Lecture 9, BCH 8102, 2021 Winter

Lipid metabolism and drug resistance: ABC transporters and structural biology

Jyh-Yeuan (Eric) Lee, PhD, Assistant Professor
Department of Biochemistry-Microbiology and Immunology
Part I: ABC Transporters
a) From bacteria to mammals
b) The engine
c) The transport
d) A long way to structural understanding

Part II: Structural studies of multidrug resistance transporters
ABCB1 (P-glycoprotein)

Part III: Structural studies of lipid transporters
ABCG5/G8 (Sterolin)
Part I:

ABC TRANSPORTERS
ATP-Binding Cassette (ABC) Proteins

- **Transmembrane domain (TMD)**
- **Nucleotide-binding domain (NBD)**

ATP binding cassette (ABC) proteins are divided into:

- **Full transporters**
  - Homo-dimer
  - Hetero-dimer

- **Half transporters**
- **Non-transporters**

ATP binding results in ADP and Pi release.
ABC coupled transport: a simple idea

ATP-binding cassette (ABC)
Evolutionary History of ABC Proteins

(Srikant, FEBS Lett, 2020)
shown to be homologous to members of the ABC1 family (Table 1). Fifty percent of the ABC exporters in the TCDB are of this type. These proteins proved to have arisen from a two-TMS hairpin precursor by intragenic triplication, as shown schematically in Fig. 1a and demonstrated statistically, first using the IC and GAP programs (Table 2) (Zhai and Saier 2002; Zhou et al. 2003) and subsequently using four additional programs (Table 3).

The sequence similarities of the three hairpin repeat elements in these six TMS proteins are demonstrated in Fig. 2a–c and Table 2 (rows A–C) where the superfamily principle (Doolittle 1981) is invoked to establish homology of the repeat units in all members of this ABC1 family.

These membrane domains are often fused to ABC domains to form 'half-sized' ABC transport proteins with the membrane (M) and cytoplasmic (C) (ATP hydrolyzing) domains fused, most frequently in the order M–C. Particularly (but not exclusively) in eukaryotes, these can be duplicated to yield full-length ABC transport systems, with a total of 12 TMSs, in an MCMC arrangement in a single, large, polypeptide chain. Additional domains can be fused to this basic structure, as occurs frequently in eukaryotes but seldom in prokaryotes (Higgins 1992, 2007). However, in all of our studies, only the six-TMS transmembrane domains were analyzed. These porters actively expel all kinds of substrates, from simple ions and sugars to drugs.

**Table 3**

<table>
<thead>
<tr>
<th>ABC Type</th>
<th>TMSsa</th>
<th>Protein 1 (organism)</th>
<th>Protein accession</th>
<th>E valueb</th>
<th>E valuec (LALIGN)</th>
<th>E valuec (GGSEARCH)</th>
<th>E valuec (GLSEARCH)</th>
<th>E valuec (PairwiseStatSig)</th>
<th>E valuec (average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC-1</td>
<td>1–3</td>
<td>Clostridium leptum</td>
<td>DSM 753 ZP_02078939</td>
<td>3.0</td>
<td>6.0</td>
<td>3.0</td>
<td>2.0</td>
<td>6.0</td>
<td>5.0</td>
</tr>
<tr>
<td>ABC-1</td>
<td>1–3</td>
<td>Colwellia psychrerythraea 34H</td>
<td>YP_267051</td>
<td>5.9</td>
<td>4.5</td>
<td>5.9</td>
<td>7.9</td>
<td>4.9</td>
<td>5.9</td>
</tr>
<tr>
<td>ABC-1</td>
<td>1–3</td>
<td>Caulobacter crescentus</td>
<td>CB15 NP_419501</td>
<td>5.8</td>
<td>6.0</td>
<td>5.8</td>
<td>3.0</td>
<td>6.0</td>
<td>5.0</td>
</tr>
<tr>
<td>ABC-1</td>
<td>1–3</td>
<td>Rhodoferax ferrireducens</td>
<td>T118 YP_523966</td>
<td>2.6</td>
<td>5.4</td>
<td>2.6</td>
<td>4.9</td>
<td>4.9</td>
<td>3.9</td>
</tr>
<tr>
<td>ABC-2</td>
<td>1–3</td>
<td>Aeromonas hydrophila subsp. hydrophila ATCC 7966</td>
<td>YP_855895</td>
<td>5.5</td>
<td>5.5</td>
<td>5.5</td>
<td>4.9</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>ABC-2</td>
<td>1–3</td>
<td>Unknown homologue</td>
<td>ZP_01811308</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>ABC-2</td>
<td>3–6</td>
<td>Streptomyces avermitilis MA-4680</td>
<td>NP_825831</td>
<td>5.8</td>
<td>6.0</td>
<td>5.8</td>
<td>2.0</td>
<td>6.0</td>
<td>5.0</td>
</tr>
<tr>
<td>ABC-2</td>
<td>3–6</td>
<td>Burkholderia sp. 383</td>
<td>YP_368972</td>
<td>5.8</td>
<td>5.8</td>
<td>5.8</td>
<td>2.0</td>
<td>5.8</td>
<td>3.0</td>
</tr>
<tr>
<td>ABC-2</td>
<td>3–6</td>
<td>Unknown homologue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*E values reflect the probability that the alignment occurred by chance*

Evolutionary Origin of ABC Exporter TMD

(Wang et al, J Membr Biol, 2009)
Evolutionary History of ABC Proteins

(Srikant, FEBS Lett, 2020)
**Human ABC proteins: 48 members, 44 transporters**

### Table 1. Human ATP-binding cassette proteins.

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Human ABC protein</th>
<th>Physiological role (known and probable)</th>
<th>Disease</th>
<th>Structure</th>
<th>Select citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC1 B</td>
<td>ABCB1</td>
<td>Efflux of xenobiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCB2</td>
<td>Peptide transport associated with antigen processing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCB3</td>
<td>Multidrug resistance</td>
<td>Immune deficiency</td>
<td>Oldham et al. 2016</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCB4</td>
<td>Phospholipid excretion into bile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCB5</td>
<td>Efflux of xenobiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCB6</td>
<td>Porphyrin transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCB7</td>
<td>Transport substrate involved in the mitochondrial iron homoeostasis</td>
<td>X-linked sideroblastic anemia with ataxia</td>
<td>Aikens et al. 1996</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCB8</td>
<td>Mitochondrial iron and glutathione export; efflux of xenobiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCB9</td>
<td>Yeast and mammalian lipid transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCB10</td>
<td>Involvement in bone homeostasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCB11</td>
<td>Bile salt absorption into bile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>ABCC1</td>
<td>Multispecific organic ion transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCC2</td>
<td>Renal and biliary elimination of organic anions</td>
<td>Dublin-Johnson syndrome</td>
<td>Martin et al. 2013</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCC3</td>
<td>Organic anion transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCC4</td>
<td>Nucleotide transport; anion-coupled drug efflux</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCC5</td>
<td>Nucleotide and glutamate conjugate transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCC6</td>
<td>Transport of organic anions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCC7</td>
<td>Epithelial chloride channel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCC8</td>
<td>Modulation of associated potassium channels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCC9</td>
<td>Efflux of xenobiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCC10</td>
<td>Anionic hydrophilic solute transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. adrenoleukodystrophy-related proteins, ADD, 4 members</td>
<td>ABCD1</td>
<td>Long and very long chain fatty acid transport</td>
<td>Adrenoleukodystrophy</td>
<td>Tammur et al. 2001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCD2</td>
<td>Branched chain fatty acid transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCD3</td>
<td>Possible role in vitamin E2 transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCD4</td>
<td>Cholesterol and phospholipid transport</td>
<td>Tangier disease; familial high-density lipoprotein deficiency</td>
<td>Qian et al. 2017</td>
<td></td>
</tr>
<tr>
<td>ABC2 A</td>
<td>ABCA1</td>
<td>Phospholipid transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCA2</td>
<td>Phospholipid transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCA3</td>
<td>Phospholipid transport; transport of retinoid</td>
<td>Neonatal surface deficiency</td>
<td>Conners et al. 1991</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCA4</td>
<td>Transport of retinoid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCA5</td>
<td>Nucleotide and glutamate conjugate transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCA6</td>
<td>Role in macrophage lipid homeostasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCA7</td>
<td>Phospholipid and sphingolipid transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCA8</td>
<td>Cholesterol and retinolide transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCA9</td>
<td>Role in macrophage lipid homeostasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCA10</td>
<td>Role in macrophage lipid homeostasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCA11</td>
<td>Sphingolipid transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCA12</td>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. five members</td>
<td>ABCG1</td>
<td>Cholesterol and phospholipid transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCG2</td>
<td>Efflux of xenobiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCG3</td>
<td>Cholesterol transport</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCG4</td>
<td>Cholesterol and plant sterol efflux</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCG5</td>
<td>Cholesterol and plant sterol efflux</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCG8</td>
<td>Cholesterol and plant sterol efflux</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. one member</td>
<td>ABCF1</td>
<td>Role in translation initiation and ribosome recycling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCF2</td>
<td>Regulation of innate immune response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABCF3</td>
<td>Role in cell volume regulation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** The human ABC proteins from the subfamilies ABCA-C can be classified into two groups: transporters and non-transporters. Information on the physiological functions and disease phenotypes was obtained from www.genesandcancer.org and https://www.ncbi.nlm.nih.gov/entrez/query.fcgi, respectively.

(Xavier et al, BCB, 2019)
ABC transporter structures have come a long way …

Crystal structure of HisP
- ATP-binding subunit
- Right structure, wrong model


Crystal structure of Rad50
- ATP-binding cassette
- Right structure, right model

(Hopfner et al, Cell, 2000)
ABC transporter structures have come a long way ...

Crystallographic dimer ≠ biological dimer

(Yuan et al, J Biol Chem, 2001)
ABC transporter structures have come a long way ...

ATP sandwich model

(Smith et al, Mol Cell, 2002)
ABC transporter structures have come a long way ...

Crystal structure of MsbA
- Full transporter structure
- Wrong model

Crystal structure of BtuCD
- Full transporter structure
- Correct model

(Chang & Roth, Science, 2001; retracted 2006)

(Locher et al, Science, 2002)
ABC-coupled transport: a simple idea

(Locher, Nat Struct Mol Biol, 2016)
ABC-coupled transport: not that simple!
ABC-coupled transport: not that simple!

(Thomas et al, FEBS Lett, 2020)
So, ...

- High-degree of structural diversity in the transmembrane domains of ABC transporters.

- The structural variability (likely) determines the functional diversity of ABC transporters.

- Transport mechanism is (likely) individually distinct.
Part II:

STRUCTURAL STUDIES OF MULTIDRUG RESISTANCE TRANSPORTERS
Multidrug Resistance (MDR) in Mammalian Cells

Biochimica et Biophysica Acta, 455 (1976) 152–162
© Elsevier/North-Holland Biomedical Press

BBA 77508

A SURFACE GLYCOPROTEIN MODULATING DRUG PERMEABILITY IN CHINESE HAMSTER OVARY CELL MUTANTS

R. L. JULIANO a, c and V. LING b, c

aResearch Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, bThe Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario and cThe Department of Medical Biophysics, University of Toronto, 500 Sherbourne Street, Toronto, Ontario (Canada)

(Received April 23rd, 1976)
Multidrug Resistance (MDR) in Mammalian Cells

Daunorubicin-resistant Chinese Hamster Ovary Cells Expressing Multidrug Resistance and a Cell-Surface P-Glycoprotein

Norbert Kartner, Michael Shales, John R. Riordan, and Victor Ling

Ontario Cancer Institute, Princess Margaret Hospital and Department of Medical Biophysics, University of Toronto, Toronto M4X 1K9 [N. K., M. S., V. L.], and Research Institute, The Hospital for Sick Children and Departments of Biochemistry and Clinical Biochemistry, University of Toronto, Toronto M5G 1X8 [J. R. R.], Ontario, Canada

6th Annual San Antonio Breast Cancer Symposium

Multidrug resistance

V. Ling, J. Gerlach, and N. Kartner
Department of Medical Biophysics, University of Toronto and the Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario, Canada M4X 1K9
P-glycoprotein (Pgp/MDR1/ABCB1): the first mammalian ABC transporter reported.

Fig. 1. Immunofluorescent detection of multidrug-resistant CHO cells. (A) Mixture of sensitive (AuxB1) and resistant (CHRC5) CHO cells photographed using phase contrast microscopy. (B) The same field using fluorescence microscopy revealing resistant cells heavily labeled with an antibody specific for multidrug-resistant CHO cells. The antibody was produced by injecting rabbits with plasma membranes from CHRC5 cells. It was then extensively absorbed with glutaraldehyde-fixed, sensitive (AuxB1) cells. Binding to azide-poisoned cells was detected with a fluorescein-labeled, swine antibody to rabbit immunoglobulins.

The identical molecular weight and immunological cross-reactivity observed in mammalian cells from different species indicated that P-glycoprotein is a highly conserved membrane component. In addition, it demonstrates that increased expression of P-glycoprotein is a good indicator of the multidrug resistance phenotype.

The intimate association of P-glycoprotein with multidrug resistance is also demonstrated by other studies. The level of P-glycoprotein expression correlates with the degree of drug resistance (1-4). P-glycoprotein expression is greatly reduced in revertant cells (4, 24) and is expressed in cells transfected with DNA from multidrug-resistant cells (12). Furthermore, increased expression of P-glycoprotein correlates with increased numbers of DMs and increased multidrug resistance in a mouse line (16). We speculate, therefore, that gene amplification results in over-expression of P-glycoprotein and expression of the multidrug resistance phenotype.

Whether or not over-expression of P-glycoprotein alone is sufficient to mediate the multidrug resistance phenotype is still not known. Molecular cloning of the P-glycoprotein gene and transfer of the purified gene into drug-sensitive cells to test for function may provide a definitive answer to this question. Different changes associated with multidrug resistance have been reported (10, 15, 19). How these changes relate to over-expression of P-glycoprotein is not known. At present, P-glycoprotein is the best molecular indicator of the multidrug resistance phenotype.

Concluding remarks

The questions of whether or not multidrug resistance mutations do in fact occur in human cancers, and whether they play a role in the development of therapy-resistant disease are important ones with wide implications. For example, if chemotherapy with the current repertoire of antineoplastic agents is indeed limited by the occurrence of multidrug resistance mutations, then new drugs will be re-
P-glycoprotein (Pgp/MDR1/ABCB1): the first mammalian ABC transporter reported.

(Ling et al, Breast Cancer Res Treat, 1984)
MDR1 (ABCB1), MRP1 (ABCC1) and BCRP (ABCG2)

(Liu et al, Oncotarget, 2016)
MDR1 (ABCB1), MRP1 (ABCC1) and BCRP (ABCG2)

(Gomez-Zepeda et al, Pharmaceutics, 2019)
P-glycoprotein as the model system for MDR

(Senior et al, FEBS Lett, 1995)
P-glycoprotein as the model system for MDR

(Sharom, Front Oncol, 2014)
P-glycoprotein as the model system for MDR

Low-resolution EM models:

(A) (B)

(Rosenberg et al, J Biol Chem, 1997)
P-glycoprotein as the model system for MDR

(Lee et al, J Biol Chem, 2002)
P-glycoprotein as the model system for MDR

(Lee et al, J Biol Chem, 2008)

(Aller et al, Science, 2009)
Conformational Landscape of P-glycoprotein

(Frank et al, Mol Pharmacol, 2016)
Conformational Landscape of P-glycoprotein
Dynamics of P-glycoprotein
hydrogen-deuterium exchange mass spectrometry (HDX-MS)

(Kopcho et al, Sci Rep, 2019)
Dynamics of P-glycoprotein

Double electron electron resonance (DEER) spectroscopy

(Dastvan et al, Science, 2019)
A wide variety of chemically diverse compounds could reverse MDR in resistant cancer cells via inhibition of P-gp function/expression.

*(Dong et al, Drug Resist Updat, 2020)*
Beyond P-glycoprotein

ABCG2/BCRP/MXR

ABCC1/MRP1

(Robey et al, Nat Rev Cancer, 2018)
Part III: STRUCTURAL STUDIES OF LIPID TRANSPORTERS
Canalicular ABC Transporters

(Saad et al, Int J Mol Sci, 2021)
ABC Transporters in the Brain

(Pereira et al, J Alzheimers Dis, 2018)
Transporting Cholesterol (A Simple View)

Guts
- Dietary sterols
- ABCG5/G8
- Bile
- Cholesterol

Plasma / Tissues
- Liver
- ABCA1
- Apolipoproteins
- Peripheral tissues
- ABCA1, G1
- Cholesterol
- HDL
- LDL
- LDLR
- PCSK9

Small intestine
- FORWARD
- REVERSE
Cholesterol: a Risk Factor of Cardiovascular Diseases
ABCG5/G8: patients, genetics, animal model
ABCG5/G8: human nutrition

Dietary sterols

Animal (60%)

Plant (40%)

ABSORPTION:

~50%

< 5%

Cholesterol

β-Sitosterol

Campesterol

Sitosterol

Campesterol
ABCG5/G8: patients

**Sitosterolemia**  
(Bhattacharyya & Conner, 1974, JCI)

---

**Table 1.** Plasma sterol concentration

<table>
<thead>
<tr>
<th>Patient Age</th>
<th>Sex</th>
<th>Cholesterol (mg/dL)</th>
<th>Cholestanol (mg/dL)</th>
<th>Campesterol (mg/dL)</th>
<th>Campestanol (mg/dL)</th>
<th>Sitosterol (mg/dL)</th>
<th>Sitostanol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y7</td>
<td>Ke.</td>
<td>C. (n = 10)</td>
<td>Ke. C. (n = 10)</td>
<td>T. C. (n = 10)</td>
<td>R. C. (n = 10)</td>
<td>c. L.</td>
<td>M.</td>
</tr>
<tr>
<td>24 F</td>
<td>18 F</td>
<td>22 F</td>
<td>16 M</td>
<td>52 M</td>
<td>7 M</td>
<td>32 F</td>
<td>30 F</td>
</tr>
<tr>
<td>245 ± 39</td>
<td>202 ± 25</td>
<td>233 ± 12</td>
<td>249 ± 39</td>
<td>134 ± 12</td>
<td>202 ± 12</td>
<td>207 ± 12</td>
<td>368 ± 12</td>
</tr>
<tr>
<td>6.7 ± 1.1</td>
<td>4.7 ± 1.0</td>
<td>3.8 ± 1.4</td>
<td>7.5 ± 2.4</td>
<td>1.6 ± 0.6</td>
<td>1.2 ± 0.6</td>
<td>2.5 ± 0.6</td>
<td>3.6 ± 0.6</td>
</tr>
<tr>
<td>11 ± 2.3</td>
<td>12 ± 2.3</td>
<td>10 ± 2.3</td>
<td>13 ± 1.5</td>
<td>13 ± 1.5</td>
<td>12 ± 1.5</td>
<td>10 ± 1.5</td>
<td>29 ± 1.5</td>
</tr>
<tr>
<td>20 ± 2.3</td>
<td>14 ± 4.1</td>
<td>21 ± 8.3</td>
<td>20 ± 5.5</td>
<td>27 ± 6.5</td>
<td>18 ± 6.5</td>
<td>27 ± 6.5</td>
<td>19 ± 6.5</td>
</tr>
</tbody>
</table>

**Mean ± SD:** 258 ± 96  

---

In separate experiments, the A5-sterols, cholesterol, campesterol, and sitosterol were separated from their 5a-dihydro derivatives cholestanol, 5a-campestanol, and 5a-sitostanol by argentation thin-layer chromatography, and were then quantitated as their trimethylsilyl ether derivatives by gas-liquid chromatography on 180 cm x 4 mm glass columns packed with 3% QF-1 (Applied Science Lab, State College, PA). The retention times relative to 5a-cholestane of the trimethylsilyl ether derivatives are: cholesterol 1.73, cholestanol 1.85, campesterol 2.52, 5a-campestanol 2.66, sitosterol 3.03, and 5a-sitostanol 3.17.

It is necessary to separate the unsaturated sterols from their 5a-saturated derivatives by argentation thin-layer chromatography because only small differences exist between the unsaturated and 5a-saturated sterol peak retention times on QF-1 (2). However, quantitative results by the two independent methods agreed within ± 10%.

Lipoproteins were separated by the method of Havel, Eder, and Bragdon (14). After separation of the low density and high density lipoprotein fractions, the proportions of free and esterified sterols were measured in each fraction (11).

**RESULTS**

Plasma concentrations of campesterol and sitosterol (Table 1) were markedly elevated in all fourteen clinically affected subjects, consistent with previous findings that this is the major biochemical determinant in establishing the diagnosis of this condition (1-10). Plasma cholesterol levels were elevated in seven of fourteen subjects, which was a finding of importance. Approximately 80% of the unsaturated sterols were cholesterol and 16% were plant sterols. Of the remaining plasma sterols, cholestanol concentrations were increased in all subjects and the mean percentage of total sterols was 80.5 ± 1.3. The percentage of respective A5-derivative was 5.0 ± 0.9 for campesterol and 1.3 ± 0.1 for sitosterol.

**14 patients:**

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Sitosterolemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitosterol (mg/dL)</td>
<td>0.3 ± 0.3</td>
<td>35 ± 16 (50-120x)</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>187 ± 29</td>
<td>258 ± 29</td>
</tr>
</tbody>
</table>

(Salen et al, J Lipid Res, 1985)
ABCG5/G8: genetics

Autosomal recessive & rare genetic disorder

(2p21)

(Berge et al, 2000, Science; Lee et al, 2001, Nat Genet; Lu et al, 2001, AJMG)
ABCG5 and ABCG8 are half ABC transporters.

A: Walker A motif
   (GxxGxGKS/T)
B: Walker B motif
   (φφφφDE)

S: ABC signature motif
   (φSGGQ/E)
φ: hydrophobic amino acids
ABCG5/G8: genetics

Last stop of reverse cholesterol transport (RCT)

Trans-intestinal cholesterol efflux (TICE)
ABCG5/G8 promotes biliary and intestinal sterol secretion (liver/small intestine specific).
ABCG5/G8: animal model

-/-

(+/+)

(Yu et al, 2002, PNAS)

(G5G8\textsuperscript{-/-})

bile extraction

-G5G8

mG5:

mG8:

Calnexin:

Precursor

Mature

(Graf et al, 2003, JBC)
ABCG5/G8: animal model

Functional asymmetry

(Zhang et al, 2006, JBC; Wang et al, 2011, JBC)
Large-scale Purification of Human G5G8

Tandem Affinity Chromatography: (Pichia pastoris yeast)

- Ni-NTA
- CBP
- Gel filtration

hG5: RGS-His$_2$-G-His$_6$
- hG8: 3C
- CBP

3C cleavage site
CBP: calmodulin-binding peptide

Ni-NTA: (2-3 mg/L)
CBP: (1-2 mg/L)
Stable and Monodisperse G5G8

Analytical gel filtration:

Negative-stained TEM single particles:
(FEI Tecnai G2)
Optimization of Protein Preparation

1. **Shaker culture (4-5 days)**
   - Membrane Preparation

2. **Solubilization (β-DDM)**
   - Solubilization
   - Membrane Preparation

3. **1° Ni-NTA**
   - 1° Ni-NTA

4. **1° CBP**
   - 1° CBP

5. **Detergent Exchange**
   - Detergent Exchange
   - DDM
   - MNG
   - + Endo H
   - + 3C protease

6. **Crystallization (manual/robot set-up)**
   - Crystallization
   - (manual/robot set-up)
   - (cholesterol)

7. **Crystal growth (2 wks – 6 mths)**
   - Crystal growth
   - (2 wks – 6 mths)
   - ± Mg/ATP

8. **PD-10 (desalting)**
   - PD-10
   - (desalting)

9. **2° Ni-NTA**
   - 2° Ni-NTA

10. **Relipidation**
    - Ligands
    - ± ATPase inhibitor
    - Relipidation
    - Synthetic phospholipids (Avanti)

11. **Methylation (CH₃-Lys)**
    - Methylation
    - (CH₃-Lys)

12. **Alkylation (Cys capping)**
    - Alkylation
    - (Cys capping)

13. **Gel filtration**
    - Gel filtration

14. **Synthetic phospholipids (Avanti)**
    - Synthetic phospholipids
    - (Avanti)

15. **Maltose Neopentyl Glycol (MNG)**
    - Maltose Neopentyl Glycol

16. **DDM (Decyl Maltoside)**
    - Decyl Maltoside

17. **Detergent Exchange**
    - Detergent Exchange

18. **Alkylation (Cys capping)**
    - Alkylation
    - (Cys capping)

19. **Synthetic phospholipids (Avanti)**
    - Synthetic phospholipids
    - (Avanti)
Bicelle Crystallization (Lipid Bilayers)

G5G8 bicelle preparation

Crystal growth & X-ray diffraction

- **Long exposure**
  - 2-5 sec @ APS
  - 30 sec @ ALS

- **Radiation damage**
  - 3-5 frames (<5°)

- **Signal**
  - $I/\sigma = 1-1.5$ at 3.9-4Å
Optimization of Crystal Growth

A

B

C

50-500 µm
25-30 Å

100-300 µm
7-10 Å

50-150 µm
3.5-4 Å

Detergent chain length
C1
C1

Cholesterol
0-1 %
2-5 %
(mol)
Single-wavelength Anomalous Diffraction (SAD)

W-SAD

W: dodeca-tungsten cluster
(phosphotungstate: $\text{PW}_{12}\text{O}_{40}^{3-}$)

Keggin, Proc R Soc Lond A, 1934
Zhang & Wang, RSC Adv, 2012

(Lee et al, Nature, 2016)
Crystal Packing
# Model Building for the G5G8 Structure

<table>
<thead>
<tr>
<th><strong>Space group</strong></th>
<th>1 2 2 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell dimensions</strong></td>
<td></td>
</tr>
<tr>
<td>$a$, $b$, $c$ (Å)</td>
<td>173.6, 224.8, 253.3</td>
</tr>
<tr>
<td><strong>Resolution (Å)</strong></td>
<td>50-3.9 (3.93-3.9)</td>
</tr>
<tr>
<td>$R_{sym}$ or $R_{merge}$</td>
<td>16.1 (NA)</td>
</tr>
<tr>
<td>$&lt;I&gt;/&lt;\sigma(I)&gt;$</td>
<td>8.8 (0.15)</td>
</tr>
<tr>
<td><strong>Completeness (%)</strong></td>
<td>99.4 (84.2)</td>
</tr>
<tr>
<td><strong>Redundancy (%)</strong></td>
<td>18.9 (2.5)</td>
</tr>
</tbody>
</table>

### Refinement

<table>
<thead>
<tr>
<th><strong>Resolution (Å)</strong></th>
<th>25-3.94</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. reflections</strong></td>
<td>34,889</td>
</tr>
<tr>
<td>$R_{work}$ / $R_{free}$</td>
<td>24.5 / 32.9</td>
</tr>
<tr>
<td><strong>No. atoms</strong></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>18,151</td>
</tr>
<tr>
<td><strong>R.m.s deviations</strong></td>
<td></td>
</tr>
<tr>
<td>Bond lengths (Å)</td>
<td>0.010</td>
</tr>
<tr>
<td>Bond angles (°)</td>
<td>1.64</td>
</tr>
</tbody>
</table>

---

Merge: 19 datasets (least amorphous)
(Collect: ~200 native datasets)
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)
Triple Helical Bundle: Connecting the ATP-Binding Cassette to the Transmembrane Domain

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

G5
Walker A
Walker B
TMD
NBD

G8

<table>
<thead>
<tr>
<th>Species</th>
<th>G5</th>
<th>E-helix</th>
<th>CnH</th>
<th>CpH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo sapiens</td>
<td>133</td>
<td>SFLDYE</td>
<td>NKGLVLRLRVTRNLVRH-LRAVSQTDQSQDG</td>
<td>458</td>
</tr>
<tr>
<td>Mus musculus</td>
<td>134</td>
<td>SFLDYE</td>
<td>NKGLVLRLRVTRNLVRH-LRAVSQTDQSQDG</td>
<td>459</td>
</tr>
<tr>
<td>Gallus gallus</td>
<td>126</td>
<td>SFLDYE</td>
<td>NKGLVLRLRVTRNLVRH-LRAVSQTDQSQDG</td>
<td>451</td>
</tr>
<tr>
<td>Xenopus tropicalis</td>
<td>135</td>
<td>SFLDYE</td>
<td>NKGLVLRLRVTRNLVRH-LRAVSQTDQSQDG</td>
<td>460</td>
</tr>
<tr>
<td>Danio rerio</td>
<td>137</td>
<td>SFLDYE</td>
<td>NKGLVLRLRVTRNLVRH-LRAVSQTDQSQDG</td>
<td>462</td>
</tr>
<tr>
<td>Homo sapiens</td>
<td>152</td>
<td>SFLDYE</td>
<td>NKGLVLRLRVTRNLVRH-LRAVSQTDQSQDG</td>
<td>498</td>
</tr>
<tr>
<td>Mus musculus</td>
<td>153</td>
<td>SFLDYE</td>
<td>NKGLVLRLRVTRNLVRH-LRAVSQTDQSQDG</td>
<td>492</td>
</tr>
<tr>
<td>Gallus gallus</td>
<td>154</td>
<td>SFLDYE</td>
<td>NKGLVLRLRVTRNLVRH-LRAVSQTDQSQDG</td>
<td>487</td>
</tr>
<tr>
<td>Xenopus tropicalis</td>
<td>154</td>
<td>SFLDYE</td>
<td>NKGLVLRLRVTRNLVRH-LRAVSQTDQSQDG</td>
<td>496</td>
</tr>
<tr>
<td>Danio rerio</td>
<td>158</td>
<td>SFLDYE</td>
<td>NKGLVLRLRVTRNLVRH-LRAVSQTDQSQDG</td>
<td>498</td>
</tr>
</tbody>
</table>

**Signature**

**Walker A**

**Walker B**

**TMD**

**NBD**
Triple Helical Bundle: Connecting the ATP-Binding Cassette to the Transmembrane Domain

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

<table>
<thead>
<tr>
<th>Species</th>
<th>G5</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</td>
<td>LQEDT-VYQTLHLYALLA---FKKLGVLLRRYVRHRHNLRAVSDEEQQDQLY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mus musculus</td>
<td>134</td>
<td>LQEDT-VYQTLHLYALLA---FKKLGVLLRRYVRHRHNLRAVSDEEQQDQLY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallus gallus</td>
<td>126</td>
<td>PNDIA-ISHLYALLA---ISKLNLFLRILLSFRED-LRAISDEQSKDGLY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xenopus tropicalis</td>
<td>135</td>
<td>LQEDT-VYQTLHLYALLA---LSKYVVLTLLEPFLRLSRLD-LRAIDIQQSDKQGLY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zanio reflexus</td>
<td>137</td>
<td>LQEDT-VYQTLHLYALLA---ISKLGVLLRRYVRHRHNLRAVSDEEQQDQLY</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

G8

<table>
<thead>
<tr>
<th>Species</th>
<th>G8</th>
<th>Q-loop</th>
<th>E-helix</th>
<th>CnH</th>
<th>CpH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo sapiens</td>
<td>152</td>
<td>FGDDQ-VQTLAFIQLRNLQ---VQQTTLERQGQSNFRD-FRAMLYYELEDQLY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mus musculus</td>
<td>153</td>
<td>FGDDQ-VQTLAFIQLRNLQ---VQQTTLERQGQSNFRD-FRAMLYYELEDQLY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallus gallus</td>
<td>154</td>
<td>FGDDQ-VQTLAFIQLRNLQ---VQQTTLERQGQSNFRD-FRAMLYYELEDQLY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xenopus tropicalis</td>
<td>154</td>
<td>FGDDQ-VQTLAFIQLRNLQ---VQQTTLERQGQSNFRD-FRAMLYYELEDQLY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zanio reflexus</td>
<td>158</td>
<td>FGDDQ-VQTLAFIQLRNLQ---VQQTTLERQGQSNFRD-FRAMLYYELEDQLY</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Signature

Walker A

Walker B

TMD

NBD
The TMD polar relay connects the triple helical bundle to the TMD.
TMD Polar Relays: a General Feature?

Polypeptide processing and secretion transporter:

PCAT1 (nf → ATPγS)

Maltose transporter:

MalK2EGF (pre-T → ATP)


(Oldham et al, Nature, 2007)

(Oldham & Chen, Science, 2011)

(Lee et al, Nature, 2016)
Co-Evolution Analysis

a) Coevolving residue pairs: ≤ 8 Å (within respective TMD)
   - G5: LLS1AGVLV
   - G8: ALYN-SFYL

b) Coevolving residue pairs: > 8 Å (candidate protein interface residues)
   - G5: ALPVTGMLN
   - G8: LIIPFNVILD
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)
Teaching and learning genetics with Drosophila. 2. Mutant phenotypes of Drosophila melanogaster

(Ranganath & Tanuja, 1999)
M429V
R406Q
R389H
E146Q
G269R
C287R
Q604E/K
M622V
R550S
A540F
N437K
M429V
E423D
L501P
T400K
R405H
M429V
E423D
L501P
T400K
R405H
G269R
C287R
A259V
R184H
P231T
L195Q
A259V
R184H
P231T
L195Q
(Xavier et al, Int J Mol Sci, 2020)
Location of the residues with the disease-causing missense mutations of sitosterolemia.

ER-escape missense mutations

- G574R
- E146Q
- R419H/P
- R543S

Non-ER-escape missense mutations

- R389H
- N437K
- G574E
- L596R
- L501P
- R184H
- P231T
- R263Q
- G5
- G8

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.
Trajectory of Domain Movement

Molecular Dynamics Simulation

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

Upward movement
(TM helices)

(Lee et al, Nature, 2016)
Trajectory of Domain Movement

(Zein et al, Biochem Soc Trans, 2019)
Using disease mutations to gain mechanistic understanding
CHS-Stimulated ATPase Activity of ABCG5/G8

A

Cholesterol

Cholesteryl hemisuccinate

Cholic acid

B

Mg$^{2+}$ ATP

Mg$^{2+}$ ADP Pi

CHS

Phospholipid

Cholate

DDM
ATP Dependence of ABCG5/G8

(Xavier et al, Int J Mol Sci, 2020)
**CHS Dependence of ABCG5/G8**

*(Xavier et al, Int J Mol Sci, 2020)*

<table>
<thead>
<tr>
<th></th>
<th>V\textsubscript{max} (nmol/min/mg)</th>
<th>K\textsubscript{M} (CHS) (mM)</th>
<th>k\textsubscript{cat} (s\textsuperscript{-1})</th>
<th>k\textsubscript{cat}/K\textsubscript{M} (M\textsuperscript{-1}s\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>702.9 ± 50.7</td>
<td>0.8</td>
<td>1.74</td>
<td>2.2x10\textsuperscript{3}</td>
</tr>
<tr>
<td>G5-E146Q</td>
<td>210.0 ± 33.2</td>
<td>1.13</td>
<td>0.52</td>
<td>0.46x10\textsuperscript{3}</td>
</tr>
<tr>
<td>G8-R543S</td>
<td>237.1 ± 33.4</td>
<td>2.38</td>
<td>0.59</td>
<td>0.25x10\textsuperscript{3}</td>
</tr>
<tr>
<td>G5-A540F</td>
<td>99.8 ± 11.4</td>
<td>0.70</td>
<td>0.25</td>
<td>0.36x10\textsuperscript{3}</td>
</tr>
</tbody>
</table>
A Catalytic Model of ABCG5/G8

(Xavier et al, Int J Mol Sci, 2020)
Beyond ABCG5/G8

**ABCA1** - cholesterol

(Qian et al, Cell, 2017)

**ABCA4** - phospholipid import

(Liu et al, eLife, 2021)

**ABCB4** - phospholipid export

(Olsen et al, Nat Struct Mol Biol, 2020)
Transmembrane Domain of ABC Cholesterol Transporters: a Pathogenic Hot Spot

Polar relay

Pathogenic residues: G5G8 (red), A1 (green)

(Xavier et al, Biochem Cell Biol, 2019)
Transmembrane Domain: the Dynamic Nature (Probably at an ATP-Prehydrolytic state)

(Xavier et al, Biochem Cell Biol, 2019)
Working Model of ABC Sterol Transporters
(CELLULAR)

(Xavier et al, Biochem Cell Biol, 2019)