Deciphering the genetic basis of dystroglycanopathies: the beginning of the story.
Gregor Mendel 1865.

Between 1856 and 1863 he tested some 29,000 pea plants (i.e., *Pisum sativum*).

The three laws Mendel deduced seem common-sense now, but were radically new in his day:

1. **Law of Paired Factors**: Traits come in pairs (alleles), and each parent contributes just one of the alleles.

2. **Law of Dominance**: In a pair of genes (genotype), one allele will dominate the other and control the outward appearance (phenotype).

3. **Law of Segregation**: Traits are inherited independently.
Gregor Mendel 1865. He was cited only 3 times in 35 years!!!

After Charles Darwin (On the Origin of Species, 1859).

Blending inheritance generally accepted.

Saltationism vs Gradulamism.

By 1900, research aimed at finding a successful theory of discontinuous inheritance rather than blending inheritance led to independent duplication of his work by Hugo de Vries and Carl Correns, and the rediscovery of Mendel's writings and laws.

Willian Bateson begins to report a series of breeding experiments, conducted by his pupil, Miss E.R. Saunders, using the alpine brassica Biscutella laevigata in the Cambridge botanic gardens.

Bateson was the first to suggest the word "genetics" (from the Greek gennō, γεννώ; to give birth) to describe the study of inheritance and the science of variation in a personal letter to Alan Sedgwick, dated April 18, 1905.
There are good reasons for thinking that alkaptonuria is not the manifestation of a disease but is rather of the nature of an alternative course of metabolism, harmless and usually congenital and lifelong.

Garrod identified the condition as alkaptonuria, an exceedingly rare and essentially harmless condition believed at the time to be caused by a microbe.

Garrod collected all the cases he could, mapped out pedigrees, and published a short note on it, suggesting that the high frequency within the families of his study could hardly be due to chance.

Bateson read his paper and collaborated with him.

**Alkaptonuria was the first human condition defined as a mendelian trait.**

“There are good reasons for thinking that alkaptonuria is not the manifestation of a disease but is rather of the nature of an alternative course of metabolism, harmless and usually congenital and lifelong.”
INBORN ERRORS OF METABOLISM

The Croonian Lectures delivered before the Royal College of Physicians of London, in June, 1908

By
ARCHIBALD E. GARROD
D.M., M.A. OXON.

Fellow of the Royal College of Physicians, Assistant Physician to, and Lecturer on Chemical Pathology at St. Bartholomew’s Hospital, Physician to the Hospital for Sick Children, Great Ormond Street

"ἐν τῷ τούτῳ φορέων θαρτή τι βαμματίν." Aristotle, Ἡπὶ δύον μαθήματι, i. 5.

LONDON
HENRY FROWDE HODDER & STOUGHTON
OXFORD UNIVERSITY PRESS 20, WARWICK SQUARE, E.C.
1909

Garrod’s Tetrad:

- Alkaptonuria
- Cystinuria
- Pentosuria
- Albinism
Garrod. Human genetics.

Recessive inheritance

Carrier Father
Nn

Carrier Mother
Nn

NN NORMAL Nn CARRIER Nn CARRIER nn AFFECTED

Dominant inheritance

Affected Father
Dd

Normal Mother
DD

Dd dd Dd dd

Dd affected dd normal Dd affected dd normal

One congenital syndrome
One gene
One allele (recessive)
Human Molecular Genetics.

Timeline | DNA milestones

- Heritable material found to be DNA
- Semi-conservative DNA replication shown
- First genome sequenced (FX174)
- Successful gene therapy
- FlavrSavr tomato commercially available
- Draft human genome released

- 1944
- 1953
- 1958
- 1966
- 1977
- 1980
- 1983
- 1990
- 1995
- 1994
- 1996
- 2001

- DNA double helix structure described
- Genetic code cracked
- Sequencing methods published
- PCR developed
- Haemophilus influenzae sequenced
- ‘Dolly’ cloned

Walker 1942 WWS
Chung and Morton 1959 LGMD
Fukuyama 1960 FCMD
Raitta et al 1978 MEB
Warburg 1978 WWS
Congenital Muscular Dystrophies

One gene, one syndrome dogma

Congenital muscular dystrophy

Normal CNS

Abnormal CNS

- white matter
- polymicrogyria
- pachygyria
- hypo. cerebellum
- agyria
- hydrocephalus

Abnormal eye

- myopia
- retinal dysplasia

MDC1C/1D | Fukuyama CMD (FCMD) | muscle-eye-brain (MEB) | Walker-Warburg (WWS)

Tobias Willer (adapted from Yoshioka et al, 1997)
Mutations identification.

How do we identify the mutations?

(e) Variable-length strands

(f) Complementary to primer 2

(g) Desired fragments (variable-length strands not shown)

Separate strands and anneal primers

Complementary to primer 1

Extend primers

And so on

Control

LD122-4
The haploid human genome is 3.2 billion base pairs long and contains about 23,000 distinct protein-coding genes.

Only 1.5% of human genome codes for proteins, the rest consists of:

- non-coding RNA genes,
- regulatory sequences,
- introns,
- and noncoding DNA (once known as "junk DNA").
Causative Gene Identification.

Candidate gene approach

FKRP
POMGnT1
POMT1
LARGE
POMT2
dystroglycan

Identification of the Human Gene Mutated

Fukutin
POMGnT1

Map the region that contains it

Clinical severity

Limb-Girdle MD
LGMD

MDC1C/1D

Fukuyama CMD
FCMD

Muscle-Eye-Brain
MEB

Walker-Warburg
WWS
Recombination and positional cloning.

But.... how do we find them????

---

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Recombination.

But.... how do we find them????

Let's play a game:

• Consanguineous family
• Recombination
• Positional cloning
• Polymorphisms

Crossing-over and recombination during meiosis
Positional cloning.
Positional cloning.

They are polymorphisms
Positional cloning.

BBS11 pedigree and shared haplotype.

Chiang A P et al. PNAS 2006;103:6287-6292
FCMD and Fukutin

More than 500 genes

Localization of a gene for Fukuyama type congenital muscular dystrophy to chromosome 9q31–33


Linkage-Disequilibrium Mapping Narrows the Fukuyama-Type Congenital Muscular Dystrophy (FCMD) Candidate Region to <100 kb

Tatsushi Toda, Masashi Miyake, Kazuhiro Kobayashi, Kunihiro Mizuno, Kayoko Saito, Makiko Osawa, Yusuke Nakamura, Ichiro Kanazawa, Yasuo Nakagome, Katsushi Tokunaga, and Yutaka Nakahori

Departments of 1Human Genetics and 2Neurology, University of Tokyo, and 3Laboratory of Molecular Medicine, Institute of Medical Science, University of Tokyo, and 4Department of Pediatrics, Tokyo Women’s Medical College, Tokyo

Fig. 2 Pedigrees of 21 FCMD families showing disease status and segregation of the two closest markers, D9S508 (top) and D9S509 (bottom). Alleles for D9S509 are: A, 147 bp; B, 145 bp; C, 142 bp; D, 137 bp; E, 135 bp; F, 133 bp; G, 131 bp; H, 126 bp; I, 121 bp; J, 119 bp; K, 115 bp; L, 111 bp; M, 110 bp; N, 109 bp; O, 108 bp. Alleles for D9S509 are: 1, 116 bp; 2, 114 bp; 3, 112 bp; 4, 110 bp.

Solid symbols: affected subjects; slash, subject deceased; “,” Second-cousin marriage; A, first and half-cousin marriage; other consanguineous marriages are first-cousins.
An ancient retrotransposonal insertion causes Fukuyama-type congenital muscular dystrophy

Kazuhiro Kobayashi, Yutaka Nakahori, Masashi Miyake, Kichiro Matsumura, Eri Kondo-Iida, Yoshiko Nomura, Masaya Segawa, Mieko Yoshioka, Kayoko Saito, Makiko Osawa, Kenzo Hamano, Youichi Sakakihara, Ikuya Nonaka, Yasuo Nakagome, Ichiro Kanazawa, Yusuke Nakamura, Katsushi Tokunaga & Tatsushi Toda

Causative Gene Identification.

Candidate gene approach → Identification of the Human Gene Mutated → Fukutin

Map the region that contains it

Fukutin

Limb-Girdle MD LGMD
MDC1C/1D
Fukuyama CMD FCMD
Muscle-Eye-Brain MEB
Walker-Warburg WWS

clinical severity
FKRP and CMD1D/LGMD

Mutations in the Fukutin-Related Protein Gene (FKRP) Cause a Form of Congenital Muscular Dystrophy with Secondary Laminin α2 Deficiency and Abnormal Glycosylation of α-Dystroglycan

ARTICLE

Mutations in the fukutin-related protein gene (FKRP) identify limb girdle muscular dystrophy 2l as a milder allelic variant of congenital muscular dystrophy MDC1C

Martin Brookington, Yeliz Yuva, Paola Prandini, Susan C. Brown, Silvia Torelli, Matthew A. Benson, Chris P. Ponting, Brigite Estournet, Norma B. Romero, Eugenio Mercuri, Thomas Voit, Caroline A. Sewry, Pascale Guicheney, and Francesco Muntoni

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Chromosome 18

Dendy NT 01116

Intron 1

Exon 1

2.3kb

Exon 2

644p

Exon 3

156p

Intron 3

1.3kb

Intron 4

1.2kb

FKRP mRNA

5’UTR

FKRP

3’UTR

a

C1154A

A826G

A826G

C1154A

b

Wild Type

Patient (Heterozygote)

A826G

C1154A

Tyr269Cys

Ser385Stop

Family 1

Family 2

Family 3

Family 4

Family 5

Family 6

Family 7

Family 8

Family 9

Family 10

Family 11

Family 12

Family 13

Family 14
Causative Gene Identification.

Candidate gene approach
FKRP
Identification of the Human Gene Mutated
Fukutin
Map the region that contains it

FKRP
Limb-Girdle MD LGMD
MDC1C/1D
Fukuyama CMD FCMD
Muscle-Eye-Brain MEB
Walker-Warburg WWS

clinical severity
Clinical and genetic distinction between Walker-Warburg syndrome and muscle-eye-brain disease

B. Cormand, PhD; H. Phihko, MD, PhD; M. Bayés, PhD; L. Valanne, MD, PhD; P. Sentavuo, MD, PhD; B. Talim, MD; R. Gersheni-Boruch, MD; A. Ahmad, MD; H. van Bokhoven, PhD; H.G. Brunner, MD, PhD; T. Voit, MD; H. Topaloglu, MD; W.H. Dobyns, MD; and A.-E. Labosaki, MD, PhD
**Muscular Dystrophy and Neuronal Migration Disorder Caused by Mutations in a Glycosyltransferase, POMGnT1**

**Introduction**

Since the discovery of the Duchenne muscular dystrophy gene product dystrophin (Hoffman et al., 1987), many studies have focused on understanding the pathophysiology of muscular dystrophies and on developing therapeutic approaches. Dystroglycan is a component of the dystrophin-glycoprotein-complex (DGC) in skele-
Causative Gene Identification.

One Gene – One Syndrome Dogma
One Syndrome – One Gene Dogma

Candidate gene approach

Identification of the Human Gene Mutated

Map the region that contains it

FKRP
POMGnT1

Fukutin
POMGnT1

Clinical severity

FKRP
Limb-Girdle MD
LGMD
MDC1C/1D
Fukuyama CMD
FCMD
Muscle-Eye-Brain
MEB
Walker-Warburg
WWS
Mutations in the O-Mannosyltransferase Gene *POMT1* Give Rise to the Severe Neuronal Migration Disorder Walker-Warburg Syndrome

Daniel Beltrán-Valero de Bernabé, Sophie Currier, Alice Steinbrecher, Jacopo Celli, Ellen van Beusekom, Bert van der Zwaag, Hülya Kayserili, Luciano Merlini, David Chitayat, William B. Dobyns, Bru Comand, Ana-Elina Lehesjoki, Jesús Cruces, Thomas Voit, Christopher A. Walsh, Hans van Bokhoven, and Han G. Brunner

10 consanguineous families tested
More than one locus
More than 2 loci
Candidate gene approach
Causative Gene Identification.

One Syndrome - One Gene Dogma → WRONG!!
One Gene - One Syndrome Dogma ??

Candidate gene approach
FKRP
POMGnT1
POMT1
Identification of the Human Gene Mutated
Fukutin
POMGnT1
Map the region that contains it

FKRP
Limb-Girdle MD
LGMD
MDC1C/1D
Fukuyama CMD
FCMD
POMGnT1
Muscle-Eye-Brain
MEB
POMT1
Walker-Warburg
WWWS
clinical severity
POMT2 and WWS

ORIGINAL ARTICLE

POMT2 mutations cause α-dystroglycan hypoglycosylation and Walker-Warburg syndrome

J van Reeuwijk, M Janssen, C van den Elzen, D Beltran-Valero de Bernabé, P Sabatelli, L Merlini, M Boon, H Scheffer, M Brockington, F Muntoni, M A Huynen, A Verrips, C A Walsh, P G Barth, H G Brunner, H van Bokhoven

Causative Gene Identification.

Candidate gene approach

FKRP
POMGnT1
POMT1
POMT2

Identification of the Human Gene Mutated

Map the region that contains it

Fukutin
POMGnT1

Clinical severity

FKRP
Limb-Girdle MD
LGMD

MDC1C/1D

Fukuyama CMD
FCMD

POMGnT1
Muscle-Eye-Brain
MEB

POMT1
POMT2
Walker-Warburg
WWS

clinical severity
Mutant glycosyltransferase and altered glycosylation of α-dystroglycan in the myodystrophy mouse

Prabhit K. Grewal1, Paul J. Holzfeind2, Reginald E. Bettrn3 & Jane E. Hewitt4

Mutations in the human LARGE gene cause MDC1D, a novel form of congenital muscular dystrophy with severe mental retardation and abnormal glycosylation of α-dystroglycan

Cheryl Longman5, Martin Brockington5, Silvia Torelli1, Cecilia Jimenez-Mallebrera5, Colin Kennedy5, Nofal Khalil6, Lucy Feng5, Ravindra K. Saran5,8, Thomas Voit8, Luciano Merlini9, Caroline A. Sewry17, Susan C. Brown1 and Francesco Muntoni1,6
Causative Gene Identification.

Candidate gene approach

Identification of the Human Gene Mutated

Map the region that contains it

FKRP
POMGnT1
POMT1
POMT2
LARGE

Fukutin
POMGnT1

clinical severity

Limb-Girdle MD
LGMD

MDC1C/1D

Fukuyama CMD
FCMD

Muscle-Eye-Brain
MEB

Walker-Warburg
WWS

POMT1
POMT2
The common link. Dystroglycan glycosylation.

Post-translational disruption of dystroglycan–ligand interactions in congenital muscular dystrophies

Daniel E. Michele*, Rita Barresi*, Motoi Kanagawa*, Fumaki Saito*, Ronald D. Cohn*, Jakob S. Satz*, James Dollar†, Ichizo Nishino‡, Richard I. Kelley§, Hannu Somer¶, Volker Straub**, Katherine D. Mathews†, Steven A. Moore# & Kevin P. Campbell*
Dystroglycan function

Extracellular matrix

Plasma membrane (Sarcolemma)

myofibril

Plasma membrane (Sarcolemma)
Loss of $\alpha$-Dystroglycan functional glycosylation results in congenital muscular dystrophy

Normal Walker-Warburg syndrome (WWS)
Muscle-eye-brain disease (MEB)
Fukuyama congenital muscular dystrophy (FCMD)
MDC1C/1D
Limb girdle muscular dystrophy (LGMD)2I/2K/2M/2N

Large<sup>myd</sup> mouse

Figure generated by Tobias Willer
Dystroglycan and LGMD

A Dystroglycan Mutation Associated with Limb-Girdle Muscular Dystrophy

Yuji Hara, Ph.D., Burcu Balci-Hayta, Ph.D., Takako Yoshida-Moriguchi, Ph.D., Motoi Kanagawa, Ph.D., Daniel Beltrán-Valero de Bernabé, Ph.D., Hulya Güneşli, M.S., Tobias Willer, Ph.D., Jakob S. Satz, Ph.D., Robert W. Crawford, B.S., Steven J. Burden, Ph.D., Stefan Kunz, Ph.D., Michael B.A. Oldstone, M.D., Ph.D., Alessio Accardi, Ph.D., Beril Talim, M.D., Francesco Muntoni, M.D., Haluk Topaloğlu, M.D., Pervin Dinçer, Ph.D., and Kevin P. Campbell, Ph.D.

Causative Gene Identification.

Candidate gene approach

Identification of the Human Gene Mutated

Map the region that contains it

FKRP
POMGnT1
POMT1
POMT2
LARGE dystroglycan

Fukutin
POMGnT1

Clinical severity

FKRP dystroglycan

LARGE

Fukuyama CMD
FCMD

Muscle-Eye-Brain
MEB

Walker-Warburg
WWS

Limb-Girdle MD
LGMD

MDC1C/1D

POMT1
POMT2
Phenotype / Genotype spectrum in Dystroglycanopathy patients

**One Syndrome - One Gene Dogma → WRONG!!**
**One Gene - One Syndrome Dogma ??**

**LETTER TO JMG**

A homozygous nonsense mutation in the *Fukutin* gene causes a Walker-Warburg syndrome phenotype

D Beltrán-Valero de Bernabé, H van Bokhoven, E van Beusekom, W Van den Akker, S Kant, W B Dobyns, B Cormand, S Currier, B Hamel, B Talim, H Topaloglu, H G Brunner


**ORIGINAL INVESTIGATION**

Intragenic deletion in the *LARGE* gene causes Walker-Warburg syndrome

Jeroen van Reeuwijk · Prabhjit K. Grewal · Mustafa A. M. Salih · Daniel Beltrán-Valero de Bernabé · Jenny M. McLaughlan · Caroline B. Michielse · Ralf Herrmann · Jane E. Hewitt · Alice Steinbrecher · Mohamed Z. Sedahmed · Mohamed M. Shaheed · Abdullah Abomelha · Han G. Brunner · Hans van Bokhoven · Thomas Voit

**ELECTRONIC LETTER**

Mutations in the FKRP gene can cause muscle-eye-brain disease and Walker-Warburg syndrome


Phenotype / Genotype spectrum in Dystroglycanopathy patients

**One Syndrome - One Gene Dogma → WRONG!!**
**One Gene - One Syndrome Dogma → WRONG!!**

<table>
<thead>
<tr>
<th>LGMD</th>
<th>CMD +/- brain involvement</th>
<th>muscle-eye-brain (MEB)</th>
<th>Walker-Warburg (WWS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POMT1/2 (LGMD2K)</td>
<td>POMT1/2</td>
<td>POMT1/2</td>
<td>POMT1/2</td>
</tr>
<tr>
<td>POMGnT1 (LGMD2M)</td>
<td>POMGnT1</td>
<td>POMGnT1</td>
<td>?</td>
</tr>
<tr>
<td>Fukutin (LGMD2L)</td>
<td>Fukutin</td>
<td>Fukutin</td>
<td>Fukutin</td>
</tr>
<tr>
<td>FKRP (LGMD2I)</td>
<td>FKRP</td>
<td>FKRP</td>
<td>FKRP</td>
</tr>
<tr>
<td>LARGE</td>
<td>LARGE</td>
<td>LARGE</td>
<td></td>
</tr>
</tbody>
</table>

**mild missense mutations**

**LGMD**

**severe missense and nonsense mutations**

**WWS**

Figure generated by Tobias Willer
Walker-Warburg syndrome genes

6 known genes causing WWS:

- **POMT1** (9q34.1)
- **POMT2** (14q24.3)
- **POMGnT1** (1p34.1)
- **FKRP** (19q13.32)
- **Fukutin** (9q31)
- **LARGE1** (22q12.3)
- **dystroglycan** (3p21.31)
- **ISPD** (7p21.2)

Until 2012 only 50% of dystroglycanopathy patients can be explained with known genes and can be provided with genetic diagnosis. Preliminary linkage data suggest ~ 5 additional candidate genes that still remain unidentified.

Figure generated by Tobias Willer
## Classification of Congenital Muscular Dystrophy with Glycosylation Defects

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Motor Function</th>
<th>Eye</th>
<th>Central Nervous System</th>
<th>Intellectual Disability / Epilepsy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Walker-Warburg syndrome (WWS)</strong></td>
<td>Absent</td>
<td>Severe ¹</td>
<td>Cobblestone lissencephaly</td>
<td>Severe</td>
</tr>
<tr>
<td><strong>Muscle-eye-brain (MEB) disease</strong></td>
<td>Ambulation may be acquired</td>
<td>Common ²</td>
<td>Frontoparietal pachygyria; polymicrogyria</td>
<td>Common</td>
</tr>
<tr>
<td><strong>Fukuyama CMD (FCMD)</strong></td>
<td>Ambulation may be acquired</td>
<td>Variable / mild</td>
<td>Variable (from normal or only simplification of gyri to severe)</td>
<td>Hypoplasia, cysts, polymicrogyria</td>
</tr>
<tr>
<td><strong>Intermediate phenotypes (MDC1D, CRB-CMD)</strong></td>
<td>Ambulation may be acquired</td>
<td>Rare / mild</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td><strong>CMD with intellectual disability</strong></td>
<td>Ambulation may be acquired</td>
<td>Rare / mild</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td><strong>CMD no intellectual disability</strong></td>
<td>Ambulation may be acquired</td>
<td>None / mild</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

From Sparks et al. Gene Reviews 2012