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Moving out: from sterol transport to drug resistance – the ABCG subfamily of efflux pumps

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Abstract

The ATP binding cassette (ABC) proteins are typically ATP-driven transmembrane pumps that have been evolutionarily conserved from bacteria to humans. In humans these transporters are subdivided into seven subfamilies, ranging from A to G. The ABCG subfamily of transporters is the primary focus of this review. This subfamily of proteins has been conserved throughout evolution and plays a central role in several cellular processes, such as sterol homeostasis and multidrug resistance. Functional polymorphisms/mutations in some of these G-subfamily transporters have clinical consequences in humans.

Gene organization and evolutionary history

ABC transporter genes are vital for numerous cellular processes. The human genome encodes 48 of these genes, a number of which are medically relevant. In humans, the 48 ABC proteins are divided into seven subfamilies ranging from A to G, based on structural arrangements and phylogenetic analysis (1, 2). The ABCG subfamily consists of five members in humans: *ABCG1*, *ABCG2*, *ABCG4*, *ABCG5* and *ABCG8*. Rodents have an extra member, *Abcg3*. The *ABCG* genes are located on specific chromosomes in humans and in mice (see Table 1). Human *ABCG* genes have numerous splice variants, which are listed in Table 2. The G subfamily genes are para logs of the *Drosophila* gene – *white*, which in conjunction with its heterodimeric partners *scarlet* or *brown* plays a pivotal role in cellular uptake of eye pigment precursors (4–6).

The ABCG genes have evolved from an ancestral gene by the dual processes of ‘gene birth’ and ‘gene death’. Birth-and-death evolution is a form of independent evolution in which new genes are created by repeated gene duplication. Following gene birth, some duplicate genes may stay in the genome for a long-time, while other genes may be deleted or become non-functional in gene death (7, 8).

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An active gene duplication process occurring in the ABC transporter family in vertebrates has previously been documented (9). This process includes ancient events, such as apparent whole-genome duplication in fish, as well as more recent occurrences, such as the duplication of the *Abcg3* gene (from *Abcg2*), which is specific to rodents (9, 10).

This trend of gene birth and gene death is reflected in the evolution of the ABCG family of transporters as shown in Figure 1. The 'G' family initially evolved from an ancestral transporter gene. In rodents, *Abcg2* and *Abcg3* duplicated (gene birth) and became localized on separate chromosomes and probably evolved to have a specialized function (we do not have evidence to suggest that *Abcg3* was ever in primates). ABCG5, however, underwent duplication to give rise to ABCG8, and both remained on the same chromosome but became obligate heterodimers with a common functional role. The phylogenetic tree depicting the evolution of 'G' subfamily members is shown in Figure 2.

Characteristic structural features

Proteins are classified as ABC transporters based on the sequence and organization of their ATP-binding domain(s), also known as nucleotide-binding domains (NBDs). The NBDs include characteristic motifs (Walker A and B) found in all ATP-binding proteins. The ABC genes also contain an additional element, known as the signature motif (C-loop), located upstream of the Walker B site (12). The majority of functional ABC transporters contain two NBDs and two transmembrane domains (TMDs). The TMDs typically contain six to 12 membrane-spanning α -helices (see Figure 3) and are responsible for determining substrate specificity.

The ABCG family transporters are half-transporters containing one TMD and one NBD. They have a unique structural organization, with the NBD situated at the N-terminal half of the transporter followed by the TMD. Typically these half-transporters are required to form obligate homo- or hetero-dimers to carry out their specific functional activities (13). The structural organization for a typical ABCG transporter – human ABCG2 – is depicted in Figure 4. The structural analysis was carried out with TopPredII and the topological structure generated with TOPO2.

Localization, function and mechanics of human ABCG family members

ABCG1

Human ABCG1 is almost ubiquitous in expression, having high expression mostly in the lung, brain, kidney and spleen (16), see Table 3. The cellular localization of ABCG1 is controversial, since cell surface as well as intracellular localization has been reported (18, 19).

ABCG1 is known to play a significant role in cholesterol efflux from macrophages to extracellular lipid acceptors including high-density-lipoprotein (HDL) and phosphatidyl choline (PC) vesicles (20). *ABCG1* knockout mice fed with high cholesterol and high fat diet have been shown to accumulate cholesterol and lipids in the macrophages and the liver, while mice fed with a normal diet did not (16).

ABCG1 has also been documented to play a role in T-cell proliferation (21) and has a protective role for apoptosis in macrophages (22). No functional mutations in this transporter have yet been linked to human disease (13).

ABCG2

The *ABCG2* gene is also known as ABCP because of its high expression in the placenta (23, 24). It has been found to reduce the accumulation of anthracycline drugs in the MCF7/AdrVp tumor cell line and was named BRCP or breast cancer-resistance protein (24). Subsequently, it was also named MXR (25) because it was overexpressed in colon carcinoma cells that were resistant to the drug mitoxantrone. ABCG2 is additionally found to be localized in the plasma membrane of brain (26), liver, colon, small intestine and mammary gland (27), see Table 3.

ABCG2, unlike its other family members, plays a central role in conferring drug resistance to several substrates including, but not limited to, mitoxantrone and camptothecins (see Table 3), suggesting that it has a broad substrate specificity. It has been documented to have a number of non-synonymous (non-amino acid-changing) single nucleotide polymorphisms in humans (see Table 4).

ABCG2 has also been linked to the side population phenotype of stem cells. The side population (SP) is a subset of stem cells that has a high capacity to efflux antimitotic drugs and the fluorescent dye Hoechst 33342 (29, 30).

The cancer stem cell model of drug resistance postulates that tumors possess a population of pluripotent drug-resistant cells that can survive chemotherapeutic insult and regrow to form a drug-resistant tumor. It has been found that cancer stem cells express a number of ABC transporters including ABCG2 (31). Recently, it was discovered that ABCG2 has the capacity to efflux urate and a single nucleotide polymorphism rs2231142 in exon five of this transporter has been associated with gout (32).

ABCG3

ABCG3 is absent in primates and most other mammals but was found to be present in specific rodents, such as the mouse and rat (33). Expression analysis with reverse-transcriptase polymerase chain reaction showed that it was expressed in the mouse thymus and spleen (33). ABCG3 was also shown to have a potential aberrant NBD structure, leading to speculation on whether it could function as a transporter (33). Currently, its function is unknown.

ABCG4

The fourth member of the ‘G’ family is ABCG4. The tissue expression of ABCG4 is restricted to certain, organs, such as the brain, spinal cord, heart and thymus in humans (34), see Table 3. Subsequently, it was also identified in the neural layer of the retina (35).

ABCG4 exhibits 82 % amino acid similarity with ABCG1, suggesting that it may possess functional similarity (34, 35). It was found that, similar to ABCG1, ABCG4 facilitates the efflux of cellular cholesterol to HDL (20).

ABCG4 levels were found to be increased in microglial cells in the brain that were located adjacent to plaques in patients with Alzheimer's disease (36), however, there is currently no compelling evidence to link ABCG4 to Alzheimer's disease.

ABCG5 and ABCG8

ABCG5 and *ABCG8* genes are arranged head-to-head on chromosome two in humans (see Table 1). They were both overexpressed on liver-X-receptor (LXR) activation and were subsequently cloned (37, 38). The ABCG5 and ABCG8 half-transporters form obligate heterodimers that are expressed on the apical plasma membranes of both enterocytes and hepatocytes (39), see Table 3, where they are involved in the efflux of sterols from the enterocytes back into the intestinal lumen and can also transport plant sterols and cholesterol from the hepatocytes into the bile, limiting the absorption of these sterols into the body (40).

Mutations in this pair of transporters have been associated with sitosterolemia, a disease characterized by the accumulation of plant sterols and cholesterol, which may lead to premature atherosclerosis (41). A genome-wide association study has also found that a single nucleotide polymorphism (SNP) (D19H) in ABCG8 is a susceptibility factor for human gallstone disease (42).

Functional implications of gene birth and death in the ABCG subfamily and role in human disease

The ABCG family transporter genes exhibit high conservation in mammals. Duplication of genes has played a central role in the evolution of new gene functions and these duplicated genes may undergo certain evolutionary fates that correspond to the process of gene birth or death.

The birth and death of genes can keep the number of genes in a gene family in a state of balance. Defects in ABCG family proteins are involved in various human diseases, such as sitosterolemia, gout and clinical drug resistance; hence, studying the evolution of these transporters in other vertebrate species will aid in developing animal models for functional studies. The analysis of species-specific genes, like *Abcg3* (found only in rodents), can provide information on unique biology in individual species.

In-depth knowledge of these transporters will help us to probe disease mechanisms and design potential therapies against cancer and genetic diseases associated with ABC-transporter dysfunction.

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Figure 1.

Evolution of G subfamily transporters by gene birth and gene death. The 'G' subfamily initially evolved from an ancestral transporter gene (yellow). In rodents ABCG2 and ABCG3 duplicated and became localized on separate chromosomes. ABCG5 underwent duplication to give rise to ABCG8 (green). In primates ABCG3 was lost.

Figure 2.

Evolution of G subfamily transporters in selected mammals. The evolutionary history was inferred using the neighbor-joining method. The optimal tree with the sum of branch length=5.86717083 is shown. The percentage of replicate trees, in which the associated taxa clustered together in the bootstrap test (1000 replicates), are shown next to the branches. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). There were a total of 367 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (11). Human ABCB1 was used as the out group. (Abbreviations: Hsa, Homo sapiens ; Eca, Equus caballas ; Mus, Mus musculus ; Rno, Rattus norvegicus ; Ptr, Pan troglodytes ; Ggo, Gorilla gorilla ; Mmu, Maccaca mullata ; Bta, Bos taurus.)

Figure 3.

The domain organization in ABC transporters. ABC transporters contain transmembrane domains (TMDs) typically containing six transmembrane α helices (green) and nucleotide binding domains (NBDs, pink). NBD's contain Walker A, Walker B and C-loop functional sites. ABC full transporters contain two NBDs and two transmembrane domains. Half-transporters contain one TMD and one NBD (10).

Table 1

Chromosomal location of human and mouse ABCG subfamily genes (3).

ABC transporter	Location (human)	Location (mouse)
ABCG1	21q22.3	17 qA3.3
ABCG2	4q22	6qB3
ABCG3	-	5 qE5
ABCG4	11q23	9 qA5.2
ABCG5	2p21	17 qE4
ABCG8	2p21	17 qE4

Table 2

Splice variants of human ABCG subfamily genes (3).

ABC transporter	Number of splice variants	Transcript ID	Transcript length (bp)	Translational length (aa)	Number of exons
ABCG1	16	ENST00000398449	2998	666	15
		ENST00000398457	3119	668	16
		ENST00000347800	2923	663	15
		ENST00000343687	3037	677	15
		ENST00000361802	3034	678	15
		ENST00000398437	3475	824	16
		ENST00000450121	918	257	6
		ENST00000489035	1074	359	9
		ENST00000469119	1038	347	9
		ENST00000482161	919	291	8
		ENST00000340588	3211	786	16
		ENST00000455175	2857	668	15
		ENST00000462050	3037	-	17
		ENST00000472587	2903	-	11
		ENST00000496783	1725	-	6
		ENST00000467818	792	-	4
ABCG2	4	ENST00000237612	4479	655	16
		ENST00000515655	4276	611	16
		ENST00000505480	451	150	4
		ENST00000503830	391	130	4
		ENST00000307417	3849	646	15
		ENST00000449422	2458	646	15
		ENST00000531739	2552	646	14
		ENST00000533694	2719	-	10
ABCG4	8	ENST00000524604	496	152	4
		ENST00000534402	1454	121	3
		ENST00000416147	171	57	2
		ENST00000444613	162	54	2

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ABC transporter	Number of splice variants	Transcript ID	Transcript length (bp)	Translational length (aa)	Number of exons
ABCG5	4	ENST00000260645	2740	651	13
		ENST00000405322	2896	480	10
		ENST00000486512	3033	118	9
		ENST00000409962	2512	132	9
ABCG8	2	ENST00000272286	2665	673	13
		ENST00000458384	2661	672	13

Table 3

Substrate specificity, function and tissue distribution of human ABCG subfamily of ABC transporters (13, 17).

ABC transporter	Substrate	Function	Tissue distribution
ABCG1	Cholesterol	Cellular sterol homeostasis	Adrenal gland, lung, heart, spleen, brain and kidney
ABCG2	Broad specificity: organic anions, steroids, chlorophyll metabolites, camptothecins (topotecan), rosuvastatin, mitoxanthrone	Xenobiotic protection, multi-drug resistance	Placenta, blood-brain barrier, liver, colon, intestine, mammary gland and stem cells
ABCG4	Cholesterol	Cellular sterol homeostasis	Brain, spinal cord, heart, thymus
ABCG5	Cholesterol	Limit intestinal absorption and promote biliary excretion of sterols	Liver, intestine
ABCG8	Cholesterol, phytosterols, shellfish sterols	Limit intestinal absorption and promote biliary excretion of sterols	Liver, intestine

Table 4

Non-synonymous single nucleotide polymorphisms in human ABCG2 (28).

SNP	Base change	Amino acid change
rs2231137	A/G	V12M
rs2231142	A/C	Q141K
rs1061017	C/G	Q166E
rs12721643	A/C	I206L
rs1061018	C/T	F208S
rs3116448	C/T	S248P
rs34678167	C/T	P269S
rs41282401	C/G	D296H
rs113555375	C/T	K452R
rs112683307	A/G	L494F
rs58818712	A/C	L525R
rs45605536	A/G	A528T
rs35965584	A/G	T542A
rs9282571	A/T	F571I
rs34264773	A/T	N590Y
rs34783571	A/C/G/T	D620N/H/Y