

# Fifteen-minute consultation: The Efficient Investigation of Infantile and Childhood Epileptic Encephalopathies in the Era of Modern Genomics

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What is known about this topic:

1. The aetiology of ICEE is broad with limited diagnostic yield to date with current investigative methods.
2. The investigative processes for ICEE vary widely risking unnecessary and incomplete investigation.

What this paper adds:

1. A guide for secondary and tertiary level paediatricians on how to investigate a child with ICEE.
2. Evidence based recommendations for the stratification of investigations in ICEE.

## **Abstract:**

The investigation of children presenting with infantile and childhood epileptic encephalopathies (ICEE) is challenging due to diverse aetiologies, overlapping phenotypes and the relatively low diagnostic yield of MRI, electroencephalography (EEG) and biochemical investigations. A careful history and thorough examination remains essential, as these may identify an acquired cause or indicate more targeted investigation for a genetic disorder. Whole exome sequencing (WES) with analysis of a panel of candidate epilepsy genes has increased the diagnostic yield. Whole genome sequencing (WGS), particularly as a trio with both parents' DNA, is likely to supersede WES. Modern genomic investigation impacts on the timing and necessity of other testing. We propose a structured approach for children presenting with ICEE where there is diagnostic uncertainty, emphasising the importance of WGS or if unavailable- WES, early in the investigative process. We note the importance of expert review of all investigations including radiology, neurophysiology and

biochemistry, to confirm the technique used was appropriate as well as results. It is essential to counsel families of the risks associated with the procedures, the yield of the procedures, findings that are difficult to interpret and implication of “negative” results. Where children remain without a diagnosis despite comprehensive investigation, we note the importance of on-going multidisciplinary care.

### **Introduction:**

Infantile and childhood epileptic encephalopathies (ICEE) are a group of epilepsies, estimated to affect ~1.2/1000 live births.[1] They are typified by multiple types of seizures within the first years of life, developmental delay and resistance to anti-epileptic drugs (AED).

Diverse underlying aetiologies and overlapping phenotypes make their investigation challenging. Precision therapies are now available for selected disorders, adding impetus to identify them rapidly.[2]

In ICEE, the epileptic activity contributes to cognitive / behavioural impairments above and beyond what might be expected from the underlying pathology alone.[3] Expanding knowledge of the geno/phenotypes of genetic epilepsies led to the concept of epileptic and developmental encephalopathies (EDE). In EDE the developmental impairment occurs as a direct result of the genetic variant, in addition to the effect of excessive epileptic activity.[4]

Many will have an acquired (e.g. hypoxic ischaemic injury) or easily identifiable genetic cause (e.g. Down syndrome). Where this is not evident, investigation of other aetiologies varies, risking both unnecessary/incomplete investigation and inappropriate therapy.

Where there is no identifiable acquired cause, the evolution of trio whole exome and whole genome sequencing (WES/WGS), has increased the diagnostic yield significantly, although it remains less than 60%.[5] WES/WGS offer the potential to identify genomic variants outside epilepsy gene panels, including those which cause metabolic disorders.[5] Their higher diagnostic yield coupled with reducing cost and turnaround times makes them an increasingly cost-effective early investigative option.[5]

We propose a structured approach to children with ICEE emphasising the importance of a robust history, clinical examination and review of ictal video. Where this does not lead to targeted testing, we outline a tiered investigative pathway prioritising early genomic investigations, those with higher diagnostic yields and testing for treatable causes (figure 1, table 1). Sequencing of investigations is often altered by clinicians being opportunistic e.g. performing a lumbar puncture during general anaesthesia for MRI. We aim to improve diagnostic efficiency, facilitate early detection of treatable conditions and minimise the consequences of protracted investigation.

Before initiating this potentially lengthy diagnostic process, families must be informed of strengths / weaknesses of investigations and their results (Table 2).[6]

### **Clinical assessment:**

A meticulous history is imperative, including history of assisted conception (including donor egg, sperm or embryos) and ante/perinatal periods. A thorough family tree should be constructed (Box 1).[6] It is important to consider whether the underlying aetiology is likely to be acquired (e.g. perinatal brain injury) or genetic. Parental international travel and country of birth should be noted in view of geographical variations in infectious diseases (e.g. Zika virus) and variations in newborn screening.

A full developmental assessment of all domains should be conducted including the trajectory of developmental delay or regression. Height, weight and head circumference should be plotted on standardised growth charts. The clinician should assess for discordance between values, as well as the overall trajectory. A thorough clinical examination of all body systems should be conducted, looking for signs which may help tailor investigations. The assessment for dysmorphism should include the face, hair, nails, skin, eyes, ears, hands, feet, fat distribution and genitalia, with particular emphasis on the skin for neuro-cutaneous syndromes.[6] Clinicians should assess the level of intellectual disability and for the presence of autistic spectrum disorders. All children should be referred for ophthalmological and audiological assessment.

Abnormal clinical signs should initiate specific testing such as:

- Cutaneous stigmata or dysmorphism - referral to a geneticist;
- Spleno/hepatomegaly - referral to a metabolic specialist
- Movement disorders should prompt clinicians to consider specific metabolic and/or genomic analyses e.g. plasma oxysterols for Niemann Pick Type C, movement disorder gene panel.

### **First line testing:**

### **Electrophysiology:**

Electrophysiology- EEG- is essential in the investigation of aetiology. It may allow broader classification into a focal or generalised epilepsy and can be consistent with electro-clinical syndromes such as Lennox Gastaut or of a genetic disorder with specific investigation e.g. Dravet syndrome.

The clinician should note both a standard recording, as well as whether sleep was achieved and for older children activation techniques such as photic stimulation / hyperventilation. Where an ictal recording has not been obtained there should be consideration for repeat/longer recording.

### **Neuroimaging:**

MRI brain will have a significant yield.

We recommend consideration of MRI imaging in all children, even where the aetiology is clear on clinical grounds– e.g. tuberous sclerosis/Down syndrome. Where good quality MRI imaging

is not obtainable due to child compliance, evidence-based oral sedation or general anaesthesia may be used.[7]

The MRI sequence protocol should follow that stipulated by the regional epilepsy surgery centre. We recommend that a final conclusion is not drawn until the images have been reviewed and the sequences judged appropriate by an expert in the neuroimaging appearances of ICEE.

### **Genomic testing:**

Due to the high genetic heterogeneity of ICEE, genomic testing should be implemented early in the investigative process.[8]

Array comparative genomic hybridisation (array-CGH) can be ordered once the family have been appropriately consented. Array-CGH detects imbalances in chromosomal material. Array-CGH can detect genomic imbalances as small as 100,000 base pairs, however it is unable to detect pathogenic single or multi-nucleotide variants, triplet repeat expansions and methylation defects. Array-CGH may also miss large copy number variants in the presence of low level mosaicism and will never detect a copy number variant from a blood sample if the abnormal cell line is no longer present in lymphocytes. Array-CGH has been estimated to provide a definitive diagnosis in <5% of cases of early infantile epileptic encephalopathy (EIEE), therefore we recommend ordering next generation sequencing (NGS) and array-CGH concomitantly.[9, 10]

NGS using exome sequencing, limiting the interpretation of the exome to a targeted panel of genes known to cause ICEE, has helped to further increase the diagnostic yield although this remains <60%.[5] Clinical WES is currently not able to detect large copy number variants. We anticipate that as WGS becomes available as a clinical test, it will supersede array-CGH plus WES, since WGS is able to detect copy number variants more readily.

At present we have recommended that a “gene agnostic” trio WES/WGS is performed within second line testing based on its availability and turnaround times. Their improved diagnostic yield, coupled with the potential of early instigation of precision therapies means it may be more appropriate to initiate these as part of first line testing in some centres. The use of NGS as a research tool may provide the means to initiate testing with rapid turnaround times in some centres.[10]

We recommend storing parental DNA alongside first line investigations, as we have had difficulty obtaining both parental samples later and note the importance of trio analysis to improve diagnostic yield.[10]

As with any diagnostic assay, NGS has limitations which are outlined with suggested clinical actions and mitigations in Table 3.

### **Biochemical testing:**

Genomic and biochemical testing for metabolic conditions may yield false negative/positive results and so should be performed concurrently. Relevant biochemical/metabolic disorders, their pertinent clinical features and corresponding diagnostic tests are outlined in supplementary table 1.

First line tests aim to exclude basic and reversible metabolic derangement(s), assist in tailoring further investigation, screen for rarer metabolic conditions and prioritise those which are treatable through a single blood draw and urine collection.

Cerebral creatine deficiency syndromes may present in children with seizures, intellectual disability and sometimes movement disorders. Treatment of guanidinoacetate methyltransferase deficiency has been shown to reduce or eliminate seizures in 67% of patients, while current treatment of creatine transporter deficiency has shown improvement in only a few individuals.[11, 12] The biochemical profile of mitochondrial disorders is variable, however disturbances in plasma lactate and urine organic acid profile (UOA) may be suggestive.

Patients with biotinidase deficiency show an excellent response to oral biotin and delay in treatment leads to irreversible neurological disease.[13] Dermatitis and alopecia may be present. Since oral biotin supplementation does not affect biotinidase activity, treatment can be commenced pending the biotinidase result.

CSF studies, where indicated (Table 1), should assess for evidence of mitochondrial disease (simultaneous CSF/plasma lactate), GLUT-1 deficiency (simultaneous CSF/plasma glucose), phosphoglycerate dehydrogenase deficiency, non-ketotic hyperglycinaemia (paired CSF and plasma amino acids/serine/glycine) and vitamin dependant epilepsies

Treatment with pyridoxine/pyridoxal phosphate, folic acid and biotin should be initiated in children <1year of age, except those with infantile spasms (IS) alone (Figure 1, see supplementary table 2 for suggested dosing). Where there is no improvement, they should be stopped after 14 days, awaiting confirmatory negative biochemical/genomic analyses (Table 1). CSF should ideally be obtained prior to commencing pyridoxal phosphate/pyridoxine and folic acid (but treatment should not be unnecessarily delayed).[14][15]. For pyridoxine dependant epilepsy urinary alpha-amino adipic semialdehyde ( $\alpha$ -AASA), should remain positive post treatment, as an alternative diagnostic test.

These treatable conditions screened for using CSF analysis are unlikely to present with IS alone or >1year of age and are likely to be detected using WES/WGS.[16] Weighing this against the risks of general anaesthesia/processing difficulties, CSF analysis should be reserved as a second line test in children >1 year of age with specific caveats (see Table 1).

Urine sulfocysteine will assess for sulfite oxidase and molybdenum co-factor deficiencies. There have been reports of clinical improvement in type A molybdenum cofactor deficiency following intravenous cyclic pyranopterin monophosphate.[17, 18] This has been shown in a limited number of children to be of benefit if started early, before permanent neurological damage is established.[17, 18]

## **Second line testing:**

### **Electrophysiology:**

Clinicians should check that both an ictal recording as well as sleep has been obtained. Where this has not been achieved or where there remains diagnostic uncertainty, we recommend a repeat/prolonged recording.

### **Neuroimaging:**

Before repeating MRI, clinicians should discuss with an expert in the radiology of ICEE if all appropriate sequences were performed, their quality is sufficient and if another imaging modality is needed e.g. CT to show calcification in tuberous sclerosis.

If these are deemed unremarkable, repeat scan would be indicated where the initial MRI was performed within the first two years of life, when lack of myelin can mask radiological stigmata of ICEE. If the original scan is of high quality and was performed after the age of three years, clinicians should consider repeat MRI at 3T, which may identify subtle abnormalities e.g. cortical dysplasias, which were not evident on the original series.

### **Genetic testing:**

Trio WES or WGS improves diagnostic speed and yield over that attained with an epilepsy panel.[5, 9] ‘Trio’ describes the method of genomic analysis whereby the child’s genomic variants are compared to the parental variants using a specific bioinformatic approach. The analysis may be applied to the entire exome or genome, rather than being limited to a gene panel, hence it is sometimes referred to as ‘gene agnostic’. It allows rapid identification of de novo changes (which cannot be clarified without parental analysis) now recognised to cause a large proportion of serious paediatric genetic disorders.[19]

In many laboratories, large panel tests are performed using a bioinformatic ‘filter’ of the patient’s exome data. Although the exome is sequenced, only those genes contained within the panel would be analysed. It should be noted that when agnostic trio WES/WGS is performed, the clinical scientists will pay particular attention to known genes causing early epilepsies, but will also interrogate the remainder of the patient’s genomic data. Thus, the exact composition of specific gene panels offered by individual laboratories for ICEE/EIEE is an important factor for interpretation of WES/WGS.

We recommend referral to clinical genetics in all cases where an acquired cause is unlikely. They would review the likely implications of results, and whether variants are likely to be disease causing. At present, paediatricians are unlikely to have access to trio WES/WGS, but can order ICEE panels. Currently, clinical geneticists have limited access to trio (agnostic) WES and can initiate this testing if needed.

### **Biochemical testing:**

Where CSF studies have not already been done (Table 1) these should be performed.

Thyrotoxic and Hashimoto's encephalopathy are rare and should be associated with other clinic signs, thus thyroid function testing is included as a second line investigation unless there are clinical reasons to test sooner e.g. maternal history of Graves' disease.[20, 21]

Transferrin glycoform testing for congenital disorders of glycosylation, which often present with a multisystem process and are largely without treatment, is reserved as second line. Transferrin glycoforms are unreliable in the first three weeks of life due to the influence of maternal transferrin.

Very long chain fatty acid analysis to test for peroxisomal disorders such as Zellweger's syndrome, have been selected as second line in view of their rarity, likelihood of other clinical features predicating analysis e.g. dysmorphic features and lack of efficacious treatment. This will also detect X-linked adrenoleukodystrophy, which in a small proportion of boys can initially present with seizures alone, but are likely to have a suggestive MRI on first line testing.[22]

### **Isolated infantile spasms:**

For isolated IS, clinicians should review evidence and follow recommendations set out within the national infantile spasms consortium, noting pyridoxine dependent epilepsies are reported not to present with IS alone over 3 months of age.[16] We note the negligible yield of biochemical testing in this population and as such suggest the majority of first line biochemical investigations for ICEE be reserved as second line for isolated IS (see table 1).[16]

### **Third line testing:**

Where first and second line testing fail to identify an aetiology, we recommend careful review of results and further discussion with the family. Without new findings on history, examination and review of previous investigations, the yield will be low. There can be risks to a procedure e.g. muscle biopsy under general anaesthetic and difficulty interpreting the results. Muscle biopsy can be considered; it has a low, but not negligible risk and is unlikely to provide a specific diagnosis/therapy, with an estimated additional diagnostic yield of 1%.[23]

We recommend careful on-going review to ensure the child does not have features of a degenerative, potentially treatable, condition e.g. late infantile neuronal lipofuscinosis. We have recommended many, but not all possible metabolic investigations, and at this stage others should be considered; e.g. lysosomal enzymes to identify lysosomal storage disorders (many, but not all will be detected by trio WGS).

### **Next steps:**

In a significant minority, the aetiology cannot be identified despite extensive investigation. We recommend careful re-analysis of the case to ensure this is correct. With so many investigations, results can be mis-documented or misunderstood. Peer review, including experts in the neurophysiology, neuroimaging and genomics of ICEE is essential.

If a diagnosis is not forthcoming, we expect significant improvements in yield of both genomic and other investigations at least every 2 years.[24] Therefore, consideration to review the process in that time frame is appropriate and should include assessment of advances in technology and bioinformatics, knowledge of the genetic aetiology of conditions/specific phenotypes, the patients emerging phenotype and changes in the family history (e.g. newly affected members).[24]

Where the aetiology remains unclear, paediatricians should reassure families that they can still treat the epilepsy with evidence-based therapies. Families should receive multidisciplinary input from allied health professionals, including psychology, education and specialist therapists; as the parents should have been told prior to starting the process.[25]

### **Treatment resistance where the aetiology is identified**

Where an aetiology is identified, but treatment resistance encountered, the first step should be to challenge the diagnosis; particularly HIE. We recommend instituting appropriate first-line therapies for the relevant diagnosis whilst discussing with families the risk versus benefits of the above tiered testing. This will vary between cases for example there is over diagnosis of HIE in children with underlying disorders e.g. pyridoxine dependent epilepsy. Conversely, in children with Down syndrome presenting with infantile spasms, treatment resistance is common, other causation extremely unlikely and investigation potentially traumatic.

### **Recent advances:**

As our understanding of the genetic and molecular pathogenesis of ICEE evolves, so too does our understanding of precision therapies and the importance of early diagnosis (see supplementary table 3).[26-38] As we went to press, dietary supplementation with uridine/uridine 5' monophosphate (UMP) has been demonstrated as an effective and safe treatment for children with CAD (carbamoyl-phosphate synthetase 2, aspartate transcarbamylase and dihydroorotase) deficiency (EIEE-50).[39, 40] There are no biomarkers for the disease and the diagnosis is genetic, with many variants reported as VUS, highlighting the importance of genotype-phenotype correlation. Treated cases demonstrated significant improvement in seizure control, anisopoikilocytosis/anaemia, as well as developmental gains.[40] Treatment with uridine triacetate is licenced in the USA. Some have suggested a 6 month trial of uridine in all cases of neonatal seizures and children with developmental delay, seizures and anisopoikilocytosis/anaemia, pending negative genetic results.[40] We have not incorporated this into our standard recommendations due to the lack of long-term data. However, clinicians should engage in careful discussion with geneticists regarding CAD deficiency and related VUS, and consider uridine supplementation pending genetics, particularly in refractory ICEE or where the phenotype is indicative.

### **Conclusion:**

Investigating children with ICEE is challenging. Precision therapies emphasize the importance of early diagnosis. Assessment begins with a comprehensive history and examination, which should guide further investigation. Where an acquired cause or



recognisable phenotype is not identified, a tiered approach, prioritising higher yield and treatable conditions is needed, but investigations should be prioritised on an individual basis in the context of the clinical picture and practicality.

Families must be consented with particular reference to the interpretation of results. They should be told that a diagnosis may not be established and that where a diagnosis is made, their child may not have a modifiable disease. It is essential that families receive excellent multidisciplinary support.

ICEE gene panel testing should form part of the first line of investigations alongside array-CGH. With reducing costs/turnaround times, wider availability and higher diagnostic yield, trio WGS may soon supersede these.

When the diagnosis remains unclear there should be careful reassessment of the case and prior investigations, ensuring adequate quality and prior review by experts in the field. The process should be reviewed at least every 2 years and further analyses undertaken where there is a new, appropriate investigation or there has been an improvement in the relevant method.

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**Table 1:** First, second and third line investigations. \*The genes included within this panel should be clarified with the laboratory. †Performed first line if the child is <1 year, having a general anaesthetic, it is felt the child will tolerate the procedure well awake/under oral sedation, or the clinical picture indicates a relevant treatable condition e.g. microcephaly, intellectual disability, movement disorder and autism suggesting cerebral folate deficiency. #Perform as first line if less than 3 months. ΔPerformed as first line testing for isolated infantile spasms when clinical examination, EEG and MRI do not identify an aetiology.

First Line	Second line	Third line
<p>Genetic</p> <ul style="list-style-type: none"> <li>• Array CGH<sup>Δ</sup></li> <li>• ICEE gene panel<sup>l*Δ</sup></li> <li>• Store parental DNA samples</li> </ul>	<p>Genetic</p> <ul style="list-style-type: none"> <li>• Clinical genetics opinion</li> <li>• Trio whole exome or whole genome sequencing may be offered (this may become the first line test ordered by a specialist pediatrician in 2021)</li> </ul>	
<p>Biochemical &amp; Haematological</p> <ul style="list-style-type: none"> <li>• Full blood count</li> <li>• U+E, LFT, blood gas/bicarbonate, glucose, calcium, magnesium, ammonia, plasma lactate<sup>Δ</sup></li> <li>• Plasma amino acids<sup>Δ</sup>, biotinidase</li> <li>• Acylcarnitines</li> <li>• Urine organic acids<sup>Δ</sup></li> <li>• Urine and plasma creatine and guanidinoacetate</li> </ul>	<p>Biochemical</p> <ul style="list-style-type: none"> <li>• †CSF: glucose, lactate, amino acids (with paired plasma samples), pipecolate (and pyridoxal phosphate if less than 3 months) Neurotransmitter metabolites (including 5-methyltetrahydrofolate)</li> <li>• Serum TFT, uric acid</li> <li>• Plasma transferrin glycoforms, very long chain fatty acids</li> <li>• Urine sulfocysteine<sup>#</sup></li> </ul>	<p>Biochemical</p> <ul style="list-style-type: none"> <li>• Muscle biopsy</li> </ul>
<p>Electrophysiology</p> <ul style="list-style-type: none"> <li>• Sleep / Ictal EEG if not achieved already</li> </ul>	<p>Electrophysiology</p> <ul style="list-style-type: none"> <li>• Review and repeat EEG</li> </ul>	
<p>Imaging</p> <ul style="list-style-type: none"> <li>• MRI brain 1.5T</li> </ul>	<p>Imaging</p> <ul style="list-style-type: none"> <li>• Repeat 1.5T if original poor quality or child &lt; 3 years</li> <li>• MRI brain 3T</li> </ul>	

**Table 2:** Points for discussion with parents.

**Box 1:** Key points to include in the family tree.

Issue	
True Positives	<ul style="list-style-type: none"><li>• A true positive result may be very distressing; often no curative treatment is available</li><li>• Diagnosis can aid prognostication, provide closure to the family and help with family planning choice.</li></ul>
False Positives	<ul style="list-style-type: none"><li>• An innocent variant is mistakenly judged to be disease-causing</li><li>• This may have wide-ranging implications including incorrect decisions about treatment options for the child as well as prenatal testing in future pregnancies</li></ul>
True Negatives	<ul style="list-style-type: none"><li>• Where tests fail to identify a cause, this does not mean all disorders are excluded:<ul style="list-style-type: none"><li>○ A negative CGH or unremarkable urine organic acid may be misinterpreted by families to mean that their child cannot have a genetic / metabolic disorder (respectively)</li></ul></li></ul>
False Negatives	<ul style="list-style-type: none"><li>• Genetic testing may be initially reported as normal, but as knowledge/panels of genes tested expands, subsequently a disease-causing variant may be identified</li></ul>
Non-diagnostic results	<ul style="list-style-type: none"><li>• Results may be reported with a change of unknown significance:<ul style="list-style-type: none"><li>○ many smaller duplications in array CGH or subtle changes in organic acids</li></ul></li><li>• There is currently insufficient data to tell whether the variant is disease causing</li></ul>
Turnaround times	<ul style="list-style-type: none"><li>• More advanced genetic tests, such as whole genome sequencing, can have lengthy turnaround times</li></ul>

• At least 3 generations
• Assisted or natural conception
• Consanguinity
• Ethnicity
• Health status
• Age at death & cause
• Recurrent miscarriages
• Developmental delay
• Intellectual disability
• Seizures
• Encephalopathy

**Table 3:** Limitations of next generation sequencing and suggested mitigations.

Limitation of next generation sequencing	Suggested action or mitigation(s)
<p>Variants of uncertain significance (VUS) in genes known to cause ICEE may be reported. This occurs when the evidence for being definitely pathogenic or definitely benign is not available to the laboratory clinical scientists.</p>	<p>Review the patient’s phenotype to determine whether the reported VUS would provide a good clinical fit e.g. does the patient have evidence of tuberous sclerosis. This is common practice and is termed ‘reverse phenotyping’. Referral to a specialist may be indicated e.g. mitochondrial disorders, clinical genetics.</p> <p>Contact the reporting clinical laboratory scientist to determine whether additional genetic studies in first degree relatives could help with interpretation (e.g. if proven to be a de novo variant, will the variant classification change?)</p>

	<p>If there is a functional study available (e.g. a metabolic assay) consider ordering the relevant study</p> <p>If no further clinically available tests are possible, functional studies on a research basis may occasionally be possible by contacting the scientists who work on the specific pathway.</p>
<p>Genetic panels may be updated on an annual basis only, hence newly described genes may not be reported.</p>	<p>Specialists in ICEE should maintain close contact with the clinicians and scientists responsible for determining the composition of genetic panels.</p> <p>If a gene-phenotype correlation is new and is a likely differential diagnosis, contact the genetics laboratory and request that this gene is reported for the patient.</p> <p>For patients in whom a negative panel was reported over 2 years previously, consider ordering the newest panel or asking the laboratory for re-interpretation of the patient's whole exome or whole genome, as applicable.</p>
<p>Likely pathogenic or pathogenic variants may be reported to be present in genes with no stated phenotypic link to ICEE.</p>	<p>Clinicians should read the most recent literature on the gene-phenotype correlation, as this may reveal an expansion in the phenotypic spectrum.</p> <p>If this is truly an 'incidental finding' then a referral to clinical genetics is advised, for discussion of validity and possible implications.</p>





