SETPEG GENETIC TESTING GUIDELINES

Version 1.0, 5th October 2017

1. The Epilepsy Genetic Diagnostic & Counselling Service at King's Health Partners

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2. The specialist outpatient service

- A twice monthly dedicated half-day multidisciplinary clinic (once a month ELCH; once a month KCH)
- Accepting regional referrals from SE Thames and also extra-regional

3. Referral Criteria (any of the following):

- Patient/Family with rare or atypical Epilepsy
- Unusually early onset for the epilepsy syndrome
- Familial with multiple members affected
- Epilepsy resistant to usually effective treatment
- Neurological, behavioural, intellectual or other co-morbidities
- Seen by Genetics or elsewhere but no diagnosis yet or gene panel testing not done.

When we see the proband and family, we will evaluate phenotype, family history and existing **investigations** (**please send ahead**). We will discuss the advantages and limitations of genetic testing and suggest the appropriate testing. We will consent if needed and arrange follow-up with results and counselling.

Alternatively, you may wish to offer gene panel testing locally (below) and follow-up with us.

4. Unsuitable referrals:

- Patient/Family with common Epilepsy e.g. GGE, IFE, TLE
- A known non-genetic cause of Epilepsy is present e.g. structural, trauma etc.

More suitable for referral to local Genetics Department:

- Dysmorphic syndrome
- CGH array with result of uncertain significance
- Family only wants recurrence risks/reproductive advice.

5. How to refer:

- Write to Prof Deb Pal @ KCH or ELCH depending where patient was seen before.
- cc Regional epileptologist if appropriate
- see Figure 1 and 2 for post-referral and clinic pathways.

6. Who should I offer genetic testing to?

Questions paediatricians should ask themselves:

- Might it aid or result in a change of diagnosis or management?
- Do the parents/family want reproductive advice?
- **NOT** just because the test is available

Questions paediatricians should ask the parents:

- Why do you want genetic testing?
- How would you use the results? (Important to emphasise there can be different types of results: positive, uninformative and uncertain)

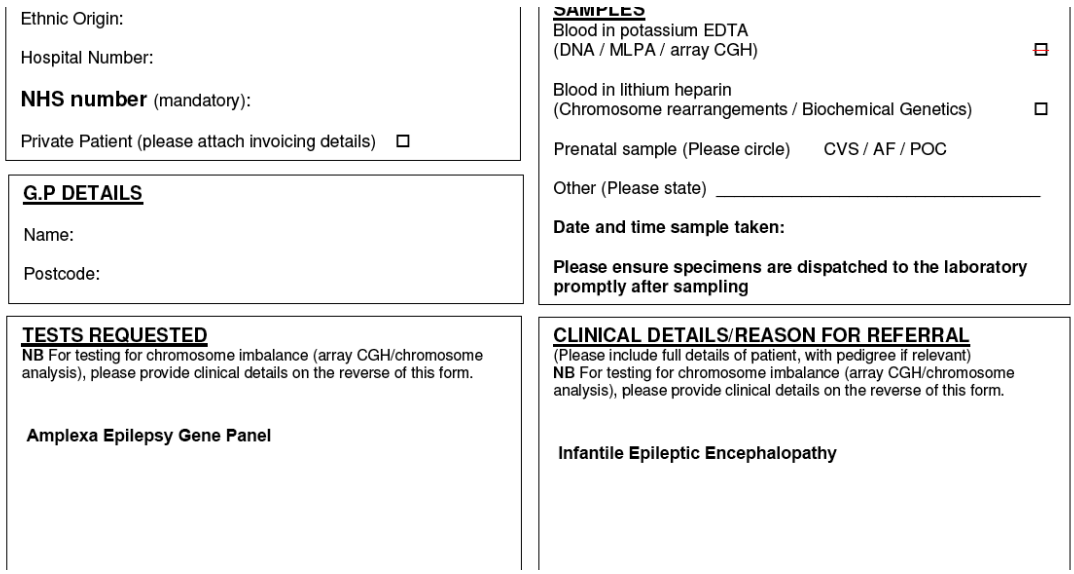
7. How do I consent families for genetic testing?

INFORMED CONSENT: process is important **and mandatory** for patients and good practice. Issues to discuss include the purpose, reliability, benefits and limitations of the test concerned as well as any possible consequences, uncertainties, and the availability of follow up support.
Forms are available on Guys Hospital Website. Check how they want to receive their results (clinic/phone)
http://www.guysandstthomas.nhs.uk/our-services/genetics/laboratories/samples-for-genetic-testing.aspx

8. Samples

Please send blood samples from **TRIO** ie proband **and both parents** to save time in interpreting results. The lab will only perform segregation in the parents if the proband has a suspicious variant.

TIPS: On the ViaPath genetic testing forms, under test requested, insert "**Amplexa Epilepsy Gene Panel**" and send it with the blood samples to ViaPath at Guys Hospital. We also have the **Amplexa EPIDASD-539 panel,** which includes all known genes for epilepsy, autism, ID and brain malformation and is more suitable if autism or ID are the dominant features and seizures are secondary. If in doubt, consult Deb Pal or Stephanie Oates.



Ethnic Origin:

Hospital Number:

NHS number (mandatory):

Private Patient (please attach invoicing details) ☐

G.P DETAILS

Name:

Postcode:

SAMPLES

Blood in potassium EDTA
(DNA / MLPA / array CGH) ☐

Blood in lithium heparin
(Chromosome rearrangements / Biochemical Genetics) ☐

Prenatal sample (Please circle) CVS / AF / POC

Other (Please state) _____

Date and time sample taken:

Please ensure specimens are dispatched to the laboratory promptly after sampling

TESTS REQUESTED
NB For testing for chromosome imbalance (array CGH/chromosome analysis), please provide clinical details on the reverse of this form.

Amplexa Epilepsy Gene Panel

CLINICAL DETAILS/REASON FOR REFERRAL
(Please include full details of patient, with pedigree if relevant)
NB For testing for chromosome imbalance (array CGH/chromosome analysis), please provide clinical details on the reverse of this form.

Infantile Epileptic Encephalopathy

At the same time, complete an Epilepsy Genetic Test Request form and send it by email to deb.pal@nhs.net. See Figure 3 for Genetic testing Pathway.

GENETICS			
Written informed consent & information sheet ? Yes			
Indication for testing (tick all that apply): Diagnostic ☒	Recurrence/Reproductive ☐		
Any suspected genes?	Genes prev tested –ve	Genes tested +ve or u/k	
Relatives tested positive ? Don't know	If so, which gene?		
CGH array No rearrangement	If any CNV(s), state which (eg 15q13.33del) :		
Family history (1st or 2nd degree only): Epilepsy? Yes	DD? Yes	ASD? No	
Psychiatric? No	Consanguineous? No		

EPILEPSY	
Epilepsy syndrome or working diagnosis: EE- Infantile	Age of onset afebrile seizures: 1 yrs 3 mo
Development prior to seizures: Abnormal	Febrile seizures? No
Main seizure type (s): Polymorphic If generalised, which types? GTCS ☐ Absence ☐ Myoclonic ☐ Tonic ☐ Atonic ☐	
Ictal EEG findings:	Interictal EEG:
Drug resistance? (2 suitable AEDs) Yes	Surgery or Device? No
MRI brain: Normal	If abnormal, summarise:

9. When to expect results

Samples are sent in a batch to Amplexa in the **3rd week of every month**. The laboratory turnaround time is **21 days** on average. **In urgent neonatal cases** the turnaround can be redued to **10 days**. Once the laboratory report is issued, the Epilepsy Genetics Team then undertakes clinical interpretation, usually in cooperation with the referring consultant. Finally, the result is fed back to the referrer and the lab report distributed to the referring consultant.

10. Diagnostic Yield

The yield from the gene panel depends on several factors, but principally influenced by age of onset. In the neonatal period, we can make a genetic diagnosis in 75% of cases; **40% overall in the first two years**; and 21% when the age of onset is over the age of two. Our initial experience is reported in Moller et al, 2016 [1] and more comprehensively (Oates, in review, 2017).

11. Choice of aCGH, EPIDASD-539 panel, exome sequencing or 100,000 Genomes.

The diagnostic yield of aCGH in patients meeting criteria for epilepsy genetic testing is currently 0% although benign rearrangements are reported quite frequently and the test costs £350. Therefore, **we do not recommend aCGH as a first-line investigation** in children with suspected genetic epilepsy who meet the testing criteria in section 3.

aCGH is appropriate when

- the child has dysmorphic features
- has autism or intellectual disability with seizures as a secondary feature
- when epilepsy has onset after the age of 2 years.
- In GGE or IFE with comorbidities or a family history
- In selected cases with infantile spasms

Exome sequencing is a more expensive alternative to gene panel testing. Whereas a gene panel is specific to epilepsy and may contain 100 or so genes curated for their causal connection to early-onset epilepsy, the exome interrogates sequence variation in >20,000 genes. Aside from the increased expense, there are several technical and ethical issues to overcome. For example, the coverage of genes on the exome is inferior to that on the gene panel (99% vs 87%) and this theoretically could result in false negative results. The extra burden of interpreting variants in 20,000 vs 100 genes is considerable, especially without the benefit of the parental exomes, and again tends towards over-reporting of variants of uncertain significance, and introduces the problem of incidental findings for example suggesting susceptibility to cancer or neurodegenerative conditions. To bypass this ethical issue, laboratories can create a "virtual" panel from the exome, in effect replicating a gene panel. If necessary, this "virtual" panel can be expanded in scope.

On balance, the **gene panel** is cheaper, has better coverage, has a faster turnaround time, and avoids ethical dilemmas and is therefore the **technology of choice**. However, the situation of panel-negative patients who are still strongly suspected of having a genetic cause is regularly reviewed and such patients may be eligible for either extended panel testing using the Amplexa EPIDASD-539, or exome analysis once informed consent has been obtained.

The **Amplexa EPIDASD-539 panel** is jointly curated by Dr Rikke Moller at the Danish National Epilepsy Centre and Amplexa Genetics. It is the most comprehensive gene panel available for neurodevelopmental disorders and s updated quarterly to include all known causative genes for autism, epilepsy, intellectual disability and brain malformation. We have been evaluating its clinical utility over 2017 and have increased the diagnostic yield of epilepsy gene panel negative cases by 10-20%. Turnaround time is about 2 months.

The **100,000 Genomes Project** is a national research project with unclear reporting lines or responsibilities and no definite timelines. Previously taken blood samples cannot be re-used for 100,000 Genomes and a fresh consent and samples are required. The project should be reserved for cases that cannot be resolved by panel or exome, or those who have more complicated rare phenotypes as listed in Section 4.

12. Is there still indication for single gene testing?

Yes, definitely but limited to few scenarios in which the **phenotype accurately predicts the genotype**. In most cases of early-onset epilepsy this condition is not met, in other words our ability to predict the epilepsy gene mutation is very poor. This is because of genetic heterogeneity, or the similarity in phenotype resulting from multiple genetic causes. This is the rationale for using a gene panel.

The two classic cases in which there are good phenotype-genotype correlation are **Dravet syndrome** with *SCN1A*; and **Glut1-D** with *SLC2A1*. In the case of Dravet syndrome, if the phenotype is classic then single gene testing, including both sequencing of all exons and MLPA to detect the 5% of copy number variation, is indicated as a first-line genetic investigation. Cases where the phenotype does not meet textbook definition should be discussed for suitability for gene panel, bearing in mind that there are several genes associated with Dravet-like phenotypes (*GABRG2*, *PCDH19*, *SCN8A*, *CHD2* etc). It is helpful to review the consensus indications for *SCN1A* testing [2].

The paediatric neurology community is still debating whether *SLC2A1* testing should replace measurement of the actual biomarker i.e. CSF: plasma glucose, and there are compelling reasons for each option. *SLC2A1* mutations are the only known cause of Glut1D, although not all Glut1D cases have a *SLC2A1* mutation; also, some variants in *SLC2A1* may be reported as of uncertain significance, consequently necessitating measurement of the biomarker. However, with increasing experience, these uncertain variants will be eliminated. On the other hand, measuring CSF glucose is an invasive and expensive procedure requiring GA and inpatient stay. If not performed according to strict protocol, the results may yield false negatives [3]. Many physicians order single gene *SLC2A1* testing in cases where the phenotype is clear-cut and suggestive of Glut1D e.g. early-onset absence epilepsy.

Final Comments

Epilepsy genetics is a fast-changing field and the implications for precision medicine are growing equally quickly. If you are in any doubt about a case or a result or simply want to discuss, please get in touch with Deb or Stephanie by email and we will do our best to contact you within 24-48 hours.

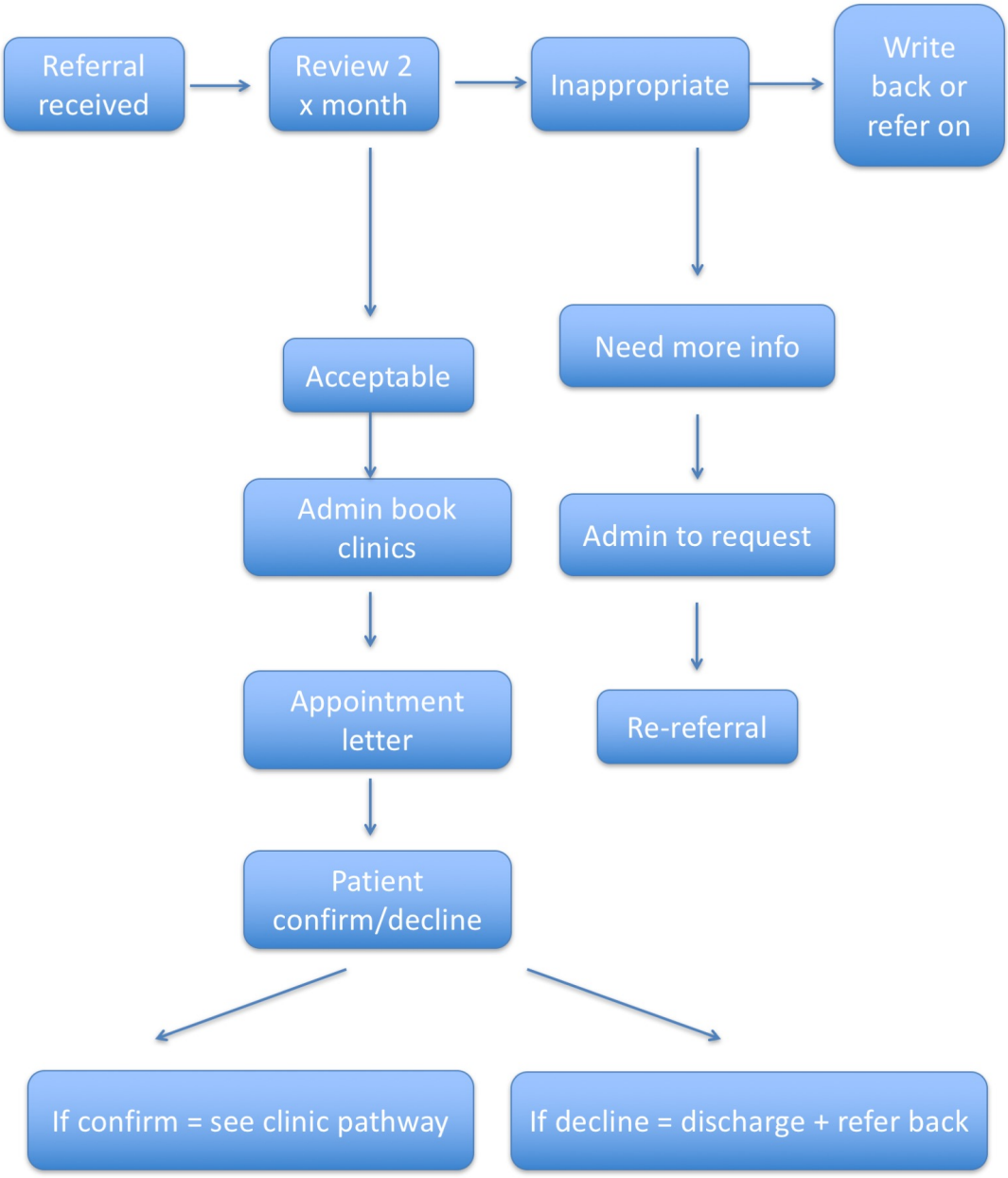
References

1. Møller, R.S., et al., *Gene Panel Testing in Epileptic Encephalopathies and Familial Epilepsies.* Mol Syndromol, 2016.

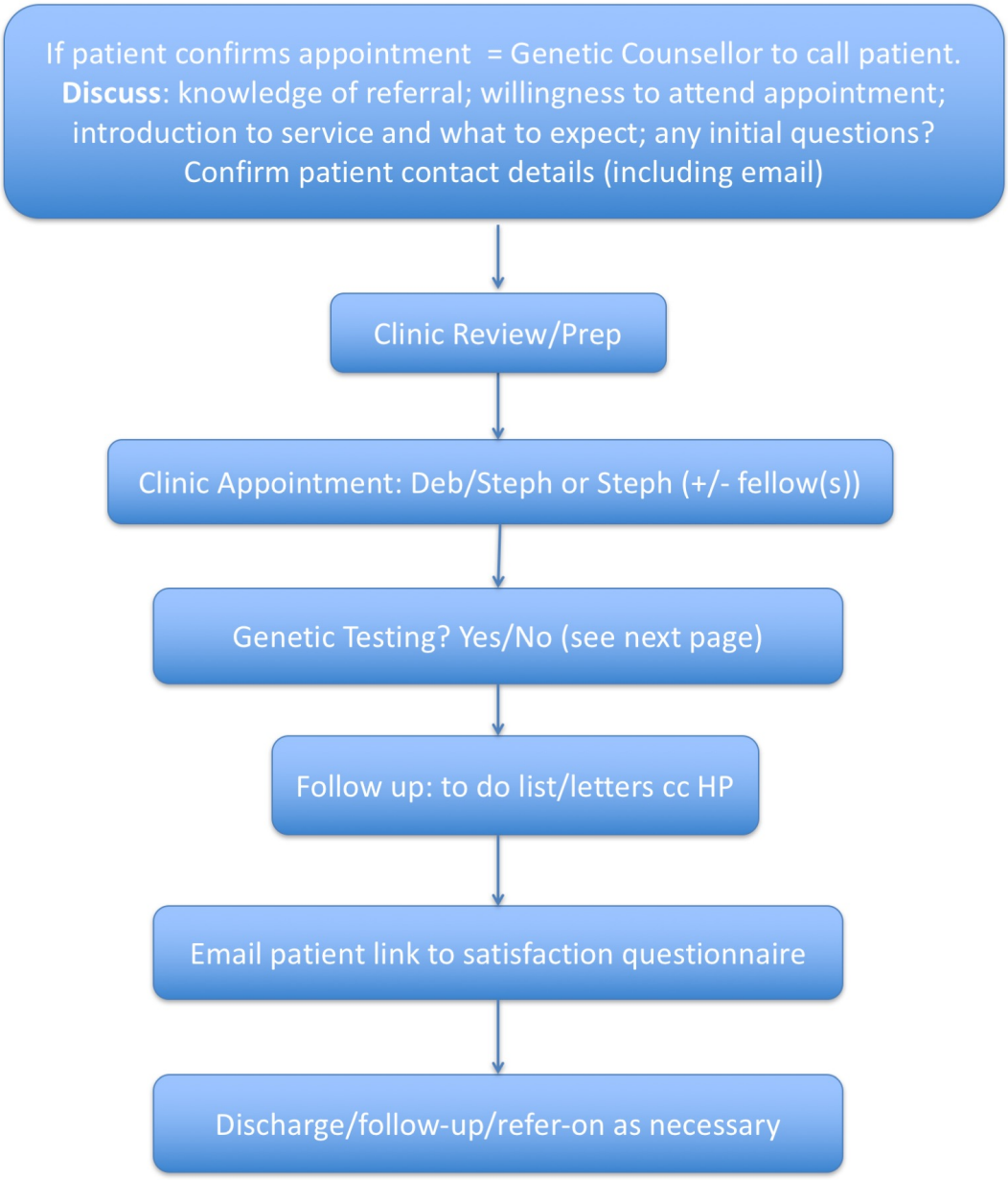
2. Hirose, S., et al., *SCN1A testing for epilepsy: application in clinical practice.* Epilepsia, 2013. **54**(5): p. 946-52.

3. Leen, W.G., et al., *Glucose transporter-1 deficiency syndrome: the expanding clinical and genetic spectrum of a treatable disorder.* Brain, 2010. **133**(Pt 3): p. 655-70.

Pre-clinic Pathway



Clinic Pathway



Genetic Testing Pathway 2