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

Submitting laboratory: Bristol RGC

1. Disorder/condition – approved name and symbol as published on the OMIM database (alternative names will be listed on the UKGTN website) If this submission is for a panel test please complete appendix 1 listing all of the conditions included using approved OMIM name, symbol and OMIM number.

Germline mutations in *GATA2* are associated with several phenotypes:
Immunodeficiency 21; IMD21
Primary Lymphedema With Myelodysplasia / Emberger syndrome
Susceptibility to Acute Myeloid Leukaemia
Susceptibility to Myelodysplastic syndrome

2. OMIM number for disorder/condition
If a panel test – see 1. above

IMMUNODEFICIENCY 21; IMD21 **614172**
LYMPHEDEMA, PRIMARY, WITH MYELODYSPLASIA **614038**
LEUKEMIA, ACUTE MYELOID; AML **601626**
MYELODYSPLASTIC SYNDROME; MDS **614286**

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

Changes in the gene *GATA2* can cause a range of potentially serious problems affecting many different parts of the body. These may be apparent soon after birth (e.g. congenital hearing loss or a form of the limb swelling called lymphoedema), or later in life through severe or frequent infections (warts, mycobacterial infections) or bone marrow problems (low blood counts, myelodysplasia or acute myeloid leukaemia). Other organs potentially affected include the blood vessels (excessive risk of blood clots or blood vessel aneurysms) and lungs. However, the disease is very variable and some family members with an altered copy of this gene may have no apparent problems.

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

Germline mutations in *GATA2* are associated with several phenotypes:

IMD21

This primary immunodeficiency, designated IMD21 or MonoMAC, is characterised by profoundly decreased or absent cellular blood components (monocytes, B lymphocytes, natural killer (NK) cells, and circulating and tissue plasmacytoid dendritic cells. CD4 lymphocytopaenia of variable degree also occurs in approximately one half of patients. Clinical features of IMD21 include susceptibility to disseminated nontuberculous mycobacterial infections, human papillomavirus (HPV) infections, chronic EBV infection, opportunistic fungal infections, and pulmonary alveolar proteinosis. Bone marrow hypocellularity and dysplasia of myeloid, erythroid, and megakaryocytic lineages are present in most patients, as are chromosomal abnormalities, including monosomy 7 and trisomy 8. Both autosomal dominant transmission and sporadic cases occur (Bigley et al., 2011; Hsu et al., 2011). Inability to control HPV results in precancerous change and squamous cell carcinoma of the vulval and perianal regions together with head and neck cancers. Unusual EBV positive tumours may also occur. Adapted from OMIMPrimary Lymphedema With Myelodysplasia / Emberger syndrome

Primary Lymphedema With Myelodysplasia / Emberger syndrome is a rare genetic disorder characterised by primary lymphoedema generally confined to the lower limbs and genitals and myelodysplasia associated with a greatly increased risk of acute myeloid leukaemia. Although most cases are sporadic, familial cases do occur and it is inherited as an autosomal dominant trait. Other symptoms may include immunological abnormalities (NK and B cell deficiency together with a low CD4/CD8 ratio), severe cutaneous warts, and congenital deafness.

Patients with the above two forms of the disease are predisposed to problems with blood vessels. These include venous clots (thrombosis), pulmonary embolism, portal vein thrombosis, and catheter-related thrombosis and arterial aneurysms.

MDS and AML are most commonly sporadic, but have recently been described in conjunction with rare inherited disorders caused by mutations in the *GATA2* gene (described above).

Susceptibility to Acute Myeloid Leukaemia

Susceptibility to the development of acute myeloid leukaemia can be caused by germline mutations in certain genes, including *GATA2*. Acute myeloid leukaemia (AML) is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells. The symptoms of AML are caused by replacement of normal bone marrow with leukemic cells, which causes a drop in red blood cells, platelets, and normal white blood cells. These symptoms include fatigue, shortness of breath, easy bruising and bleeding, and increased risk of infection. As an acute leukemia, AML progresses rapidly and is typically fatal within weeks or months if left untreated.

Susceptibility to Myelodysplastic syndrome
A predisposition to myelodysplastic syndrome can be caused by germline mutation in the *GATA2* gene. Myelodysplastic syndrome (MDS) is a heterogeneous group of clonal hematologic stem cell disorders characterized by ineffective hematopoiesis resulting in low blood counts, most commonly anaemia, and a risk of progression to AML. These symptoms include fatigue, shortness of breath, easy bruising and bleeding, and increased risk of infection.

There is almost certainly crossover between these patterns of illness so that patients/families falling predominantly into one category may share features from other categories. In time it is likely that these patients will all be classified as having “GATA2 deficiency” as an all-encompassing term.

4. Disorder/condition – mode of inheritance If this submission is for a panel test, please complete the mode of inheritance for each condition in the table in appendix 1.

Autosomal Dominant and de novo mutations

5. Gene – approved name(s) and symbol as published on HGNC database (alternative names will be listed on the UKGTN website)
If this submission is for a panel test please complete appendix 1 listing all of the genes included using approved HGNC name, symbol, number and OMIM number.

GATA binding protein 2; GATA2

6a. OMIM number(s) for gene(s)
If a panel test – see 5. above

137295

6b. HGNC number(s) for gene(s)
If a panel test – see 5. above

4171

7a. Gene – description(s)
If this submission is for a panel test, please provide total number of genes.

This gene encodes a member of the GATA family of zinc-finger transcription factors that are named for the consensus nucleotide sequence they bind in the promoter regions of target genes. The encoded protein plays an essential role in regulating transcription of genes involved in the development and proliferation of hematopoietic and endocrine cell lineages.

7b. Number of amplicons to provide this test (molecular) or type of test (cytogenetic)
(n/a for panel tests)

8

7c. GenU band that this test is assigned to for index case testing.

E

8. Mutational spectrum for which you test including details of known common mutations

(n/a for panel tests) If this application is for a panel test to be used for different clinical phenotypes and/or various sub panel tests – please contact the team for advice before completing a Gene Dossier

Missense, Nonsense, Splice Site and small insertions or deletions. An additional sequencing fragment for mutations in a conserved intronic element is also included.

Hsu et al. *GATA2* haploinsufficiency caused by mutations in a conserved intronic element leads to MonoMAC syndrome. Blood. 2013 May 9;121(19):3830-7, S1-7.

There has been one report of a large deletion (exons 3 and 4). MLPA analysis of this gene is not currently available but development will be considered based on auditing pick up rate in referred cases.

Vinh, D. C. et al Autosomal dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia. Blood 115: 1519-1529, 2010.

9a. Technical method(s) – please describe the test.

High throughput (HT) automated sequence analysis.

Gene screening by bidirectional automated Sanger sequence analysis (Beckman Biomek NX/ABI3730), the standard platform used for gene screening at this laboratory and analysis of the results with SoftGenetics Mutation Surveyor software.

9b. For panel tests, please specify the strategy for dealing with gaps in coverage.

N/A

9c. Does the test include MLPA?
(For panel tests, please provide this information in appendix 1)

No

9d. If NGS is used, does the lab adhere to the Association of Clinical Genetic Science Best Practice Guidelines for NGS?

N/A

10. Is the assay to be provided by the lab or is it to be outsourced to another provider?
If to be outsourced, please provide the name of the laboratory and a copy of their ISO certificate or their CPA number.

All components of the test will be provided in house

11. Validation process

Please explain how this test has been validated for use in your laboratory or submit your internal validation documentation. If this submission is for a panel test, please provide a summary of evidence of:
i) instrument and pipeline validation, and
ii) panel verification for the test
Please submit as appendices to the Gene Dossier (these will be included in the published Gene Dossier available on the website).
Please note that the preferred threshold for validation and verification is 95% sensitivity with 95% Confidence Intervals.

- Amplicons are described according to the GATA2 reference sequence NM_032638.4
- Primers were designed using Primer3 (http://primer3.ut.ee/) and SNP checked using www.snpcheck.net/ from NGRL Manchester.
- Three separate anonymised control DNA samples were used to check that each Region Of Interest (ROI) was correct and the sequence data was of reportable quality, using predefined laboratory standards. Analysis was undertaken using Mutation Surveyor software (Softgenetics).
- SOPs and test validation documents were produced and authorised.
- A sample from a patient with a high probability of having a *GATA2* mutation was sequenced and a previously reported pathogenic variant was found and reported.

12a. Are you providing this test already?
Yes
12b(i). If yes, how many reports have you produced?
Six
12b(ii). Number of reports mutation positive?
One
12b(iii). Number of reports mutation negative?
Five (2 x diagnostic cases, 3 x familial).
12b(iv). Please provide the time period in which these reports have been produced and whether in a research or a full clinical diagnostic setting.
This service has been available since June 2013. It was set up in response to an urgent clinical case. All tests and validation were undertaken in a full clinical diagnostic setting.
13a. Is there specialised local clinical/research expertise for this disorder?
Yes
13b. If yes, please provide details
Dr Colin Steward, Consultant in BMT for Metabolic and Genetic diseases at Bristol Royal Hospital for Children and Reader in Stem Cell Transplantation at the University of Bristol. Dr Steward has a particular interest in transplantation of genetic diseases predisposing to bone marrow failure, myelodysplastic syndrome and acute myeloid leukaemia. He was one of the team who recognised Emberger Syndrome (*GATA2* deficiency) as an important predisposing cause of MDS/AML.
14. Based on experience what will be the national (UK wide) activity, per annum, for:
Index cases: 10
Family members where mutation is known: 30
15. If your laboratory does not have capacity to provide the full national need please suggest how the national requirement may be met. For example, are you aware of any other labs (UKGTN members or otherwise) offering this test to NHS patients on a local area basis only? This question has been included in order to gauge if there could be any issues in equity of access for NHS patients. If you are unable to answer this question please write "unknown".
This laboratory has capacity to undertake testing for the estimated national need
16. If using this form as an Additional Provider application, please explain why you wish to provide this test as it is already available from another provider.
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EPIDEMIOLOGY

17a. Estimated prevalence of conditions in the general UK population

Prevalence is total number of persons with the condition(s) in a defined population at a specific time.
Please identify the information on which this is based.
For panel tests, please provide estimates for the conditions grouped by phenotypes being tested.

IMD21 <1 / 1 000 000

Emberger syndrome <1 / 1 000 000

Susceptibility to Acute Myeloid Leukaemia Unknown

Susceptibility to Myelodysplastic syndrome Unknown

All information from Orphanet

17b. Estimated annual incidence of conditions in the general UK population

Incidence is total number of new cases in a year in a defined population.
Please identify the information on which this is based.
For panel tests, please provide for groups of conditions.

Unknown – No data available

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

Please identify the information on which this is based.
n/a for panel tests.

Unknown – No data available

19. Estimated penetrance of the condition. Please identify the information on which this is based
n/a for panel tests

Penetrance is incomplete[1] although high[2]. Variable expressivity is also observed which may be caused by missense mutations retaining partial activity[3].

1. Ostergaard P, et al Nat Genet. 2011;43(10):929–931
2. Pasquet et al, Blood. 2013;121(5):822-829
3. Kazenwadel et al Blood. 2012 February 2; 119(5): 1283–1291

20. Estimated prevalence of conditions in the population of people that will be tested.
n/a for panel tests.

In cases of IMD21 Hsu et al. (Blood 118: 2653-2655, 2011) identified 12 distinct *GATA2* mutations in 20 patients (60%). Bigley et al. (Exp. Med. 208: 227-234, 2011) identified 4 *GATA2* mutations in 4 unrelated IMD21 patients (100%).

In cases of primary lymphedema and myelodysplasia, mutations were found in 100% of cases (n=8) studied by Ostergaard et al (Nature Genet. 43: 929-931, 2011)

In a study undertaken by Hoffman et al (Poster presentation ASH 2013 - *GATA2* Mutations In Pediatric Myelodysplastic Syndromes and Bone Marrow Failure) a *GATA2* mutation was identified in 5/103 (4.8%) individuals presenting with *sporadic* appearing primary MDS, aplastic anaemia or an unclassified BMF. These patients were enrolled on the Pediatric MDS and BMF Registry.

INTENDED USE (Please use the questions in Annex A to inform your answers)

21. Please tick either yes or no for each clinical purpose listed.
Panel Tests: a panel test would not be used for pre symptomatic testing, carrier testing and pre natal testing as the familial mutation would already be known in this case and the full panel would not be required.

Diagnosis	☒ **Yes**	☐ **No**
Treatment	☒ **Yes**	☐ **No**
Prognosis & management	☒ **Yes**	☐ **No**
Presymptomatic testing (n/a for Panel Tests)	☒ **Yes**	☐ **No**
Carrier testing for family members (n/a for Panel Tests)	☒ **Yes**	☐ **No**
Prenatal testing (n/a for Panel Tests)	☐ **Yes**	☒ **No**

TEST CHARACTERISTICS

22. Analytical sensitivity and specificity
This should be based on your own laboratory data for the specific test being applied for or the analytical sensitivity and specificity of the method/technique to be used in the case of a test yet to be set up. Please note that the preferred threshold for validation and verification is 95% sensitivity with 95% Confidence Intervals.

High throughput automated bidirectional Sanger sequence analysis.

Sensitivity of the sequencing assay is 99-100% for point mutations and small insertion deletions. (SCOBEC validation of unidirectional sequencing indicates a sensitivity of 99%). To the best of our knowledge, no variant has been missed using a bi-directional sequencing approach.

Of the 39 reported *GATA2* mutations there is only 1 reported large (2 exon) deletion in the gene that will not be detected this gives an overall estimated sensitivity of >95%.
Specificity > 99%

23. Clinical sensitivity and specificity of test in target population The *clinical sensitivity* of a test is the probability of a positive test result when condition is known to be present; the *clinical specificity* is the probability of a negative test result when disorder is known to be absent. The denominator in this case is the number with the disorder (for sensitivity) or the number without condition (for specificity).
Please provide the best estimate. UKGTN will request actual data after two years service.

Based on the families studied by Ostergaard et al (Nature Genet. 43: 929-931, 2011) the clinical sensitivity of *GATA2* analysis in Primary Lymphedema With Myelodysplasia / Emberger syndrome is 100% (n=8).

In cases of IMD21 Hsu et al. (Blood 118: 2653-2655, 2011) identified 12 distinct *GATA2* mutations in 20 patients (60%). Bigley et al. (Exp. Med. 208: 227-234, 2011) identified 4 *GATA2* mutations in 4 unrelated IMD21 patients (100%). Combining the cases studied by Hsu et al. and Bigley et al. gives a sensitivity of 66%.

In a series of 27 families with familial MDS/AML studied by Holme et al (British Journal of Haematology, 2012, 158, 242–248) a *GATA2* mutation was identified in 4 families, giving a sensitivity of ~15%.

Clinical Specificity:

Presumed over 95%, depending on the basis of interpretation of sequence variants as 'consensus' mutations, or as innocuous polymorphisms.

For testing of at-risk relatives: Clinical sensitivity and specificity: will both be close to 100% when the mutation is definitely pathogenic and the disease is highly penetrant.

24. Clinical validity (positive and negative predictive value in the target population) The *clinical validity* of a genetic test is a measure of how well the test predicts the presence or absence of the phenotype, clinical condition or predisposition. It is measured by its *positive predictive value* (the probability of getting the condition given a positive test) and *negative predictive value* (the probability of not getting the condition given a negative test).
Not currently requested for panel tests

Clinical validity: In an index case, finding a pathogenic mutation confirms the diagnosis in all cases.

We estimate that for index cases:

Positive predictive value (PPV) = 100% for consensus mutations

Negative predictive value (NPV) = greater uncertainty but likely to approach 100%

However, for testing family members, PPV and NPV are both effectively 100% for carrier status using consensus mutations.

25. Testing pathway for tests where more than one gene is to be tested sequentially Please include your testing strategy if more than one gene will be tested and data on the expected proportions of positive results for each part of the process. Please illustrate this with a flow diagram. This will be added to the published Testing Criteria.
n/a for panel tests

N/A

CLINICAL UTILITY

26. How will the test change the management of the patient and/or alter clinical outcome? Please describe associated benefits for patients and family members. If there are any cost savings AFTER the diagnosis, please detail them here.

Patients with GATA2 mutations may be excessively susceptible to conditioning chemotherapy and/or post-transplant complications where they undergo transplantation for myelodysplastic syndrome (possibly reflecting known or unknown antecedent chronic viral infection or respiratory compromise). However, they appear to respond well to reduced intensity conditioning chemotherapy and appropriate identification will allow appropriate selection for this approach. This typically causes less post-transplant toxicity and allows earlier discharge from hospital. Other family members may also be screened for genetic mutations and allied clinical effects such as lymphocytopaenia. Occasional, e.g. annual, blood count monitoring in these individuals who are identified at an early stage will then allow appropriate selection for bone marrow aspirate surveillance in order to try to detect myelodysplastic syndrome before it progresses to acute myeloid leukaemia – with likely consequent reduction in survival probability. They can be offered vaccination against HPV in the expectation of reducing the risk of HPV associated carcinomas. They can be offered genetic counselling and antenatal diagnostics in future pregnancies. This disease also has protean manifestations including many potential respiratory presentations and appearances, chronic viral infections, thromboembolic events/aneurysms and autoimmune manifestations such as erythema nodosum. Recognising the existence of underlying GATA2 deficiency will prevent inappropriate investigation and treatment of these complications and allow greater potential for effective therapy.

Many of these areas of early detection or prevention will carry considerable cost savings to the NHS.

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

Inappropriate investigation, management or treatment of the complications described in section 26. Lower chance of survival due to a late recognition of complications such as carcinoma or acute myeloid leukaemia. Inappropriate choice of conditioning chemotherapy for hemopoietic stem cell transplantation. Giving birth to further affected individuals with attendant risk of early morbidity and mortality.

28. Is there an alternative means of diagnosis or prediction that does not involve molecular diagnosis? If so (and in particular if there is a biochemical test), please state the added advantage of the molecular test.

No

29a. What unexpected findings could this test show? For example, lung cancer susceptibility when testing for congenital cataract because ERCC6 gene (primarily associated with lung cancer) is included in a panel test for congenital cataract.

None

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

N/A

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

N/A

31. If a panel test, is this replacing an existing panel/multi gene test and/or other tests currently carried out through UKGTN using Sanger sequencing? If so, please provide details below.

N/A

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

None

IS IT A REASONABLE COST TO THE PUBLIC?

33. In order to establish the potential costs/savings that could be realised in the diagnostic care pathway, please list the tests/procedures that would be required in the index case to make a diagnosis if this genetic test was not available.

This diagnosis is not possible without genetic testing. However, patients presenting with myelodysplastic syndrome either at young age or with suspicious family history would typically undergo the following:

1. Exclusion of Fanconi Anaemia (chromosome fragility test)
2. Exclusion of gene mutations associated with familial myelodysplastic syndrome a (i.e. sequencing of TERT, TERC, CEBPA and RUNX1)
3. Exclusion of gene mutations associated with papillomatosis where present (DOCK8, CXCR4)
4. Exclusion of Shwachman-Diamond syndrome if history of diarrhoea present (faecal chymotrypsin, hip ultrasound, SBDS sequencing)

1. Chromosome fragility testing is an essential first round test in someone presenting with cytopenia or severe aplastic anaemia in the presence of reduced cellularity on bone marrow aspirate examination. It has tended to be performed also in patients with myelodysplastic syndrome because of the potential for Fanconi Anaemia to progress to this. However, it seems very likely that one of the first line tests in a paediatric/young adult patient presenting with MDS will in future be lymphocyte subset analysis, with progression straight to GATA2 testing if this confirms low numbers of natural killer cells ± B-cells, especially where accompanied by low plasmacytoid dendritic cells. Chromosome fragility testing is therefore likely to be restricted to those with a normal pattern of lymphocyte subsets, especially where any form of congenital anomaly is present.

2. Lymphocyte subset abnormalities have been described in variants of DKC but not in RUNX1. The results in GATA2 deficiency syndromes tend to be very characteristic as shown below from a typical patient:

CD3	94	%	CD3 abs	1.31	10*9/L
CD4	50	%	CD4 abs	0.70	10*9/L
CD8	34	%	CD8 abs	0.47	10*9/L
CD56	< 1	%	CD56 abs	< 0.01	10*9/L
CD19	4	%	CD19 abs	0.06	10*9/L
Total lymphocyte count	1.39	10*9/L			

These are in stark contrast to this set from a patient with RUNX1 mutations:

CD3	71	%	CD3 abs	1.08	10*9/L
CD4	55	%	CD4 abs	0.84	10*9/L
CD8	16	%	CD8 abs	0.24	10*9/L
CD56	20	%	CD56 abs	0.30	10*9/L
CD19	7	%	CD19 abs	0.11	10*9/L
Total lymphocyte count	1.52	10*9/L			

We anticipate that lymphocyte subset analysis will facilitate targeted analysis of this single gene GATA2. More complex multiple single gene or NGS panel testing approaches for the multiple genes responsible for DKC, CEBPA and RUNX1 will only be necessary in patients who lack these characteristic lymphocyte subset anomalies or those who come up negative on GATA2 testing despite low NK cell numbers.

3. Patients with papillomatosis would also be tested first by lymphocyte subset analysis, proceeding to GATA2 mutation analysis if the results of these are suggestive, with other genes only being tested once GATA2 mutation has been excluded. DOCK8 deficiency may impact CD8 T-cell numbers and NK function defects have been described but NK and B cell numbers would not usually be affected. WHIM Syndrome affects B cell, and sometimes T-cell, but not NK cell numbers.

4. SBDS would only come into the picture if there were a history of diarrhoea or malabsorption and is likely to be excluded first by faecal chymotrypsin analysis.

	Type of test	Cost (£)
Costs and type of imaging procedures	hip ultrasound	52
Costs and types of laboratory pathology tests (other than molecular/cyto genetic test proposed in this Gene Dossier)	Chromosome Fragility Test Exclusion of *TERT, TERC, CEBPA* and *RUNX1* mutations Exclusion of Shwachman-Diamond syndrome faecal chymotrypsin SBDS sequencing	384 1,200 (approx. tests not available in UK) 30 345
Costs and types of physiological tests (e.g. ECG)		
Cost and types of other investigations/procedures (e.g. biopsy)		
Total cost of tests/procedures no longer required (please write n/a if the genetic test does not replace any other tests procedures in the diagnostic care pathway)		**2,011**

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

Number of index cases expected annually	(a) 10
Cost to provide tests for index cases if the genetic test in this Gene Dossier was not available (see Q32)	(b) £2,011
Total annual costs pre genetic test	(a) x (b) = (c) £20,110
Total annual costs to provide genetic test	(a) x cost of genetic testing for index case = (d) £4,590
Additional savings for 100% positive rate for index cases	(d) – (c) = (e) -£15,520
Percentage of index cases estimated to be negative	(f) 20
Number of index cases estimated to be negative	(f) x number of index cases = (g) 2
Costs to provide additional tests for index cases testing negative	(g) x (b) = (h) £4,022
Total costs for tests for index patient activity	(e) + (h) = (i) -£11,498
Total costs for family members	Costs for family member test x number of family members expected to test in a year (j) 30 x 190 = £5,700
If there is a genetic test already available and some of the family testing is already being provided, please advise the cost of the family testing already available	Cost for family member testing already available x estimated number of tests for family members already provided (k) 0
Total costs for family members minus any family member testing costs already provided	(j) – (k) = (l) £5,700
Additional savings for all activity expected in a year	(i) + (j) or (i) + (l) -£5,798

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

The narrative which follows explains the pattern of events which occurred. Genetic diagnosis happened subsequent to most of these but the impact which early diagnosis would have had is explained for each family member.

The proband, a 9-year-old girl, presented with acute myeloid leukaemia, characterised by monosomy 7 and trisomy 21 on cytogenetic analysis. Her clinical history was unremarkable except that she had been admitted previously for antibiotic therapy for cellulitis which complicated severe chickenpox infection.

She received chemotherapy according to the AML12 protocol but developed suspected Aspergillus pneumonia (in retrospect possibly GATA2 associated nodular lung disease as seen in other patients with this disease) which resulted in cessation of chemotherapy administration. She eventually recovered from her fungal infection and proceeded to matched unrelated donor bone marrow transplantation. However, autologous reconstitution with malignant cells occurred and the patient died due to infection in the face of pancytopenia. Had GATA2 aetiology been known it is possible that steroid therapy would have resolved the pulmonary lesion, allowing this patient to complete her chemotherapy and so enter transplant in remission, with a greatly increased chance of cure.

This patient had a suspicious history, having been previously hospitalised for cellulitis secondary to severe chickenpox infection (a manifestation of natural killer cell deficiency, one of the components of *GATA2* deficiency), and then presented with AML. With such a history she would now have lymphocyte subset analysis leading to identification of numerical natural killer cell deficiency and analysis of *GATA2*. This would have provided index identification of *GATA2* deficiency leading to diagnosis in the other family members described below.

Her brother was born prematurely (29 weeks gestation) with congenital ptosis of one eye requiring surgery at 4 years of age. At 14 years (subsequent to the death of his sister), he developed marked lymphedema of his left leg and scrotum. Lymphoscintigraphy demonstrated left lymphatic hypoplasia. At 20 years, he was borderline neutropenic and B-lymphopenic. Bone marrow examination showed no evidence of MDS and cytogenetic analysis was normal.

This patient's disease has been picked up at a time when he can be prospectively screened for the development of myelodysplastic syndrome, a major consequence of *GATA2* deficiency. If this develops, the anticipated success rates of haematopoietic stem cell transplantation would be far higher than after the invariable later development of acute myeloid leukaemia and he would most probably be able to receive a reduced intensity conditioned transplant with lower expectation of post-transplant complications and a higher chance of cure than after fully myeloablative transplantation.

The mother of these children had an extremely complicated medical history. She developed severe warts during puberty, particularly affecting her vulval and anal regions, and leading to the development of cervical, vulval, and anal intra-epithelial neoplasia. She had chronic active hepatitis of unknown aetiology which led to numerous investigations including seven liver biopsies. Bone marrow examination at 35 years showed 25% of cells with X chromosome aneuploidy 45X/47,XXX/49,XXXXX but no evidence of MDS. In her 40s she developed lymphedema in her lower limbs following a femoral popliteal bypass for a mycotic aneurysm. By 50 years bone marrow examination revealed myelodysplastic syndrome but chromosome breakage studies were normal. AML subsequently developed, claiming this lady's life at 52 years of age. She had been deemed unfit for haematopoietic stem cell transplantation when assessed at between 50 and 52 years of age due to the severity of her pathologies which by that stage included chronic active EBV infection and respiratory compromise.

The personal and family toll and healthcare costs associated with this lady's history exemplify perfectly why it is important to ascertain genetic causes in patients with suspicious forms of pancytopenia/ myelodysplastic syndrome or AML. There is for instance accumulating evidence that the respiratory and carcinomatous components of this disease can be reversed by early stem cell transplantation due to correction of the immunodeficiency leading to HPV, mycobacterial and other infections. Had this lady's disease been accurately diagnosed through family screening at the time of her daughter's diagnosis there is a high probability that her life could have been saved. She also had numerous investigations and distressing clinical interventions which would have been avoided.

A maternal cousin of the index case was born prematurely at 32 weeks gestation with tense ascites, requiring ventilation for one week. She subsequently developed lymphoedema of her right leg and swelling of her left leg by 18 months she required surgery for labial oedema at 9 years of age and developed toxic-shock septicaemia secondary to Group A streptococcal cellulitis in her affected leg. She was found to be pancytopenic and further investigations confirmed acute myeloid leukaemia with monosomy 7 and trisomy 8 on cytogenetic analysis. She was successfully treated with chemotherapy and a matched unrelated donor bone marrow transplant but continues to have recurrent cellulitis in her legs.

Identification of *GATA2* deficiency in the index case would have led to cascade screening within the family and antenatal counselling/diagnostics.

TESTING CRITERIA

36. Please only complete this question if there is previously approved Testing Criteria.
Please contact the UKGTN office if you are unsure whether testing criteria is available.

36a. Do you agree with the previously approved Testing Criteria? Yes/No

36b. If you do not agree, please provide revised Testing Criteria on the Testing Criteria form and explain below the reasons for the changes.

UKGTN Testing Criteria

Test name: **GATA2 Deficiency**	
Approved name and symbol of disorder/condition(s):	**OMIM number(s):**
Immunodeficiency 21; IMD21	614172
Lymphedema, Primary, with Myelodysplasia	614038
Leukemia, Acute Myeloid; AML	601626
Myelodysplastic Syndrome; MDS	614286
Approved name and symbol of gene(s):	**OMIM number(s):**
GATA binding protein 2; GATA2	137295

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 Haematologist	
Consultant Clinical Geneticist	
Consultant Oncologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Recurrent, unexplained severe or prolonged mycobacterial, HPV, EBV or fungal infections or myelodysplastic syndrome **AND** at least one of the following:	
Lymphocyte subset analysis showing reduced numbers of NK cells ± B cells	
Primary lymphedema	
Sensorineural deafness	
Monocytopenia	
Evidence of familial MDS/AML	
OR At risk family members where familial mutation is known.	

Additional Information:
If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.