Undiagnosed and not diagnosed: a genetic view

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Alessandra Ferlini Disclosure

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Biomarin, Sarepta, McKimcrodt))

Member of Scientific Board of
PTC Therapeutics USA
Sarepta Inc USA

Honorary Visiting Professor
University College London (UK)
Undiagnosed diseases (UDN)

**DEFINITION**

“longstanding medical condition that eludes diagnosis by a referring physician can be considered undiagnosed and may be of interest to this clinical research program” (UDN International)

**CONCEPTS**

The concept of “diagnosis”
- clinical diagnosis (phenotype-phenomics)
- genetic diagnosis (genotype)
STATE OF THE ART

New NEXT GENERATION SEQUENCING strategies

WHOLE EXOME SEQUENCING
WHOLE GENOME SEQUENCING
GENE PANELS ANALYSIS

BOTTLENECKS

incomplete accuracy for

• copy number variations
• dynamic mutations
• epigenetics changes
Introducing a genetic view: how to categorize UDNs

**PHENOTYPE DISCOVERY**

- **KNOWN** PHENOTYPE ASSOCIATED WITH **KNOWN DISEASE GENE** *(diagnostics failure)*
  
  (A) The case of Calpain-3 gene

- **NEW** PHENOTYPE ASSOCIATED WITH **KNOWN DISEASE GENE** *(phenotype discovery)*
  
  (B) The case of VERSICAN gene

**GENE DISCOVERY**

- **NOVEL** DISEASE GENE CAUSING **KNOWN PHENOTYPE** *(gene discovery)*
  
  (C) The case of POPDC1 gene

- **NOVEL** DISEASE GENE CAUSING **NOVEL PHENOTYPE** *(disease discovery)*
  
  (D) The case of MSTO gene
CASE (A)

A KNOWN GENE CAUSING A KNOWN PHENOTYPE CALPAIN 3

#253600 MUSCULAR DYSTROPHY, PELVOFEMORAL LEYDEN-MOEBIUS MUSCULAR DYSTROPHY CALPAINOPATHY

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<th>Phenotype mapping key</th>
<th>Gene/Locus</th>
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**Family-of-4 with Limb-girdle muscular dystrophy**

Female aged 48 yrs (d.o.b.: 1968)
Onset 8-9 yrs with difficulty walking and climbing stairs, slowly progressive, wheelchair bound at the age of about 20 yrs.
The muscle biopsy showed a dystrophic picture.
At present: mild weakness of the neck flexors and extensors muscles, weakness of the deltoid, supraspinatus and infraspinatus. Weakness of quadriceps, iliopsoas and femoral adductor.
The distal muscles of the limbs are less involved.
CPK: 660 U/L
ECG: normal
Normal spirometry

Male aged 41 yrs (d.o.b.: 1974)
Same clinical picture as the sister, slightly less severe disease progress.
Wheelchair bound

Previous Gene panel testing negative
TOTAL SNPs 860
Prioritization for variants IN COMPOUND HETEROZYGOSIS

HETEROZYGOUS VARIATIONS
807

HET. VARIATIONS PRESENT IN AFFECTED SISTER
416

FOR AT LEAST 2 VARIATIONS IN SAME GENE
127

COMMON VARIATIONS WITH AFFECTED SISTER
122

HETERO VARIAT FILTERED FOR CAT.1 AND 2
9

WGS ANALYSIS
Exons
Splice boundaries
5’ and 3’ UTRs
9 HETEROZYGOUS VARIATION FILTERED

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Gene-Phenotype Relationships

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<td>3</td>
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CAPN3 c.328C>T p.R110*, stop gained with Clinical significance Pathogenic (already described)
CAPN3 c.2115+2T>A, splice_donor_variant (not described)
VALIDATED

Diagnosis LGMD2A

Exon 2  c.328C>T, p. Arg110*

Exon 19 c.2115+2T>A

Exon 2  c.328C>T, p. Arg110*

Exon 19 c.2115+2T>A
CASE (B)

A KNOWN GENE CAUSING A NEW PHENOTYPE
Versican (VCAN)

# 143200
WAGNER VITREORETINOPATHY; WGVRP
Alternative titles; symbols
EROSIVE VITREORETINOPATHY; ERVR
WAGNER VITREORETINAL DEGENERATION
HYALOIDEORETINAL DEGENERATION OF WAGNER
WAGNER SYNDROME 1; WGN1

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Fam 76 CONSANGUINEOUS FAMILY FROM PAKISTAN
TEETH ABNORMALITIES AND DYSMORPHIC FEATURES IN TWO SIBLINGS

impaired tooth and periodontium development
• Loss of the maxillary central incisors
• Dental arches show a cutting-edge profile of the crest in the edentulous regions
• The root complex of exfoliated teeth is almost completely absent
FILTERS:

- **Homozygous** on patients (CONSANGUINITY), heterozygous on parents
- Exclusion of SNPs present on dbSNP132/1000 genomes/hapmap/YH project/BGI Db, Sanger Centre (UK), with frequency greater than 0.5%
- Non synonymous mutations/5’-3’ UTR, splice site variations and small indels <20bp

VCAN gene, homozygous missense mutation in exon 8 (c.7994A>T; p.His2665Leu; chromosome 5: 82836816; GRCh37 / hg19) (NM_004385.4)
VCAN gene, homozygous missense mutation in exon 8 (c.7994A>T; p.His2665Leu; chromosome 5: 82836816; GRCh37 / hg19) (NM_004385.4)
VERSICAN (VCAN) His2665 amino acid IS EXTREMELY CONSERVED AMONG SPECIES AND IS LOCATED IN THE PROTEIN C-TERMINUS DOMAIN.

VERSICAN PROTEIN FUNCTIONS

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VERSICAN: ROLE IN RAT TOOTH DEVELOPMENT SUPPORTS ITS PATHOGENIC ROLE in our PATIENTS

Expression of versican and ADAMTS during rat tooth eruption

Shinya Sone1,2, Megumi Nakamura2, Yuriko Maraya1, Ichiro Takahashi1, Ikuo Mizoguchi2, Hideo Mayanagi1 & Yasuyuki Saano1
1Division of Pediatric Dentistry, Graduate School of Dentistry, Tohoku University, Sendai, 980-8575, Japan
2Division of Craniofacial Development and Regeneration, Graduate School of Dentistry, Tohoku University, Sendai, 980-8575, Japan

VERSICAN EXPRESSION IN RAT TOOTH

IN SITU HYBRIDIZATION OF VCAN AND ADAMTS1 IN ODONTOBLASTS

VCAN IS PRESENT ALSO IN CEMENTUM, ALVEOLAR BONE AND DENTAL PULP

VERSICAN: HIGHLY EXPRESSED IN RAT TOOTH
VERSICAN IS A KNOWN GENE DISEASE

# 143200 5q14.2-q14.3 Wagner syndrome 1 143200 AD 3 VCAN 118661

WAGNER VITREORETINOPATHY; WGVRP
Alternative titles; symbols
EROSIVE VITREORETINOPATHY; ERVR WAGNER VITREORETINAL DEGENERATION HYALOIDEORETINAL DEGENERATION OF WAGNER WAGNER SYNDROME 1; WGN1

5 FAMILIES DESCRIBED
SPlice SITE MUTATIONS ONLY OCCUR

DOMINANT TRANSMISSION PEDIGREE WAGNER
CONCLUSIONS: Reduced visual acuity in both eyes EARLY SIGNS OF VITREOPATHY AND RETINOPATHY, compatible with a pauci-asymptomatic stage of WAGNER DISEASE

Bilaterally minimal peripheral vitreous condensation

(optical coherence tomography (OCT), hypereflective inner limiting membrane with hypereflective foci within the ganglion cell layer.

Visual field test showed bilaterally several points of reduced retinal sensitivity.

CONCLUSIONS: Reduced visual acuity in both eyes EARLY SIGNS OF VITREOPATHY AND RETINOPATHY, compatible with a pauci-asymptomatic stage of WAGNER DISEASE
CASE (C)

A NEW GENE CAUSING A KNOWN PHENOTYPE

BVES (POPDC1)

# 616812

MUSCULAR DYSTROPHY, LIMB-GIRDLE, TYPE 2X; LGMD2X

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FAMILY OF FOUR WITH LGMD AND AV BLOCK WES STRATEGY

GENETICS
- Autosomal recessive inheritance
- Pseudodominant pedigree (Albanian community in Southern Italy)
- PHENOTYPE
  - Limb Girdle Muscular dystrophy (LGMD)
  - Late onset (3-4th decade)
  - Mild muscle phenotype (predominantly lower limbs)
  - Severe AV block (requiring pacing)
  - High CK (up to 7000 IU/L)

variant prioritization:
- recessive model
- mutation effect (missense, nonsense, splice site)
  - population frequency < 0.01%
  - pathogenicity prediction
  - segregation analysis in the family
- **Homozygosity** on patients (4, 5), heterozygosity on parents (2, 3) according to RECESSIVE MODEL
- SNP and indel calling
- Screening for mutations in coding regions, UTR and splice sites
- Exclusion of SNPs present on dbSNP132/1000 genomes/hapmap/YH project with frequency greater than 0.05%
- HMM prediction
- Aminoacid substitution prediction with SIFT
- Annotating candidate variations with GO and KEGG databases

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**STRONGEST CANDIDATE : BVES (POPDC1)**
THE MISSENSE S201F MUTATION IS PREDICTED TO BE PATHOGENIC

located in a functional domain

protein model predicting impaired cAMP binding
Cardiac arrhythmia in popdc1 zebrafish morphants
FUNCTIONAL ASSAYS PERFORMED

- Null POPDC1 mice: described
- Immunohistochemistry and western blot in patients’ muscle
- Ligand precipitation assay testing cAMP binding capacity
- FRET current measurement to test the effect on cAMP
- HL cells transfection with POPDC1\(^{S201F}\) and patch-clamp polarization measurement
- Xenopus Oocytes POPDC1-TREK-1 interaction studies
  - Zebralish morphant for POPDC1 silencing
  - Zebralish TALEN gene editing with POPDC1\(^{S201F}\)

New type of LGMD: LGMD2X
CASE (D)

A NEW GENE CAUSING A NEW PHENOTYPE

Misato 1 (MSTO1)

OMIM# 617675
MYOPATHY, MITOCHONDRIAL, AND ATAXIA; MMYAT

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Brain MRI (hypotrophy of cerebellar vermis, enlarged cisterna magna, hyperintense signals in periventricular white matter.)

MAIN CLINICAL FEATURES
- congenital MYOPATHY
- growth and motor delay
- cerebellar hypotrophy and ATAXIA
- mental retardation
- SCOLIOSIS
- dystrophic features on muscle biopsy and high CK
NOVEL GENE AND NOVEL PHENOTYPE DISCOVERY THE MSTO GENE OMIM 617619

MUSCLE BIOPSY showing dystrophic changes, patchy fatty infiltration, fibrosis, increased variation in fibre size, empty vacuoles

Ultrastructural examination: AGGREGATES OF MITOCHONDRIA with vacuolar degeneration

R345C/F376L VARIANTS
HIGH INTER-SPECIES CONSERVATION
NOT REPORTED IN ALL GENOMIC VARIANTS DATABASES

T324I/c.996+1G>A VARIANTS
HIGH INTER-SPECIES CONSERVATION
NOT REPORTED IN ALL GENOMIC VARIANTS DATABASES
MSTO1 GENE IS A NEW GENE DISEASE CAUSING A NOVEL PHENOTYPE with MULTISYSTEMIC DISEASE DUE TO ABNORMAL MITOCHONDRIAL NETWORK

MISATO (MSTO) gene
DROSOPHILA MELANOGASTER homologous region
GTPase family
ubiquitously distributed
Localized in mitochondria

MITOCHONDRIAL MORPHOLOGY (obtained with MitoTracker red):

FILAMENTOUS mitochondrial network of fibroblasts from a control (CT)
FRAGMENTED network in patients’ cells, (family A) grown in glucose medium

NEW GENE, NEW DISEASE MMYAT
UNIFE OVERVIEW OF GENE/PHENOTYPE DISCOVERY STUDIES by WHOLE SEQUENCING (exome or genome)

PROJECT
Next-generation sequencing and gene therapy to diagnose and cure rare diseases in Regione Emilia Romagna (RER)

N=18

PROJECT
An Integrated European-Omics research project for the diagnosis and therapy in rare neuromuscular and neurodegenerative diseases

N=12

(N=1)

( Francesca Brancati L’Aquila, Italy)
• SUMMARY OF RESULTS, WES OR WGS ANALYSIS

• CASE A (KNOWN PHENO-KNOWN GENE) N= 7
• CASE B (NEW PHENO-KNOWN GENE) N=4
• CASE C (NEW GENE-KNOWN PHENO) N=2
• CASE D (NEW GENE-NEW PHENO) N=4 NEW DISEASE
• CASE E DIGENIC INHERITANCE N=2

TOTAL SOLVED 19 (59%)

STILL UNSOLVED 12 (41%)

NEW EU H2020 PROJECT: Solving the unsolved
**REFLECTIONS**

- extensively exploring all mutation types in known genes, reaching accuracy close to 95%, as for a diagnostic test
- we need implementation for CNVs identification
- designing exhaustive strategies to identify new genes (functional studies)

**AVENUES**

- Family analysis
- patients cohorts with very homogenous phenotype grouped and studies
- others?

**Challenges**

- ChiP Sequencing for detecting epigenetic causes
- RNA sequencing (appropriate source of RNA)
- Digenic inheritance or modifiers
UNIFE RESEARCH GROUP

• Marcella Neri
• Rachele Rossi
• Maria Sofia Falzarano
• Francesca Gualandi
• Rita Selvatici
• Alessandra Ferlini

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• Francesco Muntoni (UCL) London
• Eugenio Mercuri (Gemelli) Roma
• Massimo Zeviani, (MRC) Cambridge
• Davide Ghezzi (Besta) Milano