Personalized approaches to decode the genetic complexity of “simple” neurological disorders

Enza Maria Valente

University of Salerno
CSS-Mendel Institute, Rome
Mendelian Diseases:
conditions caused by impairment in a single defective gene

Gene mutations are inherited following mendelian laws:

also known as:
“monogenic (single gene) disorders”
“simple”
Are Mendelian disorders really «simple»?

...there is no such thing as a ‘single’ gene disorder. In other words, there is no obvious clear distinction between simple Mendelian and complex traits: genetic diseases represent a continuum with diminishing influence from a single primary gene influenced by modifier genes, to increasingly shared influence by multiple genes”.

Dipple and McCabe 2000
Changing paradigms...

multifactorial
  complex
  sporadic

monogenic
  simple
  familial

mild risk factor

heavy risk factor

oligogenic

Reduced penetrance

Full penetrance

BEYOND MENDEL: AN EVOLVING VIEW OF HUMAN GENETIC DISEASE TRANSMISSION

Nat Rev Genet 2002

Jose L. Badano* and Nicholas Katsanis*
Deleterious and disease alleles in the general population

Healthy individuals harbour large numbers of potentially deleterious variants

A healthy person carries on average:

- ~13600 single nucleotide variants, of which 2.3% likely pathogenic
- ~100 definite loss of function variants, of which ~20 in recessive state
- ~300-500 damaging missense variants, of which ~80 in recessive state

- The subject may be heterozygous carrier of a recessive mutation
- The disease may be clinically mild and then oversought
- The disease may have late onset
- The disease may require additional genetic or environmental factors to manifest (reduced penetrance)
Challenges in mendelian diseases

- Reduced penetrance
  - Not all individuals harbouring a particular mutation / genotype express the phenotype within a specific time period

- Variable phenotypic expression, lack of genotype-phenotype correlates
  - Mutations in the same gene (even the same mutation!) may give rise to different phenotypes

- Genetic heterogeneity
  - The same phenotype can be caused by mutations in many different genes

- Variable inheritance of the same gene mutations
  - Some mutations may be dominantly or recessively inherited
Allele dosage effect: dominant or recessive?

Mutations in the same gene

heterozygous

penetrant

Risk factor

homozygous

penetrant

Autosomal recessive «severe» phenotype
Two leading principles in genetic nosology are pleiotropism and genetic heterogeneity. Pleiotropism refers to multiple end effects of a single gene. Genetic heterogeneity refers to the existence of two or more fundamentally distinct entities with essentially the same clinical picture. Nosologists tend to be either lumpers or splitters. To the extent that he pulls together the multiple features of single gene syndromes, the medical geneticist is a lumper. To the extent that by various means he identifies heterogeneity, he is a splitter.

On Lumpers and Splitters, or the Nosology of Genetic Disease*

Victor A. McKusick, M.D.**

Fig. 1a and 1b. The resemblance of the splitter to the revered Civil War President is a clue to the author’s bias. The cartoons also indicate the splitting is harder work than lumping.
The concept of «lumping and splitting»

LUMPING
- Phenotypic variability
- Reduced penetrance

SPLITTING
- same phenotype → distinct genes
“complex” modulation of “simple” mutations

Mendelian mutation

Mutation type

Gene expression level

Epigenetic modification

Environment

Age

Gender

Allele dosage

Oligogenic mutations

Digeneic mutations

Modifier genes

Copy number variants

Allelic variants

10
Neurological disorders: examples from our research

Joubert syndrome & ciliopathies

Parkinson disease
Example 1 – Parkinson disease

Genetic factors and relative risk to develop Parkinson disease

- RR 1.3
- 1.8
- 4-5
- 7
- 9
- 20
- 100

- SNPs in dominant and other genes
- heterozygous mutations in recessive genes
- GBA, LRRK2
- SMPD1
- GTP-CH1
- mutations in SNCA (other AD genes?)
- homozygous mutations in recessive genes

Sporadic

Familial
Autosomal recessive early onset parkinsonisms

- Parkin >>> PINK1 > DJ-1
  - 50% fam
  - 10-15% spor
  - 1-8% in different populations
  - < 1%

Distinct genes, same phenotype
- early onset (<40 years) → DJ1 < Parkin < PINK1
- slow progression
- excellent and sustained response to treatment

Variable phenotypic features, same gene
- ± dystonia at onset
- ± sleep benefit, diurnal fluctuations
- ± hyperreflexia
- ± treatment-related complications (dyskinesias, behavioral problems)

video: Anna Rita Bentivoglio
Heterozygous mutations in recessive genes

PINK1 and Parkin heterozygous mutations:
- are ++ found in sporadic cases
- Parkin mutations influence age at onset
- have a mild effect on increasing PD risk

Rogaeva 04
Bonifati 05
Abou-Sleiman 06
Marongiu 08

<table>
<thead>
<tr>
<th>Mutation</th>
<th>OR PINK1</th>
<th>OR Parkin</th>
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<tbody>
<tr>
<td>0 Mutations</td>
<td>1.62 – 95%CI 0.88-2.99</td>
<td>1.86 – 95%CI 1.01-3.45</td>
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<tr>
<td>1 Mutation</td>
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<td>≥2 Mutations</td>
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Marongiu et al, Hum Mutat 2008
Parkin gene in autosomal recessive PD

- Right arm dystonia
- Unaware of disease
- No clear PD signs!

Dup ex2-3 hom

wt

- early onset PD (age at onset: 34 years)
- rigid-akinetic phenotype
- good response to L-dopa
- anxiety
- no dystonia

Dup ex2-3 het parkin

Problems with genetic counselling!!!!
### Phenotypic spectrum of synuclein mutations

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<td>cognitive impairment</td>
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<td>hallucinations</td>
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<td>pyramidal signs</td>
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<td>epilepsy</td>
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- **Very rare or absent**
- **Occasional**
- **Frequent or always present**
LRRK2 in PD: autosomal dominant or risk factor?

Mutations in *LRRK2* Cause Autosomal-Dominant Parkinsonism with Pleomorphic Pathology

LRRK2 G2019S mutation → commonest cause of autosomal dominant PD

Age-specific penetrance of *LRRK2* G2019S in the Michael J. Fox Ashkenazi Jewish LRRK2 Consortium

Reduced penetrance: 30% by age 80 years

*LRRK2* mutations in Parkinson disease; a sex effect or lack thereof? A meta-analysis

relative risk 4-5
Homozygous GBA mutations → Gaucher’s disease

Relative risk proportional to mutation severity:
- Mild mutations: RR 3
- Severe mutations: RR 21

Heterozygous GBA mutations represent the most common genetic risk factor for PD identified to date: 4-8% PD vs <1% controls – 11% PD in Italy
Example 2 – Joubert syndrome and ciliopathies
Genetic heterogeneity in JS

- >30 genes to date

All encode for proteins of the primary cilium
Genotype-phenotype correlates

JS with liver involvement
- TMEM67
- TMEM216
- OFD1
- C5Orf42
- CEPI290

JS pure or with retinal involvement
- AHI1
- ARL13B
- CC2D2A
- INPP5E
- C5Orf42

Cerebello-oculo-renal phenotype
- CEP290

O/U
several genes cause distinct ciliopathies with variable clinical overlap

not all genes have been tested for all phenotypes → further associations to come soon

Zaghloul & Katsanis, Trend in Genet 2010
Shared features among ciliopathies

- Disorders caused by genes encoding for proteins of the primary cilium and its apparatus (basal body, centrosome)
- Variable severity and multiorgan involvement
- Clinical and genetic overlap among distinct conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Bardet-Biedl</th>
<th>Meckel-Gruber</th>
<th>Joubert</th>
<th>NPH</th>
<th>Senior-Loken</th>
<th>OFD1</th>
<th>craniectodermal dysplasias, Jenue, short rib polydactylies</th>
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<tr>
<td>Cystic kidneys</td>
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<td>Hepatobiliary disease</td>
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<td>Retinal degeneration</td>
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<td>Laterality defects</td>
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<td>Intellectual disability</td>
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<td>Cerebellar vermis hypoplasia</td>
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<td>Encephalocele</td>
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<td>Polydactyly</td>
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<td>Obesity</td>
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<td>Shortening/bowing of bones</td>
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<td>Ectodermal dysplasia</td>
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Intrafamilial variability of ciliopathies

Co-Occurrence of Distinct Ciliopathy Diseases in Single Families Suggests Genetic Modifiers

Maha S. Zaki, Shifteh Sattar, Rustin A. Massoudi, and Joseph G. Gleeson

AJMG 2012

<table>
<thead>
<tr>
<th>Ciliopathy-672</th>
<th>Ciliopathy-1491</th>
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<tr>
<td>IV-1</td>
<td>IV-1</td>
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<td>IV-2</td>
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<td>IV-3</td>
<td>IV-3</td>
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<tr>
<td>IV-4</td>
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</table>

NPH          | BBS            |
polydactyly  | mild CVA       |
mild CVA     | JS             |
NPH          | JS             |
NPH          | JS             |
MCI          | polydactyly    |
OFDIV: overlap ciliopathy between OFD, SRP, JS and MKS

Mohr-Majewski syndrome ➔
OFDII (Mohr) + SRPII (Majewski)

Oro-facio-digital abnormalities
• tongue anomalies, frenula
• cleft palate/lip
• postaxial polydactyly

Skeletal abnormalities
• tibial hypoplasia and thickening
• bowing of long bones
• trident shape acetabulum

Other organs
• cystic dysplastic kidneys
• liver ductal plate proliferation
• CNS malformations

Mutations in TCTN3 also found in patients with JS and typical MTS

Thomas et al, AJHG 2012
Allelism between lethal and non-lethal ciliopathies

Meckel fetus, aborted 15th weeks g.a.

Joubert patient, 50 year old
### Correlates between the mutation type and phenotype

<table>
<thead>
<tr>
<th>Mutation Type</th>
<th>MKS</th>
<th>MKS</th>
<th>MKS</th>
<th>OFDIV</th>
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<td>2 truncating mutations</td>
<td>JS</td>
<td>BBS</td>
<td>NPH</td>
<td>JS</td>
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<tr>
<td>at least 1 missense mutation</td>
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</table>

- **C5Orf42**

  same mutations identified in patients with severe OFD phenotypes and pure JS

- **NPHP1**

  95% cases: same homozygous 250kb deletion encompassing the gene → variable phenotypes (NPH – SLS – JS with kidney involvement)
Oligogenic inheritance and mutational load

Mutations or rare variants at a second locus influence the phenotypic expression of recessive mutations at the main disease locus.

**Gene mutated**

<table>
<thead>
<tr>
<th>Gene mutated</th>
<th>None</th>
<th>AHI1</th>
<th>INPP5E</th>
<th>CEP290</th>
<th>NPHP1</th>
<th>NPHP1+AHI1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenotype</strong></td>
<td>Normal</td>
<td>JBTS</td>
<td>JBTS</td>
<td>LCA, SLS, NPHP, JBTS, BBS, MKS</td>
<td>NPHP</td>
<td>NPHP+RD</td>
</tr>
</tbody>
</table>

= Normal chromosome  = Mutated chromosome  Severity

Novarino and Gleeson, Cell 2011
Oligogenic inheritance and mutational load in ciliopathies

The oligogenic properties of Bardet–Biedl syndrome

Nicholas Katsanis*

Evidence of Oligogenic Inheritance in Nephronophthisis

Julia Hoefele,*† Matthias T.F. Wolf,* John F. O’Toole,* Edgar A. Otto,* Ulla Schultheiss,* Georges Déschênes,‡ Massimo Attanasio,§ Boris Utsch,* Corinne Antignac,¶ and Friedhelm Hildebrandt**

High NPHP1 and NPHP6 Mutation Rate in Patients with Joubert Syndrome and Nephronophthisis: Potential Epistatic Effect of NPHP6 and AHI1 Mutations in Patients with NPHP1 Mutations

Kálmán Tóth,*† Tiphaine Lacoste,*† Lydie Burglen,‡ Vincent Morinière,*† Nathalie Boddaert,§ Marie-Alice Machet,¶ Brigitte Llanas,¶ Hubert Nivet,*† Albert Bensman,‡ Patrick Niaudet,¶ Corinne Antignac,¶ Rémi Salomon,¶† and Sophie Saunder***

TTC21B contributes both causal and modifying alleles across the ciliopathy spectrum

IFT139

TTC21B recessive mutations:
- isolated NPH / NPH plus / JATD

TTC21B heterozygous mutations:
- 2.5% pts with ciliopathies (some mutated in other genes) vs 0.06% controls

in several patients with JS and other ciliopathies, heterozygous mutations are often detected in cilia-related genes

Nat Genet 2011
Even common variants may act as genetic modifiers

A common allele in **RPGRIP1L** is a modifier of retinal degeneration in ciliopathies

Hemant Khanna1,22, Erica E Davis3,22, Carlos A Murga-Zamalloa1, Alejandro Estrada-Cuzcano1, Irma Lopez3, Anneke I den Hollander1, Marijke N Zonneveld1, Mohammad I Othman1, Naushin Waseem2, Christina F Chakarova3, Cecilia Maubaret3, Anna Diaz-Fon4, Ian MacDonald2, Donna M Muzny8, David A Wheeler8, Margaret Morgan8, Lora R Lewis8, Clare V Logan9, Perciliz L Tan2, Michael A Beer2,10, Chris F Inglehearn9, Richard A Lewis11-14, Samuel G Jacobson15, Carsten Bergmann16, Philip L Beales8, Tania Attié-Bitach17, Colin A Johnson1, Edgar A Otto18, Shomri S Bhattacharya18, Friedhelm Hildebrandt16,19, Richard A Gibbs8, Robert K Koenekoop5, Anand Swaroop11,18,29 & Nicholas Katsanis2,21

Nat Genet 2009

**RPGRIP1L p.A229T**

- controls: 2.8%
- ciliop. no retinopathy: 0%
- ciliop. + retinopathy: 4.5% (p<0.001)

**AHI1** is required for photoreceptor outer segment development and is a modifier for retinal degeneration in nephronophthisis

Carrie M Louie1, Gianluca Caridi2, Vanda S Lopes3,4, Francesco Brancati5,6, Andreas Kispert7, Madeline A Lancaster1, Andrew M Schlossman1, Edgar A Otto5,9, Michael Leitges19, Hermann-Josef Gröne11, Irma Lopez12, Harini V Gudiseva13, John F O’Toole5,9, Elena Vallespin14, Radha Ayyagari13, Carmen Ayuso14, Frans P M Cremers15, Anneke I den Hollander16, Robert K Koenekoop12, Bruno Dallapiccola17, Gian Marco Ghiggeri2, Friedhelm Hildebrandt9, Enza Maria Valente5,18, David S Williams3,4 & Joseph G Gleeson1

Nat Genet 2010

**AHI1 p.R830W**

- controls: 2.8%
- isolated NPH: 1.8%
- NPH + retinopathy: 25% (p<0.001)
The revolution of next generation sequencing

- 3–4 million genomic variants
  - Common
    - >5% of population
      - Disease association database
        - Risk assessment for common diseases
  - No disease association
  - Rare
    - <5% of population
      - Coding
        - Coding variants
          - Nonsynonymous
            - Nonsense, splice site, frameshift
          - Synonymous
            - Gene information: expressed in tissue relevant to disease, known disease associations (OMIM®)
            - Pathogenic variants (carrier status or disease-causing) in Mendelian diseases, or rare variants with large effects on complex traits
        - Noncoding
          - Gene information: expressed in tissue relevant to disease, known disease associations (OMIM®)
          - Pathogenic variants (carrier status or disease-causing) in Mendelian diseases, or rare variants with large effects on complex traits

Target sequencing
Whole exome sequencing
Whole genome sequencing

Disease-causative mutations
Rare variants with large effects
Risk alleles: polymorphisms, but also rare mutations

Whole exome (and even whole genome) sequencing are likely to replace GWAS to search for genetic modifiers of the phenotype

Manolio et al, Nature 2009
GTP-CH1 rare variants and Parkinson disease risk

Parkinson’s disease in GTP cyclohydrolase 1 mutation carriers

Co-occurrence of DRD and PD in the same GTP-CH1 mutated families

WES of 1318 sporadic PD patients vs 5935 controls

↓

10 GTP-CH1 heterozygous variants in PD (0.75%) vs 1 in control (0.1%)

↓

OR 7.5, CI 2.4-25.3, p<0.001

Confirmed in an independent study (0.57% mut freq in PD)

Mencacci et al, Brain 2014
Ciliary genes rare variants in JS patients

Screening of 120 ciliary genes in over 350 JS patients

About 30% (including mutated and non mutated) carry at least one heterozygous deleterious variant in a ciliary gene (novel or rare)

Impact on disease penetrance and phenotypic expression?
Towards personalized approaches to genetic diagnosis and counselling of mendelian disorders.
Take-home messages

• The genetic basis of mendelian disorders is complex and the same genes are often implicated – as more or less strong risk factors – in familial and sporadic forms; most of these genes are found to interact, and to converge into key cellular pathways that are disrupted in the disease.

• «True monogenic» diseases are very rare: many genetic determinants represent susceptibility factors, which variably increase the risk to develop a given phenotype in a multifactorial context.

• Rare mutations, other than common polymorphisms, may represent important genetic modifiers of mendelian disease phenotypes. High throughput next-generation-sequencing approaches are bringing a tremendous acceleration in this field of research.

• Interpretation of the huge amount of genetic data generated from these studies represents the big challenge ahead.
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erc

@Salerno

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AISJAC