



**DEVELOPMENT OF A DIAGNOSTIC SERVICE FOR
LIMB GIRDLE MUSCULAR DYSTROPHY 2I
IN SCOTLAND**

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LIMB GIRDLE MUSCULAR DYSTROPHIES

Group of disorders

Autosomal dominant / recessive inheritance

Proximal weakness affecting voluntary muscles (red) mainly around the hips and shoulders –i.e. the limb girdles

Affects children and adults

Both genders equally affected

Distinct from the dystrophinopathies

Age of onset

Depends on genetic subset

Onset, progression & severity also varies among individuals

Diagnosis

Muscle biopsy

DNA analysis “Gold Standard” – gives definitive diagnosis

Prevalence

Ranges from 1/14 500 to 1/123 000 in all known forms



KNOWN FORMS OF LIMB GIRDLE MUSCULAR DYSTROPHY

Autosomal Dominant Limb Girdle Muscular Dystrophy			
Locus Name	Gene Symbol	Chromosomal Locus	Protein Product
LGMD1A	<i>TTID</i>	5q31	Myotilin
LGMD1B	<i>LMNA</i>	1q21.2	Lamin A/C
LGMD1C	<i>CAV3</i>	3p25	Caveolin-3
LGMD1D	Unknown	6q23	Unknown
LGMD1E	Unknown	7q	Unknown
LGMD1F	Unknown	7q31.1-q32.2	Unknown
LGMD1G	Unknown	4q21	Unknown

Autosomal Recessive Limb Girdle Muscular Dystrophy			
Locus Name	Gene Symbol	Locus	Protein Product
LGMD2A	<i>CAPN3</i>	15q15.1-q21.1	Calpain-3
LGMD2D	<i>SGCA</i>	17q12-q21.3	α -sarcoglycan
LGMD2E	<i>SGCB</i>	4q12	β -sarcoglycan
LGMD2C	<i>SGCG</i>	13q12	γ -sarcoglycan
LGMD2F	<i>SGCD</i>	5q33	δ -sarcoglycan
LGMD2B	<i>DYSF</i>	2p13.3-p13.1	Dysferlin
LGMD2G	<i>TCAP</i>	17q12	Telethonin
LGMD2H	<i>TRIM32</i>	9q31-q34.1	Tripartite motif protein 32
LGMD2I	FKRP	19q13.3	Fukutin-related protein
LGMD2J	<i>TTN</i>	2q24.3	Titin
LGMD2K	<i>POMT1</i>	9q34.19q31	Protein O-mannosyl-transferase 1
LGMD2L	<i>FKTN</i>		Fukutin
LGMD2M	<i>POMGNT1</i>	1p34-p33	Protein O-linked-mannose beta-1,2-N-acetylglucosaminyltransferase 1
LGMD2N	<i>POMT2</i>	14q24.3	Protein O-mannosyl-transferase 2

Types of LGMDs

21 known forms at this time

Autosomal dominant / recessive

FEATURES OF LGMD2I

LGMD2I

Autosomal recessive

Caused by mutations in the *FKRP* gene (19q13.3)

Second most prevalent LGMD (~20%)

Typical features can include

Age of onset late teens to mid 20s

Muscle weakness

Calf hypertrophy

Cardiomyopathy

Possible wheelchair dependence

Phenotype can overlap with those seen with BMD

Important to clarify diagnosis to ensure correct management and genetic counselling and avoid incorrect reproductive risk being given

LGMD2I AND THE *FKRP* GENE

LGMD2I

Gene was identified in 2001 via homology to the *FKTN* gene – causes fukuyama muscular dystrophy.

Patients affected by LGMD2I will be either homozygous or compound heterozygous for mutations in the *FKRP* gene

Majority of these mutations are missense and are located in the putative catalytic domain of the protein

Most prevalent mutation

c.826C>A;p.Leu276Ile

Carrier frequency in the European population 1 in 306

Homozygous c.826C>A;p.Leu276Ile - mild disease course

If patient compound heterozygous for common mutation and second mutation, the second mutation determines severity.

c.427C>A;p.Arg143Ser

This is the second most common mutation in the European population.

The carrier frequency is unknown

Variable clinical phenotype

Bedouin family, same mutation – different phenotype

Suggests role of modifier genes and environmental factors

LGMD 2I HAS AN OVERLAPPING PHENOTYPE WITH BMD

Referral

2007 Mother was worried about 4y son “dragging leg”

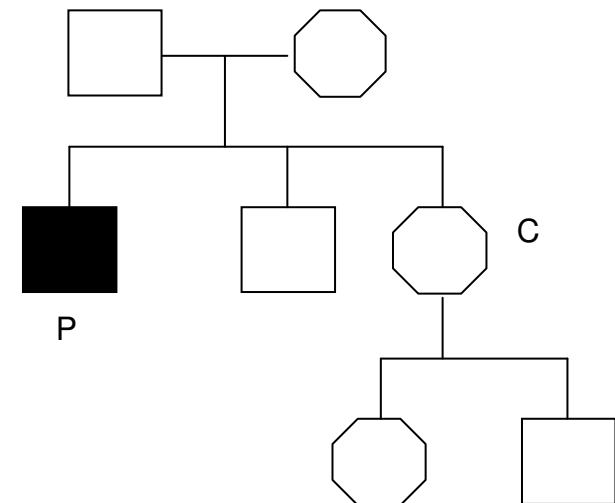
Affected Brother was clinically diagnosed as having BMD

History: 1992

Progressive proximal weakness, with hypertrophy
Onset early-mid 20s, wheelchair by 40s
SCK >3000 IU/L

Key

- Affected male
- Unaffected male
- ◻ Unaffected female
- P Proband
- C Consultand



Dr Cheryl Longman

LGMD 2I HAS AN OVERLAPPING PHENOTYPE WITH BMD - continued

ASSESSMENT OF AFFECTED BROTHER

Muscle biopsy analysis

Dystrophic biopsy - variation in dystrophin staining of individual fibres

Dystrophin immunoblot: band normal size, reduced abundance

DNA analysis

No deletion/duplication detected, point mutation testing not available at that time

Subsequent *FKRP* gene analysis

Homozygote for common mutation c.826C>A;p.Leu276Ile

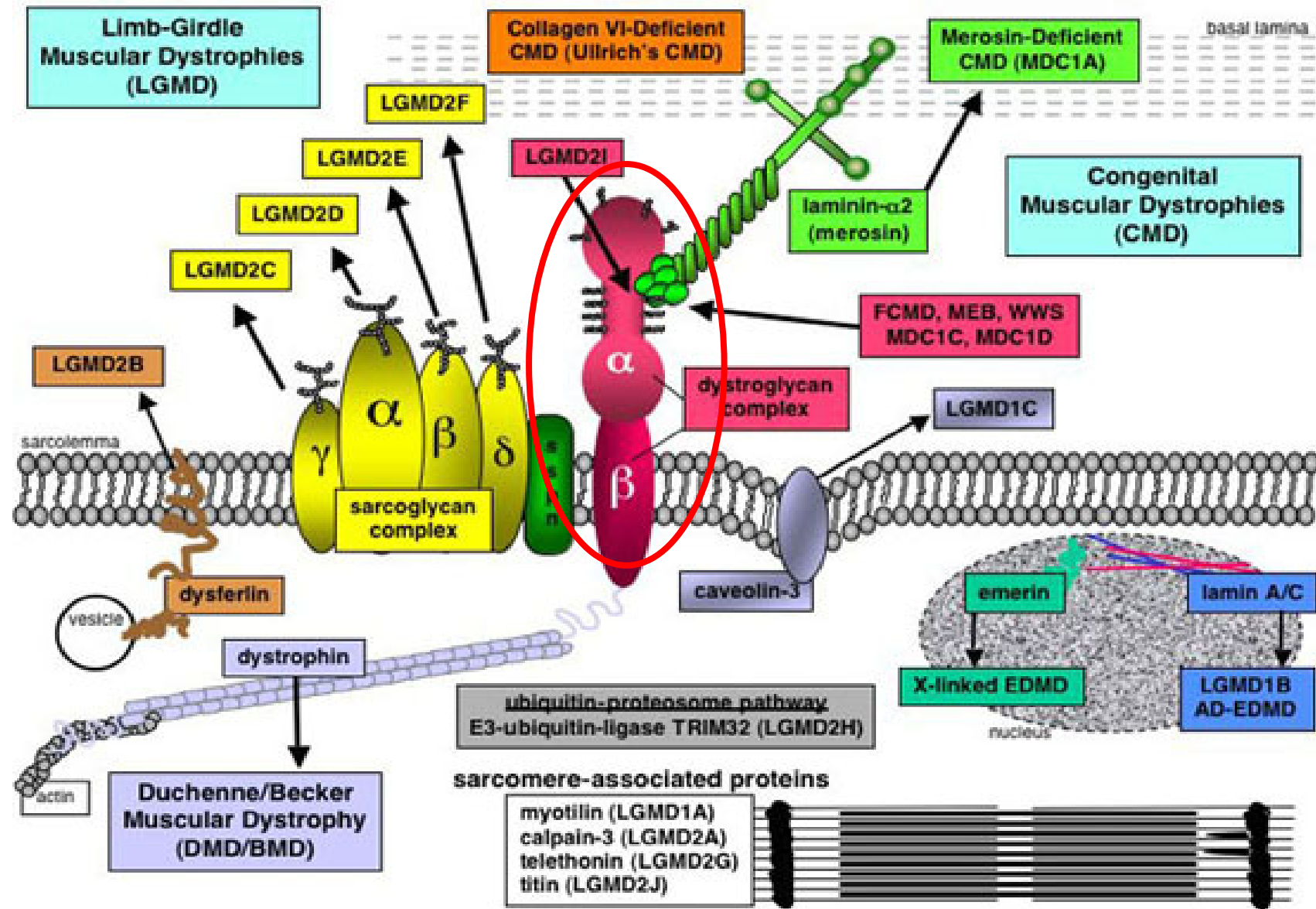
Outcome for sister

Initially given a 1 in 40 risk of being a carrier based on clinical diagnosis of BMD – this risk no longer valid.

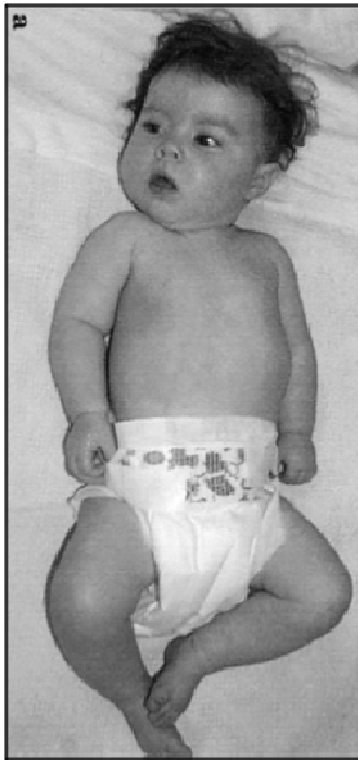
Highlights importance of distinguishing between BMD and LGMD2I

Follow up testing as appropriate

DYSTROPHIN ASSOCIATED PROTEIN COMPLEX



CONGENITAL MUSCULAR DYSTROPHY TYPE 1C (MDC1C)



Molecular cause

Also caused by mutations in the *FKRP* gene

Patients are either heterozygous for missense mutations, or compound heterozygous for missense and nonsense.

Clinical Phenotype

Severe form

Hypotonia at birth

Leg hypertrophy

Upper limb wasting

High levels of creatine kinase

Figure from Mercuri *et al.*, 2003

DEVELOPMENT OF A SERVICE

Overlap with BMD

Sporadic cases of LGMD2I – misdiagnosed as a dystrophinopathy.

Current Testing

Guy's – Congenital form

Newcastle – Adult onset

Cost

Scotland currently pays NSG, therefore it is beneficial to make this available within Scotland.

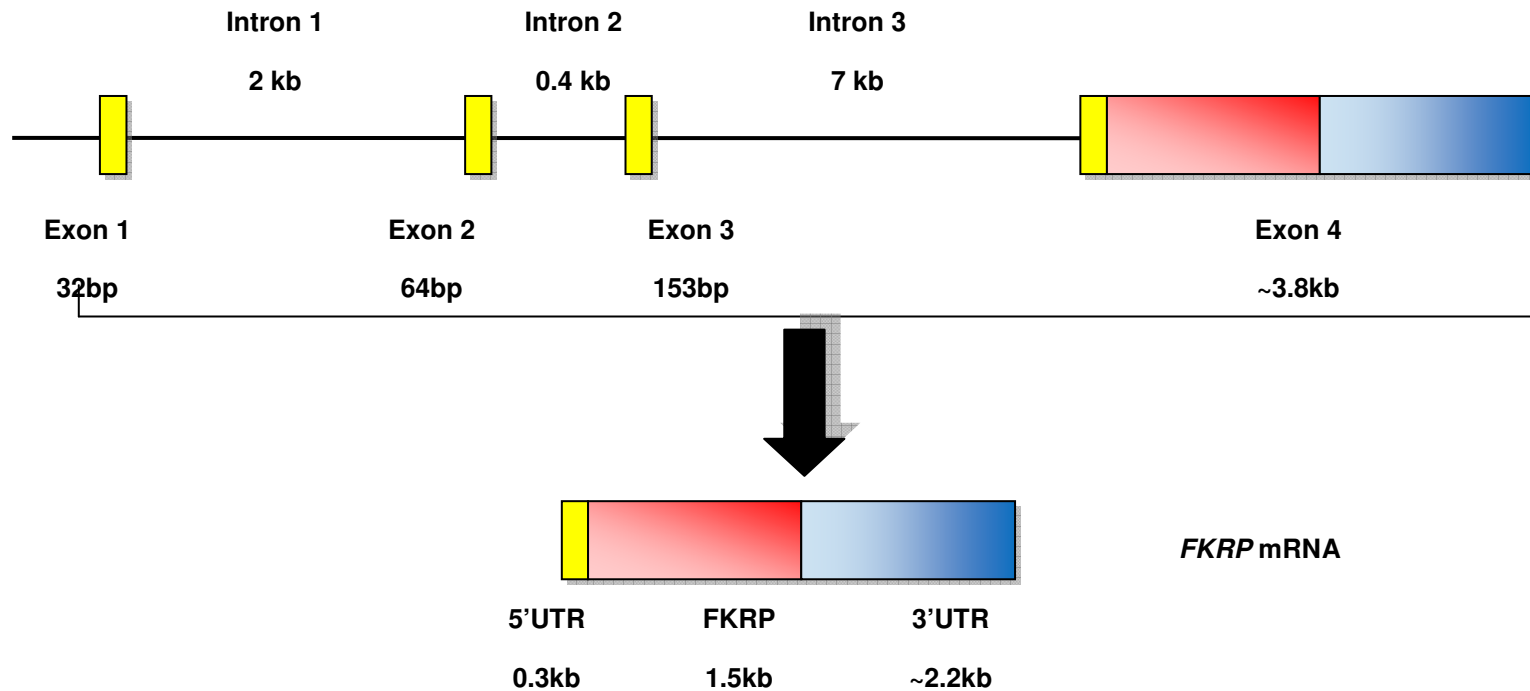
FKRP is a small gene, easy to sequence and has a quick turnaround time.

Available cohort of patients

We have a cohort of patients with a BMD-like clinical presentation who are negative for mutations in the dystrophin gene.

These patients are being reassessed by Dr Longman and if meet clinical criteria are being screened for variants within the *FKRP* gene.

FKRP GENE



4 exons

Exons 1-3 code for 5' UTR only

Exon 4 covers the remaining part of the 5' UTR, the entire protein coding region and the full 3' UTR sequence

Adapted from Brockington *et al.*, 2001

ASSAY DESIGN

Sequence

www.ensembl.org

Primer design

Five overlapping primer sets - combination of manual design and NCBI primer design tool <http://www.ncbi.nlm.nih.gov/tools/primer-blast/>

Aimed for a final product size of < 500bp
Designed so T_m for all would be 55°C

NCBI - Blast and reverse e-PCR

Indicated that primers bind only one gene and no other binding sites in genome

Secondary structures and annealing temperatures

<http://www.sigma-genosys.com/calc/DNACalc.asp>

SNP check

<http://ngri.manchester.ac.uk/SNPCheckV2/snpcheck.htm>

ASSAY METHODOLOGY

PCR / 5 amplicons



PCR template clean up
(ExoI + Shrimp Alkaline Phosphatase (Promega)
or AmPureXP (Beckman Coulter) on Biomek



Sequencing reaction using BigDye Terminators (ABI)



Sequencing product clean up
CleanSeq (Beckman coulter)



3130xl Sequencer (ABI)



Analysis using Mutation Surveyor

VALIDATION OF ASSAY

12 samples from the Glasgow lab, used as normal controls – no mutations found
9 samples from Newcastle and Guy's – all results agreed with previously reported

SAMPLE TYPE	LAB NUMBER	Variants
CONTROL		
1	D208-5144	None
2	D210-3800	Heterozygous for c.826C>A
3	D202-2418	None, but poly c.135C>T
4	D210-3802	Heterozygous for the UCV c.613C>G
5	D208-4072	None
6	D209-0873	Heterozygous for UCV c.520A>T; p.Ser174Cys
7	D205-3516	Compound heterozygote for c.826C>A; c.919T>A
8	D210-3801	Compound heterozygote for c.826C>A and the UCV c.646C>T.
9	D208-3801	Heterozygous for the UCV c.341C>G

SERVICE AVAILABLE AUGUST 2010

19 Patients tested to date

8 Normal no variants detected

9 Normal with common polymorphisms found

c.135C>T; p.(=) (3)

c.249C>T; p.(=) (2)

c.-34C>T (5)

1 Carried unclassified variant c.636G>A; p.(=)

Previously reported in Leiden – unclear pathogenicity.

Patient has no other variants.

Splice prediction software proposes no interference with splicing.

Previously reported as benign by the Newcastle laboratory.

1 Patient homozygous for c.826C>A; p.Leu276Ile

CASE STUDY PATIENT GN

Patient GN referred with a possible diagnosis of BMD

51 year old male

Initial lab referral early 2009 for dystrophin testing

Presenting with severe proximal muscle weakness

Hypertrophy of triceps (mild) and tongue (mild) and calves (mild)

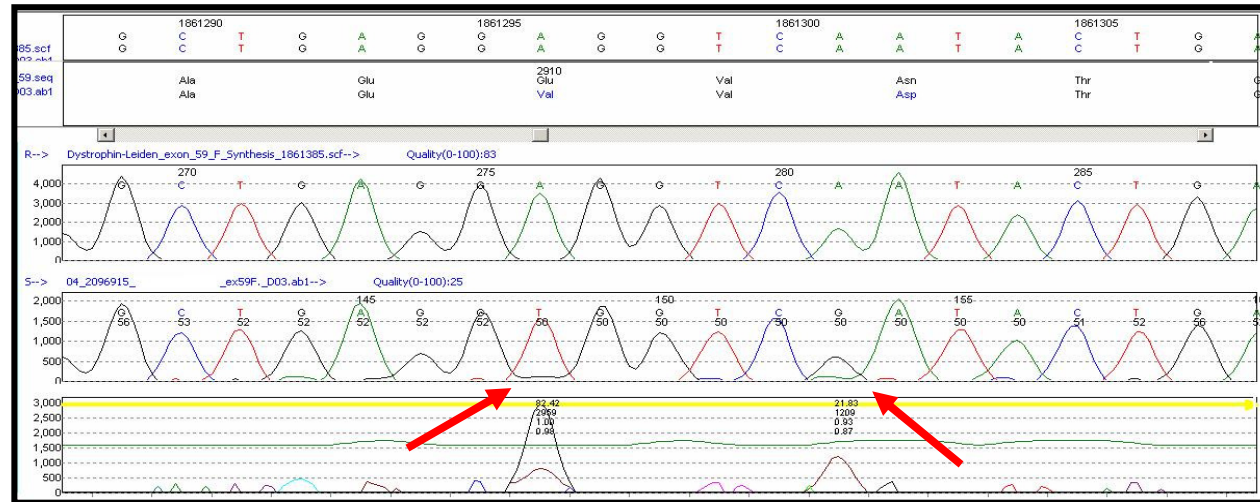
Elevated SCK > 4000

Dystrophic biopsy

Dystrophin MLPA result detected no deletions or duplications

Dystrophin point mutation analysis - identified an unclassified variant

Case study patient GN - Dystrophin analysis



Deletion-insertion variant

c.8729_8734delinsTGGTCG in exon 59. p.([Glu2910Val; Asn2912Asp]).

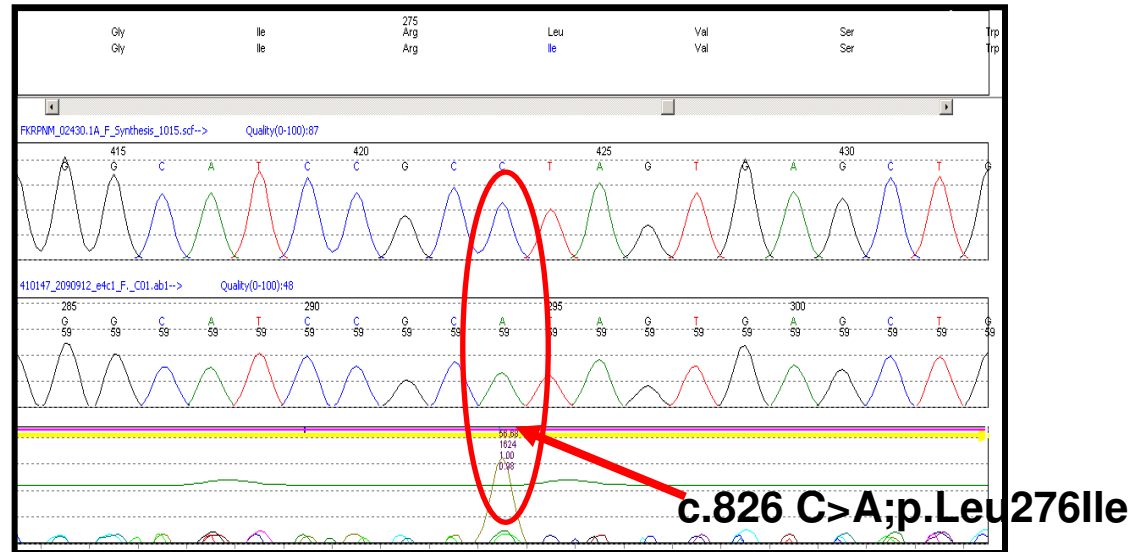
Variant reported

Previous reports in literature have indicated variant may be polymorphic. however a recent paper indicated that the variant may decrease the stability of a repeat within the rod domain of the protein (Legardinier *et al.*, 2009)

Samples requested from male family members to check pathogenicity

Therese Bradley

Case study patient GN – *FKRP* analysis



GN was recently reassessed and referred for *FKRP* mutation analysis

Patient GN was found to be homozygous for the common *FKRP* mutation and confirmed a diagnosis of LGMD2I

Can the dystrophin variant be ruled out?

This dystrophin variant has been found in one other patient (AT). AT was also tested for *FKRP* and no variants were found.

Does a combination of this dystrophin variant and *FKRP* mutation result in a more severe phenotype?

Unclear as don't know disease course of AT's muscular dystrophy compared to GN's

SUMMARY

Aim was to set up a screening service for the FKR P gene

Assay design and Validation

9 previously tested patients – all results in agreement

12 normal patients - no pathogenic variants identified

Service Available August 2010

19 patients tested

1 identified with common mutation – initially referred ? Becker

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THANK YOU
