Clinicians Viewpoint of Channelopathies: Integrating Science into Practice

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Disclosure

No financial relationship relevant to this presentation.
Phenotype to genotype

The aim of genetic studies 20-30 years ago was to connect an existing disease phenotype to a genotype.
Explosion of discoveries

Whole exome sequencing and whole genome sequencing is available to the public.

What do we do with the information?

Who is going to interpret it?
What is in my genes?
Gattaca - 1997
Genetic printout

- MST1R mutation – 60% of breast cancer by age 40
- FOSB splice truncation – 33% chance of drug addiction
- LDLR homozygous mutation – 100% elevated cholesterol
- FRB1 missence mutation – 15% lifetime chance of depression
- PLB2 frameshift – 95% chance of elevated blood pressure
- JBB42 polymorphism – 30% chance of glaucoma
- KCNJ1 mutation – 25% chance of sudden cardiac arrest
Clinicians viewpoint

= parental perspective

What does this mean to my patient?  

What is the risk of arrhythmia?  

How can I best treat it/prevent it?  

DIAGNOSIS

PROGNOSIS

THERAPY
Mendelian 1-to-1

What the genes code is visible

100% penetrance

100% predictability

Variable phenotype

Variable penetrance
Variable penetrance
Gene to function

Proteome Complexity

- **Genome** ~20-25,000 genes
- **Transcriptome** ~100,000 transcripts
- **Proteome** >1,000,000 proteins

- Alternative promoters
- Alternative splicing
- mRNA editing
- Post-translational modifications
Phenotypic plasticity

Everyone is different (1-to-0)

0% predictability

Individualized medicine
Example 1 - LQTS

Genotype: KCNH2 mutation E637K, non-conservative AA change in the pore-loop of $I_{Kr}$ (rapid inward rectifying K)
Table 3. Genotype-Phenotype Correlation in the Long-QT Syndrome

<table>
<thead>
<tr>
<th>ECG Pattern, Clinical Presentation</th>
<th>Risk Factors for Events</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LQT1</strong></td>
<td></td>
</tr>
<tr>
<td>Broad T wave</td>
<td>Exercise, swimming</td>
</tr>
<tr>
<td>Impaired QT adaptation during tachycardia</td>
<td>Cytoplasmic loop mutations (vs other localizations)</td>
</tr>
<tr>
<td>Incomplete penetrance (~60%), especially at rest</td>
<td>Dominant negative mutations (vs haploinsufficiency)</td>
</tr>
<tr>
<td>High effectiveness of β-blockers</td>
<td></td>
</tr>
<tr>
<td><strong>LQT2</strong></td>
<td></td>
</tr>
<tr>
<td>Flat, notched T waves</td>
<td>Acute emotions, loud nose, sudden awakening</td>
</tr>
<tr>
<td>Usually normal QT adaptation at fast rates</td>
<td>Pore region mutations (vs other localizations)</td>
</tr>
<tr>
<td>Reduced β-blocker effectiveness (vs LQT1)</td>
<td>Female sex</td>
</tr>
<tr>
<td><strong>LQT3</strong></td>
<td></td>
</tr>
<tr>
<td>Straight ST segment, narrow T wave</td>
<td>Rest, sleep</td>
</tr>
<tr>
<td>Reduced β-blocker effectiveness (vs LQT1)</td>
<td>Male sex</td>
</tr>
</tbody>
</table>
## Example 1 - LQTS


<table>
<thead>
<tr>
<th>Predictors of cardiac events</th>
<th>Relative risk</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>First cardiac event in childhood (&lt;7 y)</td>
<td>4.34 (2.35-8.03)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>QTc &gt; 500 msec</td>
<td>2.01 (1.16-3.51)</td>
<td>0.01</td>
</tr>
<tr>
<td>LQT2 vs LQT1</td>
<td>2.81 (1.5-5.27)</td>
<td>0.001</td>
</tr>
<tr>
<td>LQT3 vs LQT1</td>
<td>4 (2.45-8.03)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Example 1 - LQTS

Genetic testing for long QT syndrome.
Example 1 - LQTS

**DIAGNOSIS**
Consistent with LQT2

**PROGNOSIS**
Appropriate risk stratification based on the type and location of mutation

**THERAPY**
Therapy guided by mutation
Example 2 - LQTS

Genotype: KCNQ1 mutation R594Q, semi-conservative AA change in the $I_{Ks}$ (slow inward rectifying K) causing loss of function

QTc: 427 msec

QTc: 462 msec
Example 2 - LQTS

Example 2 - LQTS

Example 2 - LQTS

Example 2 - LQTS

<table>
<thead>
<tr>
<th><strong>DIAGNOSIS</strong></th>
<th>Variable phenotype, not always consistent with LQT1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PROGNOSIS</strong></td>
<td>Risk stratification may be useful based on LQT type, gender, and QTc length</td>
</tr>
<tr>
<td><strong>THERAPY</strong></td>
<td>Therapy is standard, but not tailored to phenotype</td>
</tr>
</tbody>
</table>
Example 3 - BrS

Genotype: SCN5A heterozygous mutation E1053K, non-conservative AA change in Na channel, c/w BrS1
Genotyping helps in diagnosis, but up to 40% of BrS may be genotype negative. Genotyping does not influence risk stratification and therapy due to heterogeneity of symptoms and phenotype.

Insights:
Spontaneous type 1 ECG carries arrhythmia risk

Brugada type 1 ECG pattern during fever suggests higher arrhythmia risk compared to drug-elicited BrS

S-wave in lead I – marker for SCD in BrS
Example 3 - BrS

**DIAGNOSIS**  Consistent with BrS, not always

**PROGNOSIS**  Risk stratification based on phenotype

**THERAPY**  Therapy is not guided by genotype
Example 4 – CPVT (VUS)

Genotype: RyR3 deletion S443Y fsX20 causing frame shift and truncation of the ryanodine receptor – VUS

Genotype: RyR3 mutation K2723R resulting in a conservative AA change – VUS
CPVT guidelines:

Genetic diagnosis is important, genes involved: RYR2, CALM1, CASQ, TRDN.

Exercise stress testing: bidirectional or polymorphic VT

Primary prevention and secondary prevention guided by genotyping and observed arrhythmias, SCA
Example 4 - CPVT

**DIAGNOSIS**

VUS for CPVT

**PROGNOSIS**

Documented VT/VF triggered by exercise vs. PVCs

**THERAPY**

Therapy based on phenotype
Example 5 – Na channel

Genotype: SCN5A mutation R814W, non-conservative AA change in the voltage-sensing domain of the Na_\text{v}1.5 channel
SCN5A mutations have been associated with DCM, BrS, LQT3, SIDS, CCD, AF.

Even a single mutation (E1784K) has been associated with different phenotypes: LQT3 and BrS.

R814W is a mutation in the voltage sensor domain, and has been documented in association with DCM, AF and VT, likely due to anomalous currents (window current, gating pore current).

Sodium channel mutations and susceptibility to heart failure and atrial fibrillation. Olson et al, 2005, JAMA.


Mutations in the voltage sensors of domains I and II of Nav1.5 that are associated with arrhythmias and dilated cardiomyopathy generate gating pore currents. Moreau et al, 2015, Front Pharmacol.
**Example 5 – Na channel**

<table>
<thead>
<tr>
<th><strong>DIAGNOSIS</strong></th>
<th>Not clear, phenotype c/w DCM, not c/w BrS, LQT3, AVB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PROGNOSIS</strong></td>
<td>Not clear, FH is important</td>
</tr>
<tr>
<td><strong>THERAPY</strong></td>
<td>Therapy based on phenotype, if phenotype is unclear…?</td>
</tr>
<tr>
<td></td>
<td>SCA prevention</td>
</tr>
</tbody>
</table>
Phenotypic plasticity
From gene to protein
EPIGENETICS

Modifying factors influencing expressivity, penetrance and the variable phenotype:

• Genomic imprinting
• Transcription enhancers and silencers
• Single nucleotide polymorphism in the UTRs
• Alternative splicing
• Methylation
• Post-translational modifications
• Protein folding, trafficking, turnover
• Ethnicity
• Environmental factors
Genomic imprinting

KCNQ1 gene encoding a potassium channel

Human KVLQT1 gene shows tissue-specific imprinting.
Lee et al, 1997, Nat Gen.
Genomic imprinting

Variable biallelic expression in the cardiomyocyte

Human KVLQT1 gene shows tissue-specific imprinting.
Lee et al, 1997, Nat Gen.
RNA modulation

5’UTR    exon    intron    intron    exon    3’UTR

Enhancers / silencers    long non-coding RNA    micro RNA
RNA modulation

5’UTR  exon  intron  intron  exon  3’UTR
RNA modulation
SNPs

Single nucleotide polymorphisms in non-coding regions

SNPs

Variants in the 3’ UTR of the KCNQ1-encoded Kv7.1 potassium channel modify disease severity. Amin et al, 2011, Eur Heart J.
Variants in the 3’ UTR of the KCNQ1-encoded Kv7.1 potassium channel modify disease severity. Amin et al, 2011, Eur Heart J.
Alternative splicing
Alternative splicing

SCN5A exon 6 can be expressed in splice variants
Canonical exon 6 – common adult variant
Fetal exon 6A – a fetal splice variant with 7 AA altered in
the voltage-sensing domain of the \( \text{Na}_{\text{V}1.5} \) channel with
slower activation and inactivation and greater currents

LQT3 severity has been associated with higher ratio of
exon 6A in fetuses and infants

Muscular dystrophy 1 patients can develop AF, CCD, VT
– found to have exon 6A splice variants without mutation

SIDS, VT, SUDS without genetic mutation?
Epistatic effects of potassium channel variation on cardiac repolarization and atrial fibrillation risk. Mann et al, 2012, J Am Coll Cardiol..
Post-translation modification

Hydrophobic groups for membrane localization:
- myristoylation: attachment of myristate, a C14 saturated acid
- palmitoylation: attachment of palmate, a C16 saturated acid
- isoprenylation or prenylation: the addition of an isoprenoid group (e.g. farnesol and geranygeraniol)
- farnesylation
- geranylgeranylation
- glycosylation: glycosylphosphatidylinositol (GPI) anchor formation via an amide bond to C-terminal tail

Cofactors for enhanced enzymatic activity:
- lipoylation: attachment of a lipoate (C8) functional group
- flavin moiety (FMN or FAD) may be covalently attached
- heme C attachment via thioether bonds with cysteine
- phosphopantetheinylation: the addition of a 4'-phosphopantetheinyl moiety from coenzyme A, as in fatty acid, retinylidene Schiff base formation

Modifications of translation factors:
- diphthamide formation (on a histidine found in eEF2)
- ethanolamine phosphoglycerol attachment (on glutamate found in eEF1a)

Smaller chemical groups:
- acylation, e.g. O-acylation (esters), N-acylation (amides), S-acylation (thioesters)
- acetylation: the addition of an acetyl group, either at the N-terminus or at lysine residues
- formylation
- alkylation: the addition of an alkyl group, e.g. methyl, ethyl
- methylation: the addition of a methyl group, usually at lysine or arginine residues. The reverse is called demethylation
- amide bond formation
- amidation at C-terminus
- amino acid addition
  - arginylation: a tRNA-mediation addition
  - polyglutamylation: covalent linkage of glutamic acid residues to the N-terminus of tubulin and some other proteins
  - polyglycylation: covalent linkage of one to more than 40 glycine residues to the tubulin C-terminal tail
- butyrylation
- gamma-carboxylation dependent on Vitamin K
- glycosylation: the addition of a glycosyl group to either arginine, asparagine, cysteine, hydroxlysine, serine, threonine, tyrosine, polysialylation, addition of polyasialic acid, PSA, to NCAM
- malonylation
- hydroxylation
- oxidation (e.g. of thyroglobulin)
- nucleotide addition such as ADP ribosylation
- oxalation
- phosphate ester (O-linked) or phosphoramidate (N-linked) formation
- sulfation, the addition of a sulfonic group to a tyrosine

Phosphorylation
- Hydroxylation
- Carboxylation
- Palmitoylation
- Glycosylation
- Glycation
- Ubiquitination
- SUMOylation
- Neddylation

More than 200 PTM discovered:

- Phosphorylation
- Hydroxylation
- Carboxylation
- Palmitoylation
- Glycosylation
- Glycation
- Ubiquitination
- SUMOylation
- Neddylation

Non-enzymatic additions in vivo:
- glycation: the addition of a sugar molecule to a protein without the controlling action of an enzyme
- carbamylation: the addition of isocyanic acid to an N-terminus of either lysine, histidine, arginine, or cysteine
- carboxylation: the addition of carbon monoxide to other organic/inorganic compounds

Non-enzymatic additions in vitro:
- biotinylation, acylation of conserved lysine residues with a biotin appendage
Post-transcriptional modification

Glycosylation alters the voltage dependent gating of \( \text{Na}_v1.5 \) channels.

Glycosylation of \( I_{Ks} \) regulatory subunit encoded by \( \text{KCNE1} \) at threonine-7 is essential for trafficking and membrane localization.

S-nitrosylation of ryanodine receptor leads to progressive activation and increased Ca release.

MOG1 enhances \( \text{Na}_v1.5 \) channel trafficking and membrane localization.

“Cellular” environment

Gender: male or female in long QT syndrome

Hormones: estrogen

Fever: enzyme kinetics, Brugada syndrome

Drugs: drug induced long QT syndrome or BrS

Glucose: TS

Oxidative stress / NO availability
Clinician viewpoint

Is somewhat different from the viewpoint of the policymakers:

Policymakers – create guidelines based on established associations, scientific evidence and trends.

Clinicians – deal with families 1-on-1 integrating science into practice, and making up for the gaps of science using judgment and individualized medicine.
Ideally I would love to tell a patient that
Based on your mutation in gene X, the surrounding
SNPs, the predicted genomic imprinting, alternative
splicing during different time-points in your life, and
additonal factors due to ethnicity and gender:

your ECG will look like A, your risk of SIDS is B%, risk of
arrhythmia and SCA in teenage years is C%, and by
age 40 rises to D%.
Therefore I suggest strict q3h feeding until age 1 year,
immediate attention and medication of fever or
vomiting/diarrhea, starting medication Y at age 2 weeks,
adding medication Z and receiving an ICD by age 10.
## Genotype-phenotype

<table>
<thead>
<tr>
<th>Section # – Disease</th>
<th>Diagnostic</th>
<th>Prognostic</th>
<th>Therapeutic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section I – LQTS</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Section II – CPVT</td>
<td>+++</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Section III – BrS</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Section IV – CCD</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Section V – SQTS</td>
<td>+/−</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Section VIII – ACM/ARVC</td>
<td>+</td>
<td>+/−</td>
<td>–</td>
</tr>
</tbody>
</table>

## Channelopathy Prognosis (SCA) Therapy

<table>
<thead>
<tr>
<th>Channelopathy</th>
<th>Prognosis (SCA)</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>LQTS</td>
<td>+++ (symptoms, scoring, QTc, TWA, TdP)</td>
<td>+ (symptoms, TdP)</td>
</tr>
<tr>
<td>SQTS</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>BrS</td>
<td>++ (symptoms, ECG)</td>
<td>-</td>
</tr>
<tr>
<td>CPVT</td>
<td>++ (symptoms, EST)</td>
<td>+</td>
</tr>
<tr>
<td>ARVC</td>
<td>++ (symptoms, scoring, MRI, ECG, Holter)</td>
<td>+</td>
</tr>
</tbody>
</table>
Future trends

Laboratory studies replicating and confirming suspected phenotype-genotype correlations.

Studies conducted in strictly stratified patient subgroups analyzing genotype, SNPs, RNAs and clinical data (symptoms, ECG, Holter, ICD recording).

Scoring algorithms for BrS, CPVT and SQTS integrating genotype and phenotype data.

Potential therapeutic targets in transcription modifiers and post-translational modification.
Thank you!