2 of ARX, associated with early infantile encephalopathic seizures, X-linked mental retardation and Partington syndrome (OMIM variants 300382.0001 and 300382.0002) will be detected dideoxy sequencing. Testing for these expansions in familial cases can be performed by fluorescent sizing PCR. Deletion/duplication analysis at exonic level is performed by MLPA (www.mlpa.com) in two lissencephaly-related genes (PAFAH1B1, DCX) using MRC-Holland kit P061, and in the ARX gene using MRC-Holland kit P189 (CDKL5 kit). Large genomic defects in the other genes would not be identified, however, large deletion/duplication mutations appear to be very rare (except in PAFAH1B1 and ARX).
9. Technical method(s)	PCR amplification of coding and flanking (minimum 20bp) intronic sequence. Next generation sequence analysis using Illumina GAII platform. Confirmation of mutations (where necessary) by dideoxy sequence analysis or sizing PCR. Detection of deletion/duplications of whole exons in PAFAH1B1, DCX and ARX using MLPA. Known mutations will be tested by dideoxy sequencing of a small genomic amplicon including the mutation locus, using pre-designed & validated primers to order.
10. Validation process Please explain how this test has been validated for use in your laboratory	Next generation sequencing workflow has been validated and applied to provision of our BRCA and HNPCC cancer services. The approach has been validated in a pilot study of 55 cases (**Morgan et al, 2010, Human Mutation 31:484-491**). An additional study of 50 cases analysed in parallel with Sanger sequencing was fully concordant for 485 variants (100% sensitivity). See details below of statistical analysis (section on analytical sensitivity and specificity). All long range primers were checked for SNPs and genomic specificity/uniqueness using the Manchester NGRL "SNPcheck" algorithm and NCBI BLAST. A

	minimum sequence coverage threshold was set at depth of 50 sequence reads. Diagnostic Next Generation sequencing pathways for BRCA and HNPCC genes have been established in service for 2 years. Approximately 1600 screening reports have been produced. Additional pathways for a multi-gene panel of pheochromocytoma & paraganglioma genes, and for Marfan syndrome have since been successfully introduced. This workflow was also audited by the peer assessors during our most recent laboratory CPA assessment (May 2011).
11a. Are you providing this test already?	Yes
11b. If yes, how many reports have you produced?	N/A
11c. Number of reports mutation positive	N/A
11d. Number of reports mutation negative	N/A
12. For how long have you been providing this service?	N/A
13a. Is there specialised local clinical/research expertise for this disease?	Yes
13b. If yes, please provide details	Paediatric Neurology unit at Leeds General Infirmary, led by Dr John Livingston, have particular clinical interest and expertise in paediatric cerebral malformation disorders, with particular knowledge of rare recessive disorders of this type (due to large autozygous population locally, of Pakistani origin), and act as gatekeepers for our local referral stream (outwith Genetics).
14. Are you testing for other genes/diseases/conditions closely allied to this one? Please give details	Our current portfolio of neurology/seizure disorders is limited to a full service for GLUT1 deficiency syndrome (SLC2A1 gene).
Your current activity If applicable - How many tests do you currently provide annually in your laboratory?	
15a. Index cases	N/A
15b. Family members where mutation is known	N/A
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?	
16a. Index cases	Up to ~500 samples per year on basis of current work streams (52 weeks/2 x 20 patients).
16b. Family members where mutation is known	Up to ~300

Based on experience how many tests will be required nationally (UK wide) per annum? Please identify the information on which this is based	10 samples locally for these genes sent to external providers in the last two years. As we service ~ 5% of the UK population, this would extrapolate very roughly to **100 samples per year** (speculative referrals and exclusion tests are, however, likely to be more prevalent in our experience in cases where the disease is tested in the UK by an accredited laboratory, so this estimate may be conservative). Overall incidence of lissencephaly is estimated as high as 40 per million births (**GeneReviews: LIS1-Associated Lissencephaly/Subcortical Band Heterotopia, PubmedID 20301752**). This would suggest ~30 referrals per year with classic lissencephaly alone. Overall incidence of polymicrogyria remains unknown, although cortical dysplasia of some kind affects around 1 in 2,500 births (~300 births/yr in the UK).
17a. Index cases	~100 (see above)
17b. Family members where mutation is known	~100 (estimate). Majority of cases arise *de novo*, so scope of cascade testing is limited. However, we envisage considerable interest in carrier testing in females of reproductive age in families with X-linked disease (ARX & DCX gene), and some scope for confirmations in families with milder seizure/MR phenotypes. Additionally, some testing of parents may be indicated in cases with variants of unknown significance, in order to exclude the possibility of the variant being carried by a healthy parent (i.e. benign). Family/carrier testing will be relevant for autosomal recessive mutations, which are anticipated to make up a small minority of cases.
18. 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.** 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. It is appreciated that some laboratories may not be able to answer this question. If this is the case please write "unknown".	Cardiff currently offer single-gene services for a proportion of the genes covered in this panel. The screening services they offer (and prices quoted on UKGTN website) are: ARX (£550 + £90 for polyalanine analysis) DCX (£250) TUBA1A (£250) GPR56 (£400) PAFAH1B1 (£400) No national providers (as far as we know) screen TUBA8, TUBB2B or NDE1, although the literature evidence suggests that mutations in these genes are relatively rare.

EPIDEMIOLOGY	
19. Estimated prevalence of condition in the general UK population Please identify the information on which this is based	Overall incidence of lissencephaly is estimated as high as 40 per million births (**GeneReviews: LIS1-Associated Lissencephaly/Subcortical Band Heterotopia, PubmedID 20301752**), which is described as a possible "underestimate", and as low as ~12 per million births (**de Rijk-van Andel et al, 1991, Neuroepidemiology, 10(4): 200-204**). No published estimates are available for polymicrogyria or ARX-associated conditions, although cortical dysplasias (of which polymicrogyrias are a significant proportion) are estimated to affect ~1 in 2500 births (**Villard L, 2004, "Polymicrogyria", Orphanet encyclopedia** http://www.orpha.net/data/patho/GB/uk-PMG.pdf). No UK-specific data have been published on the prevalence of these disorders. We have a subset of services for and local research expertise in the Yorkshire Pakistani population (esp. Bradford), which practices a high proportion of consanguineous marriages (~70%); we would expect an elevated prevalence of the autosomal recessive disorders in this local group (polymicrogyria (GPR56 and TUBA8 genes) and LIS4 (NDE1 gene)), data shows a 16-fold increase in incidence of recessive conditions in similar populations (www.borninbradford.nhs.uk, **Bundey & Alam, 1993, Eur J Hum Genet 1:206-219**).
20. Estimated gene frequency (Carrier frequency or allele frequency) Please identify the information on which this is based	N/A; disorders are X-linked or dominant in majority of cases, and frequently arise *de novo*. Recessive disorders are very rare (largely arising in cases of autozygosity) and no carrier frequencies have been published. See above for estimates of prevalence.
21. Estimated penetrance Please identify the information on which this is based	No specific examples of incomplete penetrance have been described for the autosomal genes in this panel. Examples of apparent non-penetrance have been described in X-linked subcortical band heterotopia caused by heterozygous mutations in the DCX gene in females (**Aigner et al, 2003, Neurology 60(2):329-332, Demelas et al, 2001, Neurology 57(2):327-330**), which is rare and has no published figures for non-penetrance, and in Proud syndrome, in female carriers of ARX mutations, which generally exhibits a less severe phenotype than in males and which showed penetrance of 35% (significant developmental abnormalities) in a small sample size (n=25; **Marsh et al, 2009, Brain 132(6):1563-1576**).
22. Estimated prevalence of condition in the target population.	Largely unknown. Testing panel has not been used previously to the best of our knowledge, and no large-

The target population is the group of people that meet the minimum criteria as listed in the Testing Criteria.	scale, controlled screening studies have been performed on most of the genes involved. 29 out of 29 patients with bilateral frontoparietal polymicrogyria (BFPP) had GPR56 mutations (**Piao et al, 2005, Ann Neurol 58(5):680-687**). 40-70% of patients with a definitive diagnosis of lissencephaly type I have PAFAH1B1/TUBA1A/DCX mutations (**German Society of Human Genetics (gfh)/EuroGentest "Indication for Criteria of Disease: Type 1 (Classic) Lissencephaly"** http://www.eurogentest.org/web/files/public/unit3/ClinicalUtilityGeneCards/Indication%20criteria%20-%20Lissencephaly.pdf). Cardiff laboratory (presumably based on empirical data) report that 5% of patients with classic lissencephaly who are PAFAH1B1 negative, and 15-20% of patients with lissencephaly and cerebellar hypoplasia or absent corpus callosum have TUBA1A mutations (lissencepahly type III), see UKGTN gene dossier for TUBA1A (LIS3). TUBA8 and NDE1 mutations have (thus far) exclusively been reported in consanguineous births from autozygous populations of Asian origin. There are several such populations in the UK, in particular, the Pakistani-origin populations of Bradford (www.borninbradford.nhs.uk) and Birmingham (**Bundey & Alam, 1993, Eur J Hum Genet 1:206-219**), in which consanguineous births have been shown to have a 16-fold increase in recessive disorders compared to non-consanguineous ones (**Bundey & Alam, 1993**). Prevalence within these populations is likely elevated above the negligible level inferred in non-consanguineous ones.

INTENDED USE

23. Please tick the relevant clinical purpose of testing		
Diagnosis	☒ **Yes**	☐ **No**
Treatment	☐ **Yes**	☒ **No**
Prognosis & management	☒ **Yes**	☐ **No**
Presymptomatic testing	☐ **Yes**	☒ **No**
Carrier testing for family members	☐ **Yes**	☒ **No**
Prenatal testing	☐ **Yes**	☒ **No**

TEST CHARACTERISTICS

24. 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.

Statistical confidence in sensitivity of Illumina sequencing approach

Data

1) Total number of known variants by 'Gold standard' (Sanger sequencing) re-tested by clonal sequencing: 485

Total number confirmed by clonal sequencing: 4852) Number of unique variants (i.e. each variant counted once only) by 'Gold standard' (Sanger sequencing) re-tested by clonal sequencing: 78

Total number confirmed by clonal sequencing: 78

Analysis

Determined by binomial confidence interval method (see http://statpages.org/confint.html). Using 95% confidence interval (2.5% in each tail).1) Total number of variants (n=485)

As no variants have been missed, binomial distribution predicts we can be 95% confident that the false negative rate is below 0.76%.

However, it may be unrealistic to assume all variants are equally detectable, so considering each variant once only is a more cautious approach.

2. Number of unique variants

The most cautious approach to validating clonal sequencing sensitivity would be to test a different variant each time, since re-testing a variant which is already known to be detectable is of limited value. Therefore, a repeat analysis was carried out counting only the total number of unique variants (n=78). Again, as no variants have been missed, binomial distribution predicts we can be 95% confident that the false negative rate is below 4.62%.

Standard assumptions apply including:

- Quality standards for validation work are the same for subsequent patient tests.
- Testing criteria and methods remain unchanged – or modifications do not have an impact on sensitivity.
- Samples tested (or the range of variants tested in validation) are representative of patients (or variants) that will be tested.

Specificity of Illumina sequencing

Common artefacts have been observed on numerous occasions at single nucleotide runs (As and Ts). Where these are detected at a significant level Sanger sequencing is used to exclude the presence of a variant.

Sensitivity and specificity of MLPA dosage analysis

It is predicted that the sensitivity of MLPA should be greater than 95% where the deletion or duplication includes one or more of the test probes included in the kit.

Using the MLPA technique, false positives for apparent single exon deletions have been observed for some kits. These may be due either to point mutations at the ligation site or artefacts relating to reaction conditions. In all cases an alternative method is used to confirm the presence of a mutation.

25. 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 disease is known to be absent. The denominator in this case is the number with the disease (for sensitivity) or the number without condition (for specificity).

When classical lissencephaly is diagnosed positively by a combination of cranial MRI (showing definite agyria/pachygryria spectrum with gradient) and abnormal EEG (electroencephalography) scan/overt

seizure phenotype, clinical sensitivity of testing the PAFAH1B1, DCX and TUBA1A genes is empirically estimated at 40-70% (**German Society of Human Genetics (gfh)/EuroGentest "Indication for Criteria of Disease: Type 1 (Classic) Lissencephaly"** http://www.eurogentest.org/web/files/public/unit3/ClinicalUtilityGeneCards/Indication%20criteria%20-%20Lissencephaly.pdf). Addition of ARX (which causes lissencephaly and hydranencephaly with abnormal genitalia) will likely increase this sensitivity in probable X-linked cases and/or those with abnormal genitalia. Addition of NDE1 will likely only affect sensitivity in consanguineous families, as all reported instances have been autozygous, although the gene was only implicated in 2011.

Clinical sensitivity in polymicrogyria cases is unknown, and complicated by the difficulty of conclusively clinically diagnosing it; studies have largely focussed on specific presentations of disease and individual genes. In a small sample size (n=12) with bilateral frontoparietal polymicrogyria of apparent autosomal recessive inheritance, clinical sensitivity for GPR56 gene testing was ~75% (**Piao et al 2004, Science, 303(5666):2033-2036**).

Clinical specificity is assumed to be ~100%; no specific cases of unaffected patients with mutations have been described (although in theory variable penetrance and the possibility of skewed X-inactivation in females may reduce specificity in the X-linked recessive phenotypes associated with ARX and DCX).

26. 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).

Positive predictive value of a pathogenic mutation(s) is ~100%; the only cases of reduced penetrance are females with ARX and DCX mutations (no statistically useful studies of this exist).

Negative predictive value in families with a known mutation(s) is assumed to be effectively 100%. In families where the index case is not tested, we would dissuade testing an apparently healthy individual.

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

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.

All genes will be tested concurrently; process involves long-PCR amplification (in 96-well plates) of amplicons which include all coding exons of the 8 genes, and analysed in parallel by the Illumina sequencing method described in 24.

Results for certain genes can, in specific circumstances, be "masked" at the analysis stage. We envisage this only occurring in cases where specific inheritance is indicated in a large pedigree (e.g. in cases of clear X-linked inheritance, ARX and DCX may be analysed in isolation) or where the clinical picture starkly implicates one gene (likely only the case in the ARX-associated phenotypes).

CLINICAL UTILITY

28. How will the test add to the management of the patient or alter clinical outcome?

A positive test result will uncover the cause of the child's problems. Most of these children have a complex phenotype which may include developmental delay and seizures. A positive result removes the need for further clinical investigation. The parents can be advised of the likely clinical course and risk of recurrence in other children and to family members. Management and prognosis differs depending on diagnosis. There is considerable overlap in the clinical phenotype seen in these disorders, it is not possible to achieve a precise diagnosis without molecular testing.

Molecular testing can specifically implicate a gene and thus provide 1) prognostic and management guidance, 2) recurrence risks, 3) reproductive choices and information (e.g. prenatal diagnosis, decisions regarding marriage in consanguineous families and carrier testing in non-consanguineous

families for autosomal recessive disorders), 4) presymptomatic testing in some cases (specifically, non-symptomatic female carriers of DCX mutations have an elevated risk of adult-onset epilepsy).

29. How will the availability of this test impact on patient and family life?

Identification of the causative mutation clarifies the diagnosis for the patient and family. The risk of a recurrence in future pregnancies can be determined (although this may not be accurately estimated in cases of *de novo* mutations, it is known to be low) and the risks to the extended family calculated. Appropriate genetic counselling can be offered, the results of such a positive test greatly informs this process. Prenatal testing can be offered to at-risk couples. Detection of recessive alleles in consanguineous families may inform future reproductive and marriage decisions.

30. Benefits of the test

Please provide a summary of the overall benefits of this test.

There is considerable phenotypic overlap between the disorders resulting from mutations in these genes, they are a common cause of inherited seizure disorders. They carry different recurrence risks and the clinical outcome is variable. The only way to make a specific diagnosis is through molecular methods.

There are significant benefits associated with testing this panel of genes in parallel (see also case study below); current testing of the lissencephaly genes listed on UKGTN (ARX, DCX, PAFAH1B1 and TUBA1A) in series could take 32 weeks and cost £1540. This service would produce equivalent results in 8 weeks at a cost of £530. In addition, the scope and sensitivity of the polymicrogyria-associated genes is greater than the current UKGTN offering (GPR56) by adding TUBB2B and TUBA8, and an additional recessive lissencephaly gene which has been implicated in several cases in consanguineous Asian families (NDE1) is added. Additionally, in cases where the definition of the cerebral malformation is difficult to resolve by imaging techniques (i.e. differentiation of the various cortical malformations is not totally specific by MRI or CT scan; polymicrogyria is frequently misdiagnosed as pachygyria or lissencephaly), detection of mutation in a specific gene can clarify this diagnosis, further informing prognosis and management.

31. 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.

There is no biochemical or clear clinical means of diagnosing these disorders of cerebral malformation. Lissencephaly and polymicrogyria can be diagnosed by MRI or CT scan (to identify the malformation) and the seizure components of the disorders can be diagnosed clinically and/or by EEG but none of this diagnosis is specific to the precise disorder/genetic cause or the mode of inheritance, merely to the type of malformation and general symptoms, and misdiagnoses of the various types of cortical malformation are not uncommon.

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

None apparent.

33. The Testing Criteria must be completed where Testing Criteria are not already available.

If Testing Criteria are available, do you agree with them Yes/No

If No: Please propose alternative Testing Criteria AND please explain here the reasons for the changes. N/A

34. Savings or investment per annum in the diagnostic pathway based on national expected activity, cost of diagnostics avoided and cost of genetic test. Please show calculations.

Assuming 100 cases per year meeting appropriate testing criteria for lissencephaly and/or polymicrogyria-related disorders (see question 16), with a roughly 50:50 split of phenotype between lissencephaly and polymicrogyria spectrum (supported by the proportion of our local referrals sent for external testing of each gene).

Of 50 lissencephaly cases, assuming 100% are tested for LIS1 (PAFAH1B1), 80% for LIS3 (TUBA1A), and 50% for the X-linked lissencephaly genes (DCX & ARX) [based on estimate of referral rates from local Consultant Paediatric Neurologist] the following costs currently apply:

LIS1: £400 x 50 = £20,000
LIS3: £250 x 40 = £10,000
DCX: £250 x 25 = £6,250
ARX: £550 x 25 = £13,750
TOTAL = £50,000

Testing 50 lissencephaly cases using this panel at the price of £530 per test:
Cerebral malformation test: £530 x 50 = £26,500

Giving a saving of £23,500 on laboratory testing for a hypothetical 50 annual lissencephaly cases.

Of 50 polymicrogyria-related cases, assuming 100% are tested for GPR56 and 50% are tested for TUBB2B (in Germany or USA), the following costs apply:

GPR56: £400 x 50 = £20,000
TUBB2B: £450 x 25 = £11,250
TOTAL = £31,250

Testing 50 polymicrogyria cases using this panel at the price of £530 per test:

Cerebral malformation test: £530 x 50 = £26,500

Giving a saving of £4750 on laboratory testing for a hypothetical 50 annual polymicrogyria cases.

This genetic test does not remove the requirement for neurological investigations or involvement pre- or post-diagnosis or (as far as we can ascertain) significantly impact the cost of management and treatment.

There is an implication in terms of the costs of consultants' time (i.e. offering of a broad panel of LIS/PMG/ARX-related disorders in one test obviates the need to clarify the diagnosis so extensively at the clinical end, likely reducing time spent discussing the case & prioritising genes for testing) which should be an effective reduction in staff time costs, although this is not easy to estimate.

There is an additional implication in the offering of genes not currently offered in the UK (NDE1, TUBA8, TUBB2B) for rare cerebral malformation syndromes; this is difficult to quantify with limited prevalence data on the linked disorders, however, due to rarity and the continuity of the spectrum covered by this panel, we envisage that any additional costs implied would be low.

35. List the diagnostic tests/procedures that would no longer be required with costs.

Costs and type of imaging procedures	
Costs and types of laboratory pathology tests (other than molecular/cyto genetic proposed in this gene dossier)	
Costs and types of physiological tests (e.g. ECG)	
Cost and types of other investigations/procedures (e.g. biopsy)	
Total cost tests/procedures no longer required	**£**

The diagnostic tests/procedures applied in cases of lissencephaly/polymicrogyria/ARX-related disorders/infantile seizure syndromes are all neurology-based, with emphasis on imaging techniques (e.g. MRI/CT/EEG scans). The introduction of this panel of genes would not obviate the need for these procedures, as they are involved in coming to the initial clinical diagnosis and managing the disorder following diagnosis.

36. REAL LIFE CASE STUDY

In collaboration with the clinical lead, describe a real case example to illustrate how the test would improve patient experience.

Patient A (born December 2008) was referred to Genetics following involvement from local paediatric neurology department, with microcephaly, schizencephaly-related seizures, agenesis of corpus callosum in mid-2009. The patient was already known to the laboratory and the family to Clinical Genetics due to an unrelated diagnosis of cystic fibrosis, confirmed by molecular testing. Initial request was to store DNA and test for 22q deletions.

Patient A was seen by a local Clinical Genetics Consultant in September 2009. A putative diagnosis from neurology of subcortical band heterotopia had been made by this point. The doctor authorised sending of a DNA sample to the Cardiff molecular genetics lab for DCX testing in October 2009, at a cost of £450. A negative result was received in February 2010 (report dated 28/01/10).

A further diagnosis of asymmetric polymicrogyria was suggested in early 2010, and, following consultation with paediatric neurology, the doctor authorised sending the patient's DNA to the Cardiff molecular genetics lab for TUBA1A testing in May 2010, at a cost of £350. Part of this delay was due to discussions regarding the correct diagnosis and most likely genetic cause of this patient's neurological problems.

A heterozygous missense change, c.1202A>C (p.Lys401Thr), was detected in TUBA1A, which was previously unreported but thought to affect a region of the gene with possible pathogenic significance (report dated 21/07/10). In order to determine whether this variant occurred de novo in patient A, her parents, patients B&C, were sent for testing in March 2011, following communication with the family to explain the situation. The variant was not detected in either parent, and determined to be the likely cause of the clinical symptoms in patient A, with a 1-2% risk of germline mosaicism in both parents. The family thus received a definitive diagnosis and was offered future prenatal testing if necessary.

The time from initial diagnosis of a possible cortical/cerebral malformation to final diagnosis (September 2009 to March 2011) was well over a year, due to difficulties involved in producing a clear clinical diagnosis during this period and thus deciding on which genes to test, which is currently desirable due to the relatively high costs of screening a wide range of possible targets. The cost to the department of the screening of the two genes implicated was £800.

The service we propose would detect the TUBA1A variant during the parallel screening of the 8 cerebral malformation genes. The result would be available within 8 weeks, at a cost of £530. The dramatically reduced time from initial presentation to a clear clinical diagnosis would reduce anxiety for parents, enable the condition to have a clear "name" at an early stage, and facilitate management decisions for the patient. In addition, prenatal testing could have been offered to the couple more rapidly.

The screening of the majority of the known cortical malformation/polymicrogyria genes (and all the known genes with significant numbers of known cases in the literature) would give additional assurance in this case regarding the pathogenicity of the detected variant, by excluding the possibility of mutations in alternative targets.

An additional saving would have been made in terms of the time and energy of clinicians in Genetics and Paediatric Neurology of arriving at a diagnosis and implicating the genes that were eventually tested; although the family did not receive additional unnecessary outpatient consultations or home visits that would otherwise have been avoidable, there was considerable conjecture and discussion among Clinical Genetics staff (including the two Consultant Clinical Geneticists involved) and the laboratory, and contact with the family made through telephone calls and letters that could otherwise have been entirely avoided by putting the proband's sample through the Cerebral Malformation Panel pipeline at the time of first diagnosis.

37. For the case example, if there are cost savings, please provide these below:

PRE GENETIC TEST

Costs and type of imaging procedures	
Costs and type of laboratory pathology tests (other than molecular/cyto genetic proposed in this gene dossier)	£800
Costs and type of physiological tests (e.g. ECG)	
Cost and type of other investigations/procedures (e.g. biopsy)	
Cost outpatient consultations (genetics and non genetics)	£225
Total cost pre genetic test	**£1,025**

POST GENETIC TEST

Costs and type of imaging procedures	
Costs and types laboratory pathology tests (other than molecular/cyto genetic proposed in this gene dossier)	£530
Cost of genetic test proposing in this gene dossier	
Costs and type of physiological tests (e.g. ECG)	
Cost and type of other investigations/procedures (e.g. biopsy)	
Cost outpatient consultations (genetics and non genetics)	
Total cost post genetic test	**£530**

38. Estimated savings for case example described £

£270 for genetic tests.
£225 costs applied pre-genetic test for estimated 5 hours of Clinical Genetics/Paediatric Neurology Consultant time discussing the patient in attempt to arrive at a diagnosis at £45/hr.
Neurology imaging procedures and consultations have not been taken into account as the availability of the genetic test is unlikely to have impacted on this area.
Total £495.

UKGTN Testing Criteria

Approved name and symbol of disease/condition(s): See Table 1	**OMIM number(s):** See Table 1
Approved name and symbol of gene(s): See Table 2	**OMIM number(s):** See Table 2

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 Geneticists	
Consultant Paediatric Neurologists	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Lissencephaly (agyria, pachygyria, subcortical band or laminar heterotopia) diagnosed by cerebral imaging **OR**	
Polymicrogyria diagnosed by cerebral imaging **OR**	
Hydranencephaly or agenesis of corpus callosum (with abnormal genitalia)* **OR**	
Partington syndrome* **OR**	
Early infantile epileptic encephalopathy*	

Additional information:

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

* = consider referral for isolated ARX gene testing; see separate UKGTN gene dossier & testing criteria

Gene Tests Required:	**Tick if required or specify****
ALL GENES (PAFAH1B1 (LIS1), TUBA1A, DCX, ARX, GPR56, TUBB2B, TUBA8, NDE1):	
Selected genes (please specify)	

** = data for genes unspecified will be collected as part of the testing process but will NOT be interrogated, recorded or reported

Table 1

Approved name and symbol of disease/condition(s):	OMIM number(s):
Epileptic Encephalopathy, Early Infantile, 1; EIEE1	308350
Hydranencephaly with Abnormal Genitalia	300215
Lissencephaly, X-Linked, 1; LISX1 & 2 (XLAG)	300067 & 300215
Subcortical Laminar Heterotopia, X-Linked	300067
Mental Retardation, X-Linked, with or without Seizures, ARX-Related (MRXARX/MRX29)	300419
Partington X-Linked Mental Retardation Syndrome	309510
Corpus Callosum, Agenesis of, with Abnormal Genitalia (Proud syndrome)	300004
Lissencephaly 1, 3 and 4 (with microcephaly)	300004, 611603, 614019
Subcortical Laminar Heterotopia	607432
Polymicrogyria, Bilateral Frontoparietal	606854
Polymicrogyria, Asymmetric	610031
Polymicrogyria with Optic Nerve Hypoplasia	613180

Table 2

Approved name and symbol of gene(s):	OMIM number(s):
aristaless related homeobox; ARX	300382
Doublecortin; DCX	300121
platelet-activating factor acetylhydrolase 1b, regulatory subunit 1 (45kDa); PAFAH1B1	601545
G protein-coupled receptor 56; GPR56	604110
tubulin, alpha 1a; TUBA1A	602529
tubulin, beta 2B class IIb; TUBB2B	612850
tubulin, alpha 8; TUBA8	605742