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**The St George’s Classification Algorithm of Primary Lymphatic Anomalies**

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**Introduction**

Primary lymphoedema (PL) and lymphatic malformations arise from faults in lymphatic development. In 1998 at St George’s Hospital (SGH), London, UK a clinic dedicated to Primary Lymphoedema was established. At that time phenotypes were not differentiated from one another except according to time of onset i.e. at birth, at puberty, and later onset. Any inherited primary lymphoedema with onset at birth would be called Milroy disease by default. As the only dedicated clinic in the UK for primary lymphoedema and with a referral network from throughout the UK, a research-based approach was introduced. Patients with similar clinical characteristics were grouped into cohorts and DNA was collected.

In 1995, the first gene shown to be involved inlymphatic development was *FLT4* (*VEGFR3*)1 creating the possibility of finding causal genes for human disease. Primary lymphoedemas result from defects in genes involved in lymphatic vessel development. *VEGFR3* was the first lymphoedema gene to be identified in humans in 20002 following our publication of a gene for Primary Congenital Lymphoedema mapping to the chromosome 5q35.3 region in 19993.

Through a process of rigorous phenotyping in the clinic, a second cohort of patients with primary lymphoedema was identified associated with distichiasis. Again, a locus was found by our group4 with the gene, *FOXC2*, discovered by an American group5. Because the St George’s group provided essential linkage data for both discoveries a series of research grants were obtained from the British Heart Foundation and this enabled a translational approach combining both basic and clinical research.

**A translational approach**

A clinical approach for the classification of PL was developed, based on phenotype. The St George’s expertise and reputation from published research prompted increasing referrals of inherited lymphoedema from throughout the UK. Patients were carefully categorised, DNA collected and interrogated for gene mutations.

With careful phenotyping of patients by the clinical team (Professor Sahar Mansour, Dr Kristiana Gordon and Professor Peter Mortimer) and gene analysis by Dr (now Professor) Pia Ostergaard and Professor Steve Jeffery, causal mutations were identified.

Knowing the genotype has enabled further investigation of the patients in order to define the full clinical characteristics of each type of primary lymphoedema as well as the mechanisms leading to the disease e.g. lymphatic valve failure.

**Lymphoedema versus Lymphatic malformations**

Interest in Lymphatic malformations (LM) inevitably followed as many of these birthmark anomalies can be associated with lymphoedema but not always. Lymphatic malformations represent a structural abnormality of lymphatic vessels. Often the abnormality occurs in isolation with no communication with the main lymph conducting channels. In such circumstance swelling is due to the structural lymphatic vessel fault with the lymph trapped within it (atruncular lymphatic malformation). Conversely, if the malformation interferes with the main lymphatic channels then lymphoedema can coexist because lymph is now trapped in the tissues, not just within the malformation (truncular lymphatic malformation)6.

Lymphatic malformations are predominantly due to post-zygotic mosaic mutations for which the phenotype can be variable but include lymphatic abnormalities. Somatic mutations are unlikely to be found in blood DNA. Genes such as *PIK3CA* and mutations within the RAS/MAPKinase pathway cause lymphatic malformations, so a biopsy of the affected tissue is needed to provide a molecular diagnosis.

**A clinical algorithm**

It is so important to remember that primary lymphoedema is not just one disease, and there is much variation in the clinical manifestations. For many years the classification of primary lymphoedema was based on the age of presentation of the swelling with little consideration for associated clinical characteristics.

The St George’s Lymphoedema team began developing a classification system for primary lymphoedema more than 15 years ago. Careful phenotyping i.e. looking at patterns of the swelling and other health problems led to the identification of five subgroups which shared the same broad category of primary lymphoedema.

These are:

1. Lymphoedema associated with other genetic syndromes, such as Noonan or Turner syndrome (where the lymphoedema is not the overriding feature of the syndrome).

2. Lymphoedema with systemic, or internal, lymphatic problems. For example, pleural effusions, pericardial effusions, ascites, chylous reflux, protein losing enteropathy/intestinal lymphangiectasia or *in utero* swelling (fetal hydrops).

3. Lymphoedema that is congenital, so present at birth or within a few months of life (but no systemic involvement and the lymphoedema is the predominant problem).

4. Lymphoedema that occurs later in life, after 1 year of age (but no systemic involvement and the lymphoedema is the predominant problem).

5. Lymphoedema that may be associated with lymphatic malformations, vascular malformations or segmental overgrowth problems.

These 5 groups of primary lymphoedema are presented in the classification algorithm as colour-coded sections, along with the individual subtypes, including the known genes. The algorithm was first published in 20107.

Over the years patients with similar phenotypes have been allocated to one of the five classification categories. Their DNA samples have been analysed together to try and identify mutations common to the cohort, and this has proved very successful. Once a new gene has been discovered then close scrutiny and crosschecking of the clinical signs, natural history and inheritance patterns is performed to further refine an accurate phenotype for that genotype. This helps us in clinic to know what other health problems we need to screen for, for example cardiac problems or leukaemia, and also how best to manage the patient.

We consider the algorithm as a “work in progress’ and it should be used a living, dynamic system that is constantly changing and being updated as new phenotypes and causal genes are identified. The SG’s classification pathway was updated in 20138 and again in 20209.

The algorithm is designed to help clinicians categorise their patients and guide genetic testing, where possible. It involves “criteria matching”, that is, it uses specific findings for classification, and these are obtained through a process of taking a history, examination findings, and where possible incorporating results of investigation such as mutation testing and lymphoscintigraphy.

**Making a molecular diagnosis**

Making a molecular diagnosis is extremely helpful in the patient’s management as it can help determine what other problems the patient is at risk of developing. Possible other health problems could include varicose veins, hydrocele, immunodeficiency, myelodysplasia or leukaemia, congenital heart disease, scoliosis or spinal cysts, learning difficulties, eye abnormalities, renal abnormalities and systemic lymphatic abnormalities (heart/lung/gut). Such pathologies are specific to the subtype. For example, a boy with Milroy disease might develop hydroceles in the future, but is definitely not at an increased risk of leukaemia.

A molecular diagnosis will inform on inheritance patterns and likely prognosis but also guide the clinician on screening of diseases (as listed above) they are at risk of developing. The algorithm can help with this.

Knowing the gene fault can help determine mechanisms of disease. For example, a mutation in *FOXC2* leads to lymphatic valve dysfunction and so reflux of lymph as the main cause for the lower limb lymphoedema. It may also help with management. Understanding that reflux in both lymphatic vessels and veins is the mechanism with *FOXC2* disease, explains why these patients respond well to intensive Decongestive Lymphoedema Therapy and why swelling rebounds quickly afterwards. Interestingly dealing surgically with the veins does not appear to help control swelling, indicating the dominant role of the lymphatics in the control of oedema.

Identifying genes can inform on their biological function and perhaps open up possibilities for targeted treatment of the lymphoedema. Treatment for lymphoedema remains physically based with surgery an alternative in some patients. Drug therapy is emerging as an option in select patients providing the gene is known. We can offer drug therapy for patients with confirmed mutations in the mTOR pathway such as *PIK3CA* (sirolimus) where disease appears to be still progressing10. In the RASopathies e.g. Noonan syndrome, caused by germline mutations in genes of the Ras-MAPK pathway, MEK-inhibitors offer promise to those with progressive lymphatic failure11.

**How do you use the algorithm (Figure 1)?**

You start in the lower grey box and then move through the pathway working out which colour section your patient fits into depending on what problems they have.

**Syndromic**: The first question (blue box) is whether the patient fits into a known syndrome or appears to be ‘syndromic’ i.e. a constellation of characteristics often including dysmorphic features. Testing for chromosomal abnormalities is usually worthwhile particularly if there is dysmorphism or learning difficulties.

**Systemic involvement**: If the patient does not have an underlying genetic syndrome, you move on to the pink box below and consider if there are associated internal/systemic lymphatic problems such as pleural or pericardial effusions. Another pointer to the possibility of systemic involvement is the presence of fetal hydrops antenatally. Therefore, it is always important to enquire in the history if there was any extra fluid present *in utero*.

If you were a referred a young patient with lower limb lymphoedema and have excluded syndromes or systemic involvement from your history and examination, then you look at the green and purple sections. These mostly relate to lower limb lymphoedema but do include genital and arm involvement too. The green section refers to congenital swelling, present at birth, whilst the purple sections classify swelling that comes on after the first year of life.

**Congenital**: Pedal lymphoedema presenting at birth but with no ‘syndromic ‘ or ‘systemic’ features would make one consider Milroy disease and testing for mutations in *VEGFR3* is worthwhile. If a mutation is found in the *VEGFR3* gene, then the diagnosis of Milroy disease is confirmed. The next step for the clinician is to use the information gathered to advise on natural history, prognosis and risks, and to guide management. Testing the parents will show whether the mutation in VEGFR3 has been inherited (10 % of carriers are asymptomatic) or *de novo* (new in the child). If inherited, you can explain that future offspring and the patient’s future children have a 50% chance of inheriting the condition, but can reassure the patient and their family that the swelling will remain confined to the lower limbs, although there may be a risk of varicose veins when older, and a third of affected males develop hydroceles.

For **late onset lymphoedema** (purple box) there are 3 main diagnoses to consider: Lymphoedema distichiasis syndrome (LDS), due to mutations in the *FOXC2* gene, which may have varicose veins, congenital heart disease, cleft palate, spinal cysts, renal problems; Emberger syndrome, due to mutations in the *GATA2* gene, with widespread warts and monocytopenia/pancytopenia predisposing to myelodysplasia and acute myeloid leukaemia; and Meige disease which is the commonest subtype but no associated health problems for which no causal gene is yet known. One could ask why LDS and Emberger are not listed under syndromic? The answer is that the lymphoedema is the dominant feature in both whereas under syndromic the lymphoedema is not considered a dominant feature.

The algorithm tells you to look for distichiasis first. If present, the diagnosis can only be lymphoedema distichiasis syndrome. Distichiasis refers to an extra aberrant eyelashes arising from the inner eyelid. Even one of these aberrant eyelashes is sufficient to consider this diagnosis. This abnormality is usually present at birth despite the lymphoedema not manifesting until later in life usually late childhood/puberty but sometimes not until the 5th decade. This variability in age of onset of the swelling in LDS illustrates why distinguishing between the old terms of “praecox” and “tarda” forms of primary lymphoedema may not be useful. A diagnosis of LDS should prompt a search for any congenital heart or renal abnormalities.

A late onset predominantly asymmetric lower limb lymphoedema with genital involvement should make one consider Emberger syndrome. Other characteristic features would include viral warts because of the underlying immunodeficiency. This phenotype illustrates why a blood test should be carried out. A low monocyte count would point to Emberger and the finding of a mutation in *GATA2* confirms the diagnosis. A lifesaving bone marrow transplant should be considered to avoid leukaemia. The family should be screened for the same *GATA2* mutation before being considered as a bone marrow donor. It can literally be a life-saving diagnosis to make, when death from leukaemia can be prevented.

Lower limb lymphoedema of late onset, particularly in a female, without any associated features suggest Meige lymphoedema. Here, the gene is not known and so diagnosis is largely by exclusion of other late onset types.

Care must be taken to enquire about, and examine for, hand or upper limb lymphoedema as it can be easily missed. Only by asking for swelling on the back of the hand, and not just fingers, will upper limb lymphoedema be detected and, even then, 4 limb quantitative lymphoscintigraphy with quantification might be necessary for confirming the diagnosis. *GJC2* mutations cause 4 limb lymphoedema, with varicose veins but no other abnormalities and no systemic involvement.

**Mutation testing**

Screening for a molecular diagnosis in all forms of primary lymphoedema might seem an easy option but, without careful phenotyping, the detection rate will be low.

We audited our primary lymphoedema clinic and of the 234 new patients seen in one year, we only found a causative mutation in 42% of the patients tested (25% overall including those not tested) (Figure 2). We had not offered testing to a third of the patients because they had a phenotype of primary lymphoedema where the gene was not known, and we suspected the result would be negative (e.g. Meige disease).

These results have been replicated by other primary lymphoedema clinics worldwide, confirming the low pick-up rate and indicating that there are many more causal genes to be discovered. We believe that matching a phenotype to a likely gene reduces wasteful and expensive testing.

Improvements in technology for genetic testing using next-generation sequencing have led to the introduction of testing for mistakes in several genes at once (the Lymphoedema gene panel). However, this has also led to an increase in the detection of ‘Variants of uncertain significance’ (VUSs). We believe the algorithm helps enormously for the interpretation of these VUSs. Some centres are very quick to send off DNA for sequencing of all the patient’s genes, but may struggle to interpret the results when the report says “there is a variant in Gene A, but we don’t know if this is significant”. Correlation of where the patient fits into the algorithm will help the clinician decide if this gene variant or mistake is “real” or not. In other words, does the gene mistake match the clinical diagnosis. It is almost like using the algorithm in reverse, and so we believe it is a useful tool for all lymphoedema clinics, even those that rely heavily on investigation results before examining the patient thoroughly first.

**Impact of the algorithm**

This transformational approach pioneered at St George’s (SG’s) has revolutionised the understanding and classification of primary lymphatic anomalies (i.e. primary lymphoedema and lymphatic malformations). The combination of careful phenotyping and genotyping has enabled the evolution of the SG’s classification pathway which acts as an algorithm in the clinic to guide a specific diagnosis and management.

The impact of these developments has been to advise patients on what they can expect to happen from their disease and how best to avoid and manage the complications. The option of prenatal diagnosis and preimplantation genetic diagnosis is now possible.

The gene panel for primary lymphoedema, developed at St George’s, is now available for UK use. Genomics England Limited (GEL) used information from the St George’s lymphoedema gene panel for their 100,000 genomes project (www.genomicsengland.co.uk) for patients with primary lymphoedema.

The St George’s classification pathway has been adopted by a number of lymphoedema clinics in the UK and by the Primary and Paediatric Lymphoedema Working Group (PPL-WG), part of the Vascular European Reference Network (VASCERN). The new Orphanet classification for primary lymphatic anomalies is based on the St George’s classification and dovetails with the ISSVA classification for vascular anomalies (<https://www.issva.org/UserFiles/file/ISSVA-Classification-2018.pdf)>.

**Conclusion**

The model of clinical care used by St George’s has vindicated the benefit of combining basic with clinical science in a symbiotic relationship.

As more causal genes have been discovered so a molecular (genomic) diagnosis can be made more often. Knowing the gene permits knowledge of associated features e.g. venous or heart disease, informs on natural history of the disease, and enables understanding of gene function. Another benefit of a molecular diagnosis is that the genotype can be rigorously evaluated by investigation to refine the features of the phenotype.

Phenotyping using the algorithm improves the chances of finding yet more causal genes through research.

Investigation of the genotype can reveal likely mechanisms of disease. Understanding mechanisms provides the opportunity for targeting new therapies.

The St George’s classification algorithm has evolved as more genes have been discovered. It remains ‘work in progress’ and is designed to help clinicians phenotype their patients more accurately.

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Figure 1: **St George’s classification algorithm for primary lymphatic anomalies** (Published **in** J Med Genet. 2020 Oct;57(10):653-659).

Figure 2: Audit of genetic testing results for 234 new patients seen in the primary lymphoedema clinic over a one year period. A causal gene was only identified in 58 patients (25%).