Structural Role of ABC Sterol Transporters

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Cholesterol: a Risk Factor of Cardiovascular Diseases
Transporting Cholesterol

Guts:
- Dietary sterols
  - Small intestine

Plasma / Tissues:
-Liver
- Apolipoproteins
- HDL
- LDL
- Peripheral tissues

Cholesterol:
- Guts
- Plasma / Tissues

Arrows indicate the flow of cholesterol:
- **FORWARD**: Dietary sterols to Liver, then to Plasma / Tissues, and finally to Peripheral tissues.
- **REVERSE**: From Peripheral tissues to HDL, LDL, and back to Liver, then to Guts.

The diagram illustrates the transport processes of cholesterol in the body.
ABCG5/G8

ABCA1

HDL

Bile

Cholesterol

Dietary sterols

Guts

Liver

Apolipoproteins

Peripheral tissues

ABCA1, G1

Cholesterol

LDLR

Small intestine

Plasma / Tissues

Transporting Cholesterol

FORWARD

REVERSE
Function and structure of ABCG5/ABCG8

- What’s known & what’s not known
- Summary, questions, & future directions
ABCG5 and ABCG8 in the Guts

**Small Intestine**

- **DIET**
- **Enterocyte**
- **ABCG5G8**
- **NPC1L1**

**Liver**

- **Bile Canaliculus**
- **Hepatocyte**
- **ABCG5G8**

- **Plant Sterols**
- **Cholesterol**
Heterodimeric ABCG5/ABCG8

Transmembrane domain

Nucleotide-binding domain
Expression of Human ABCG5/ABCG8
Host: *Pichia pastoris* Yeast

Left: *Saccharomyces* (1.0 OD$_{600nm}$ units/mL)
Right: *Pichia* (400 OD$_{600nm}$ units/mL)

(Cereghino & Cregg, FEMS Microbiol, 2000)

(Shutter et al, Nature Biotechnology, 2009)
(Ina Urbatsch, Texas Tech HSC)
Optimization of Protein Preparation

Shaker culture (4-5 days) → Membrane Preparation → Solubilization (β-DDM) → 1° Ni-NTA → 1° CBP → Detergent Exchange

DDM (DoDecyl Maltoside) → MNG (Maltose Neopentyl Glycol) → + Endo H + 3C protease

Crystallization (manual/robot set-up) (cholesterol) → ± Mg/ATP → Crystal growth (2 wks – 6 mths)

± ATPase inhibitor → Ligands → Relipidation → Synthetic phospholipids (Avanti) → Methylation (CH₃-Lys) → Alkylation (Cys capping)

PD-10 (desalting) → 2° Ni-NTA → Gel filtration → 2° CBP
Bicelle Crystallization (Lipid Bilayers)

G5G8 bicelle preparation

- Bilayer (DMPC + cholesterol)
- Micelle (CHAPSO) (DHPC)
- G5G8 (MNG)

Bicelle

Crystal growth & X-ray diffraction

- Long exposure 2-5 sec @ APS 30 sec @ ALS
- Radiation damage 3-5 frames (< 5°)
- Signal (I/σ = 1-1.5 at 3.9-4Å)

• Scale bar: 100 µm

• Crystal structure (3.6 Å, 3.9 Å, 5.61 Å, 7.91 Å, 15.75 Å)
The G5G8 reveals new TMD structural fold for the ABC transporter superfamily.

(Hollenstein et al, Curr Opin Struct Biol, 2007)
(Lee et al, Nature, 2016)
• Function and structure of ABCG5/ABCG8

✓ What’s known & what’s not known

• Summary, questions, & future directions
ABCG5 and ABCG8 share high structural similarity.

**Domain features**

- ECD: extracellular domain
- TMD: transmembrane domain
- NBD: nucleotide-binding domain
- CnH: connecting helix
- CpH: coupling helix

**Structural similarity:**

RMSD (Cα) ~ 2Å (~28% sequence identity)

(Lee et al, Nature, 2016)
How does the catalytic NBD communicate with sterol transporting TMD?
How does the catalytic NBD communicate with sterol transporting TMD?

- CnH: connecting helix
- CpH: coupling helix
- ★: conserved polar residues

<table>
<thead>
<tr>
<th></th>
<th>Q-loop</th>
<th>E-helix</th>
<th>CnH</th>
<th>CpH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo sapiens</td>
<td>133 LQSTD-VEHTLYTALLA-...FSKLVGLRIRRVRNHVRN-LRAVSDQESQGDLY 458</td>
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<td>Danio rerio</td>
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<tr>
<td>G8</td>
<td>*</td>
<td>*</td>
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<td></td>
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<td>Homo sapiens</td>
<td>152 PDECQ-VEHTLAFAQNLPL-...VQQFTTLLIRRQISHDNYRD-ERAMLYYLEDGLEY 487</td>
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<td>Mus musculus</td>
<td>153 RDEHQ-VEHTLAFAQNLPL-...IEQSTTLIRRQISHDNYRD-ERAMLYYLEDGLEY 487</td>
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<td>Gallus gallus</td>
<td>154 RDEER-VEHTLLFTLRNLPL-...LQKFTVLSRRQVSNDFDRD-ERAMLYYLDLEMGY 492</td>
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<td>Xenopus tropicalis</td>
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<tr>
<td>Danio rerio</td>
<td>158 RDEDR-VEHTLAFVAKLRLPL-...VQFQTTLIRRQVFNYRD-ERAMLYHELEDGLEY 498</td>
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</table>
Interactions within the Triple Helical Bundle

CnH: connecting helix
CpH: coupling helix
E: E-helix
A: Walker A motif
B: Walker B motif
Sig: Signature motif
Interactions within the Triple Helical Bundle

CnH: connecting helix
CpH: coupling helix
E: E-helix

A: Walker A motif
B: Walker B motif
Sig: Signature motif
The TMD polar relay connects the triple helical bundle to the TMD.
How do sterols move across the lipid-bilayer membranes on the TMD?

Vestibules at the TMD-membrane interface

Sterol binding/entry site?
How do sterols move across the lipid-bilayer membranes on the TMD?

(Lee et al, Nature, 2016)
• Function and structure of ABCG5/ABCG8

• What’s known & what’s not known

✓ Summary, questions, & future directions
Summary

• New TM structural fold for ABC transporters
  ABC2 superfamily: G5, G8, G2, A1, …
• Triple helical bundle b/w NBD and TM helices
• Polar relay
• Sterol “binding” sites
Questions:

• Impact on medical conditions?
Localization of Missense Mutations and Polymorphisms

Polymorphisms:
- Q604E
- C600Y
- M622V
- I523V

Missense mutations:
- G5
  - R50Y
  - D19H
  - C54Y
- G8
  - E340Q
  - G269R
  - L36P

- G5
  - E146Q
- G8
  - R419P/H
  - N437K
  - R543S
  - L572P
  - R550S
  - R389H
  - L596R
  - R50Y
  - D19H
  - C54Y
  - L501P
  - L405H
  - R184H
  - P231T
  - R263Q
  - L195Q
Location of the residues with the disease-causing missense mutations of sitosterolemia.

**ER-escape missense mutations**

- R419H/P
- G574R
- R543S
- E146Q

**Non-ER-escape missense mutations**

- G574E
- R389H
- N437K
- R184H
- P231T
- R263Q
- L501P
- L596R

**Color:** conserved (multiple sequence alignment (MSA) value ≥ 7)

**White:** less/non-conserved (MSA < 7)
Disease-causing mutations cluster in the conserved functional domains in G5G8.
Questions:

• Impact on medical conditions?

• Energy-coupled conformational changes?
Molecular Dynamics Simulation
(Junmei Wang, Pittsburgh)

Inward movement
(CpH/CnH/E-helix bundle)

Upward movement
(TM helices)
ABC Sterol/lipid Transporters
(Nucleotide-free apo states)

ABCG5/G8

ABCA1
Questions:

• Impact on medical conditions?
• Energy-coupled conformational changes?
• Extracellular domains/loops?
• Sterol/lipid translocation pathway?
Cholesterol Binding/Translocation?
Homology Model of ABCG1

Substrate exit:
(Drosophila White)

Sterol-Sensing:
(Ingrid Gelissen Lab)

G581 (TMH5)

TMH5

TMH6

<table>
<thead>
<tr>
<th>Protein</th>
<th>Start</th>
<th>Sequence</th>
<th>End</th>
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</thead>
<tbody>
<tr>
<td>ABCG1</td>
<td>562</td>
<td>LQVATFVGVP-TAIPVLLFSGFFVS--//--LYLDFIVLGIFFISLRLIA\textcolor{red}{Y}FVLR\textcolor{red}{Y}KI</td>
<td>674</td>
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<tr>
<td>ABCG2</td>
<td>534</td>
<td>VSVATL\textcolor{red}{L}MTI-C\textcolor{red}{F}VFMMIFSGLL\textcolor{red}{V}N--//--LWKNHVA\textcolor{red}{L}ACMIVIFLTIAYLK\textcolor{red}{L}FLK</td>
<td>652</td>
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<tr>
<td>ABCG4</td>
<td>531</td>
<td>LQVATFVGVP-TAIPVLLFSGFFVS--//--LYMDFFLVGIF\textcolor{red}{F}LAL\textcolor{red}{R}LLAYLVRL\textcolor{red}{R}YRV</td>
<td>642</td>
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<tr>
<td>ABCG5</td>
<td>527</td>
<td>PNIVNSSVALL\textcolor{red}{S}IAGVLVGSG\textcolor{red}{F}LR\textcolor{red}{N}--//--FTMNFLILYSFIPALVILGIVVFKIRD</td>
<td>646</td>
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<tr>
<td>ABCG8</td>
<td>556</td>
<td>FHMASFFSNA-L\textcolor{red}{N}SFYL\textcolor{red}{L}AGGF\textcolor{red}{M}IN--//--LYAIYLV\textcolor{red}{I}VGLSGFGMVL\textcolor{red}{Y}V\textcolor{red}{S}LRFIK</td>
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<tr>
<td>White</td>
<td>570</td>
<td>TSMALSVGPP-V\textcolor{red}{I}IPPLL\textcolor{red}{F}GGFFLN--//--LPLDYVGLAILIVSFR\textcolor{red}{V}L\textcolor{red}{L}ALRLRA</td>
<td>683</td>
</tr>
</tbody>
</table>
Questions:

• Impact on medical conditions?

• Energy-coupled conformational changes?

• Extracellular domains/loops?

• Sterol/lipid translocation pathway?

• Functional oligomeric states?

Previous works on A1, G2, Pdr5, …