Targeting Cardiac Sodium Channel Trafficking as Therapy for Rare Inherited Cardiac Arrhythmias

Qing Wang, Ph.D., M.B.A., FAAAS

Key Laboratory of Molecular Biophysics of the Ministry of Education

Huazhong University of Science and Technology

qkwang@mail.hust.edu.cn

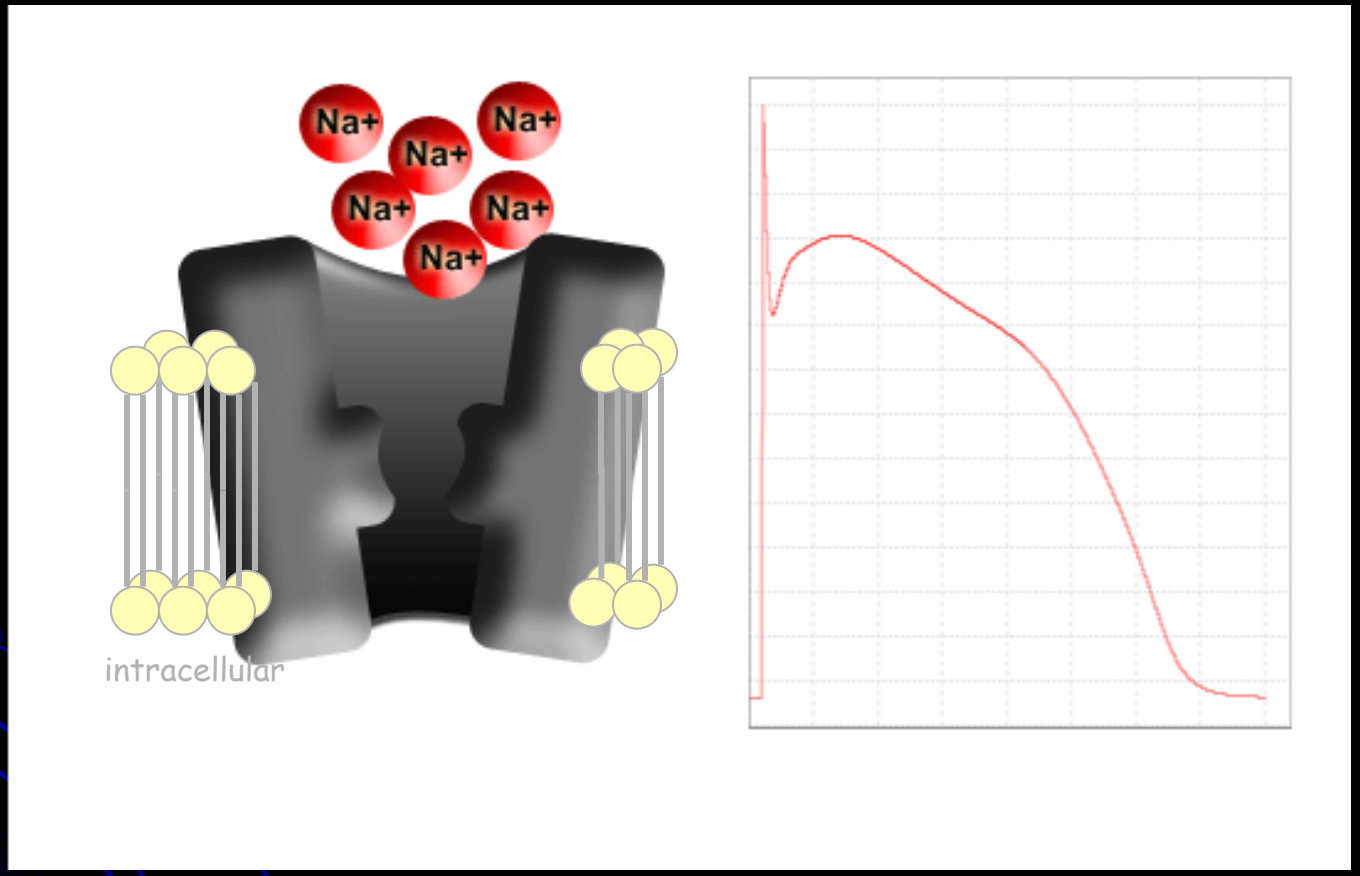
Cleveland Clinic and Case Western Resreve University

wangq2@ccf.org

Disclosures

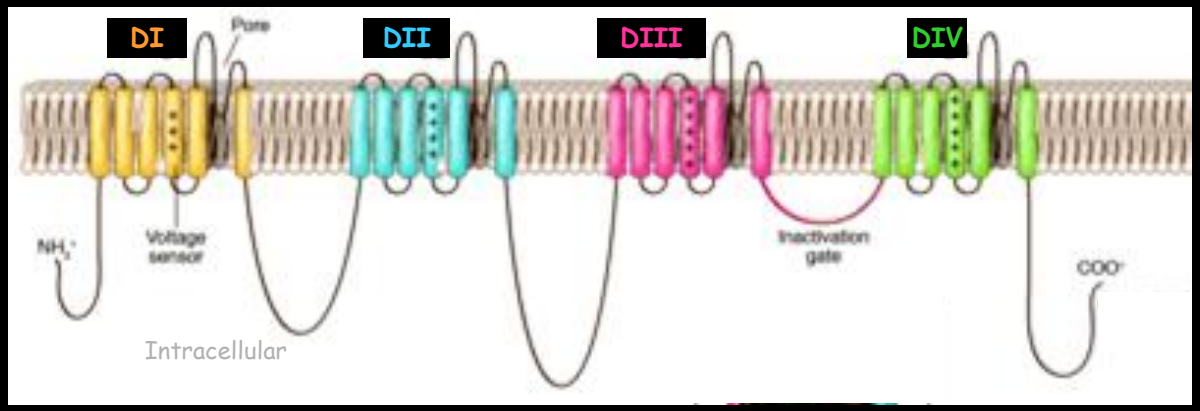
- Gilead Sciences
  - Royalty fees for transgenic mice overexpressing wild type and mutant cardiac sodium channels
  - Royalty fees for patents on arrhythmias genes KCNQ1 and SCN5A

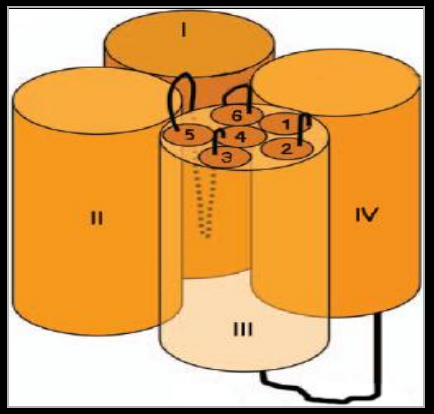
Cardiac Sodium Channel



Cardiac sodium channel Cardiac Action Potential

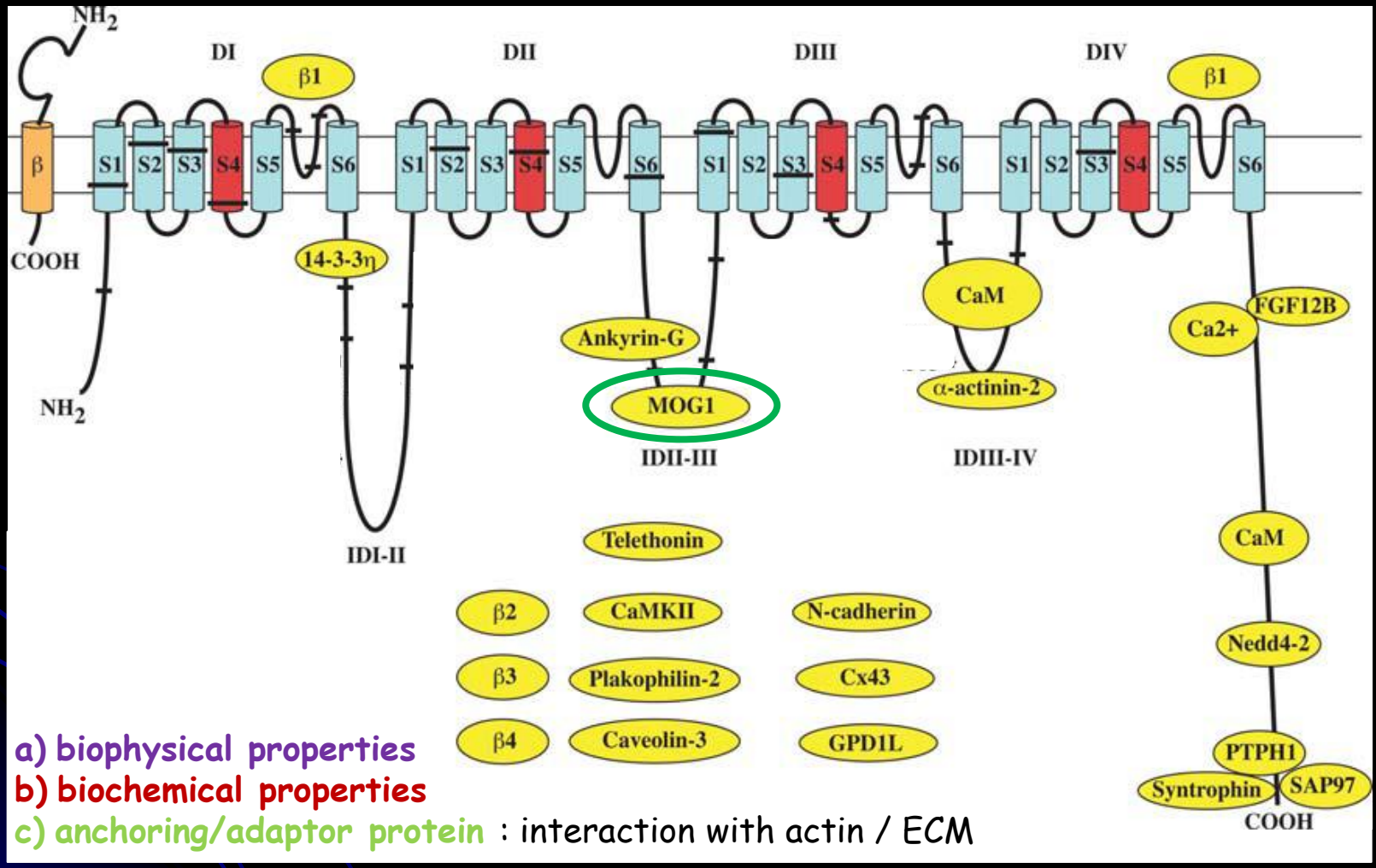
$Na_v1.5$: α subunit of Cardiac Sodium Channel





- Encoded by *SCN5A* gene at 3p21
- 4 homologous domains each with 6 transmembrane segments
- 3 intracellular loops
- Fourfold symmetric arrangement

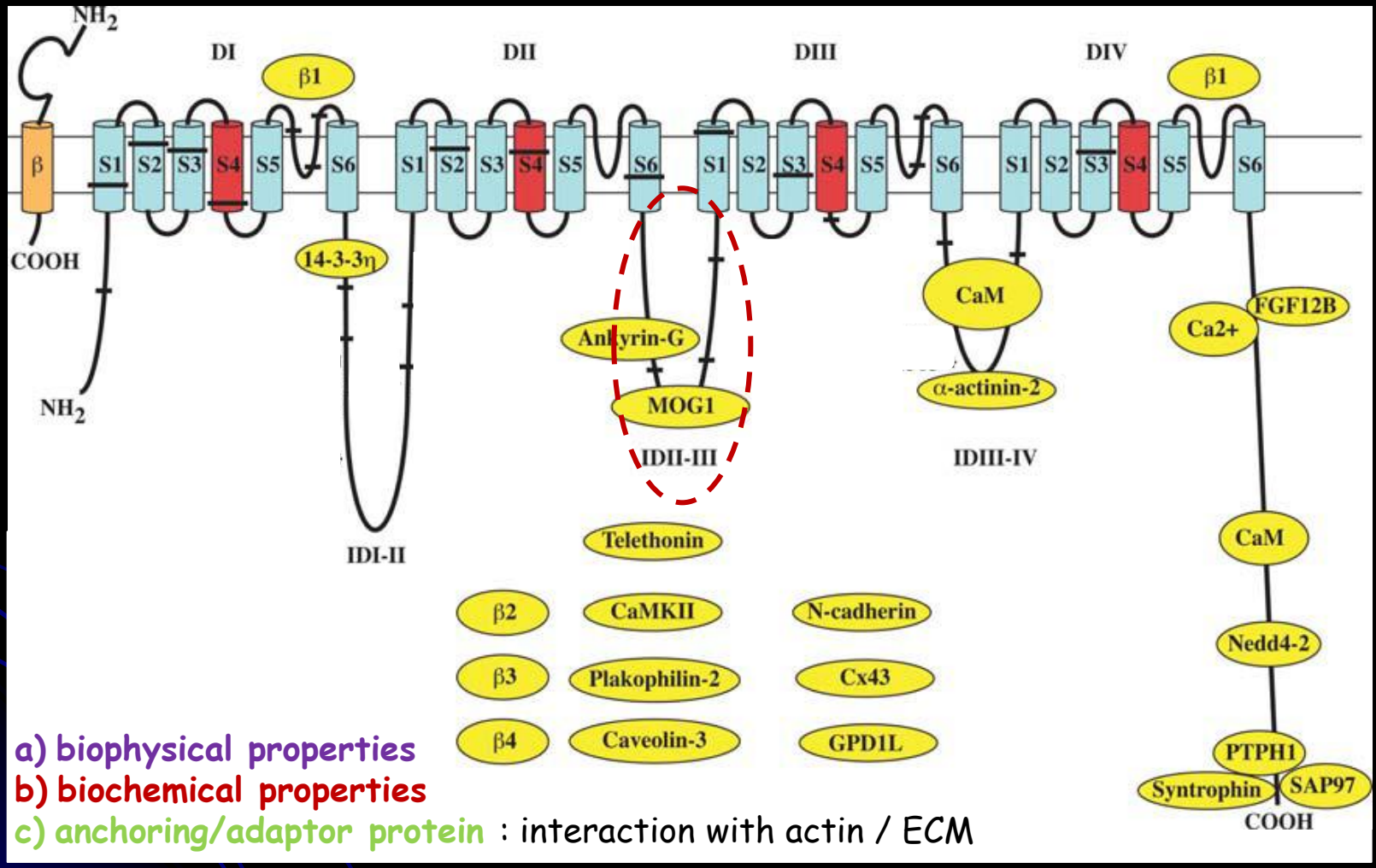
$Na_v1.5$ associated Proteins



a) biophysical properties
b) biochemical properties
c) anchoring/adaptor protein : interaction with actin / ECM

Rook MB *et al* Cardiovasc Res., 2012

$Na_v1.5$ associated Proteins

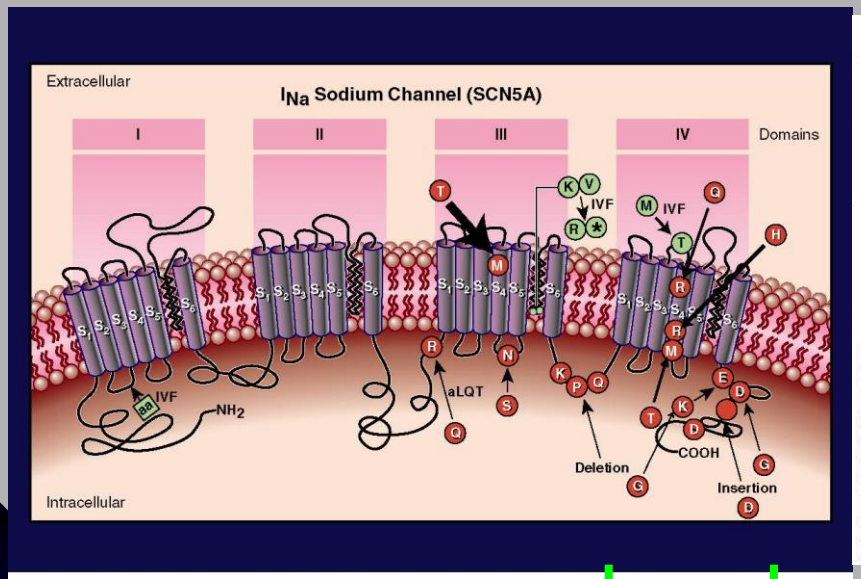


a) biophysical properties
b) biochemical properties
c) anchoring/adaptor protein : interaction with actin / ECM

Rook MB *et al* Cardiovasc Res., 2012

1995: One of the First Two Genes Causing Long QT Syndrome

Cardiac Sodium Channel Gene SCN5A



Cell, Vol. 80, 805–811, March 10, 1995, Copyright © 1995 by Cell Press

SCN5A Mutations Associated with an Inherited Cardiac Arrhythmia, Long QT Syndrome

Qing Wang,[1,2,3] Jiaxiang Shen,[1,2,3] Igor Splawski,[2,3] Donald Atkinson,[1,2,3] Zhizhong Li,[2,3] Jennifer L. Robinson,[4] Arthur J. Moss,[5] Jeffrey A. Towbin,[6] and Mark T. Keating[1,2,3,7]
[1]Howard Hughes Medical Institute

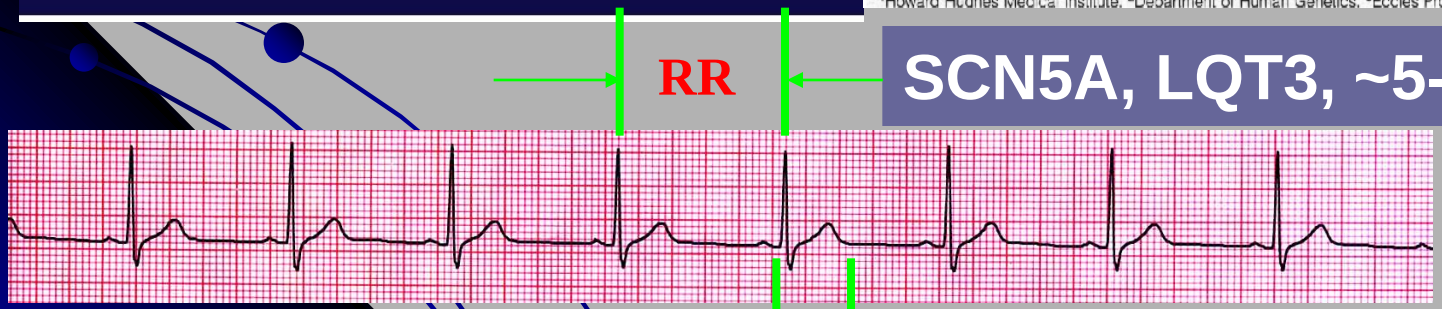
LQT (Romano–Ward … common and is not associated … abnormalities. A disorder ven… QT can also be acquired, usu… macologic therapy (Schwartz et …).

…olecular Genetics, 1995, Vol. 4, No. 9 1603–1607

Cardiac sodium … mutations in patients with long QT s… an inherited cardiac arrhythmia

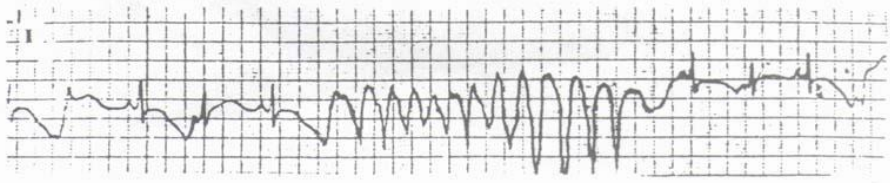
Qing Wang[1,2,3], Jiaxiang …, Zhizhong Li[2,3], Katherine Timothy[5], G.Michael Vincent[4,5], Silvia G.Priori[6], Peter J.Schwartz[6] and M… T.Keating[1–4,*]
[1]Howard Hughes Medical Institute, [2]Department of Human Genetics, [3]Eccles Program in Human Molecular Biology and Genetics,

Wang et al. Cell 1995, March



SCN5A, LQT3, ~5-10% of LQTS

$QTc = QT/(RR)^{1/2}$



Discovery of the First Gene for Brugada Syndrome

Qiuyun Chen et al. 1998, Nature 392:293-6

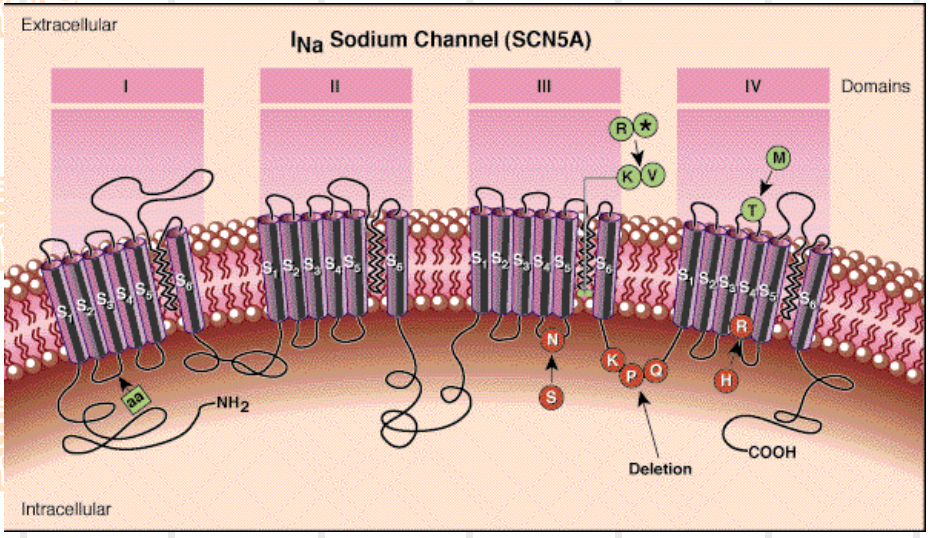
Nature 1998;392:293-296

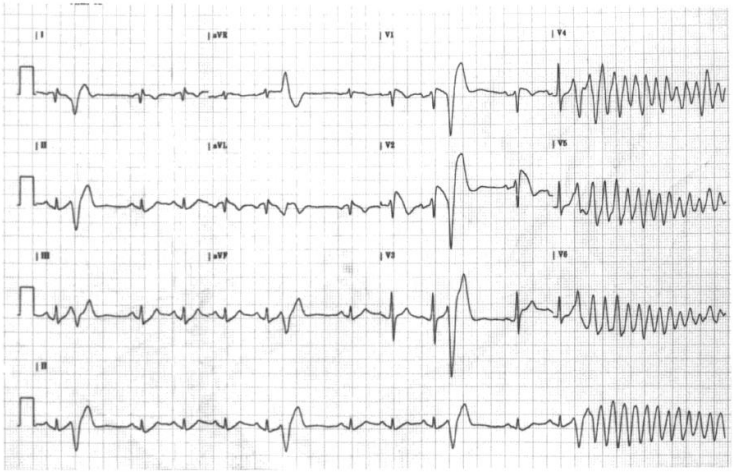
letters to nature

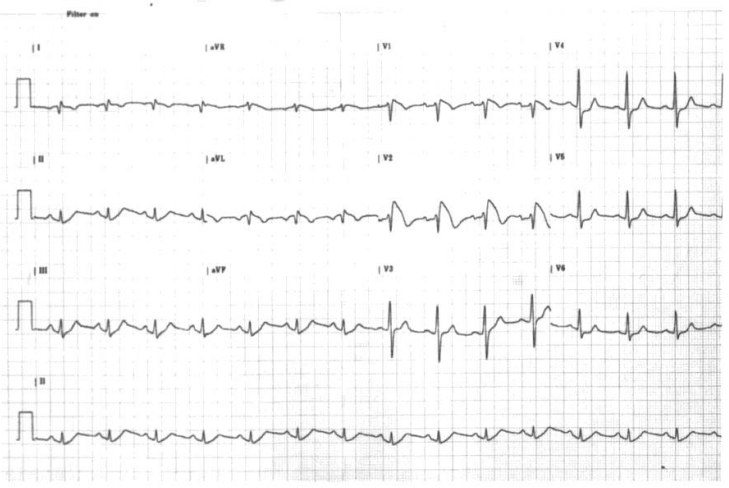
Genetic basis and molecular mechanism for idiopathic ventricular fibrillation

Qiuyun Chen*†, Glenn E. Kirsch†‡, Danmei Zhang*, Ramon Brugada§, Josep Brugada‖, Pedro Brugada¶, Domenico Potenza#, Angel Moya^, Martin Borggrefe**, Günter Breithardt**, Rocio Ortiz-Lopez*, Zhiqing Wang*†, Charles Antzelevitch‡‡, Richard E. O'Brien*, Eric Schulze-Bahr**, Mark T. Keating§§, Jeffrey A. Towbin*†††‖ & Qing Wang*

DNA sequence analyses to identify mutations in known ion channel genes, including the cardiac sodium channel gene *SCN5A*. Two aberrant SSCP conformers were identified in all affected members in family K005, one in exon 21 of *SCN5A* (data not shown) and the other in exon 28 (Fig. 1a, b)[18]. Neither of the SSCP anomalies was seen in unaffected individuals (Fig. 1b) or in DNA samples from more than 150 control individuals (data not shown). DNA sequence analysis revealed two C-to-T base substitutions, one in exon 21 and the other in exon 28 (Fig. 1c): these mutations lead to substitution of an arginine by a tryptophan at codon 1,232 (represented as R1232W; data not shown), which lies in the extracellular loop between transmembrane segments S1 and S2 of domain III, and to substitution of a highly conserved threonine by a methionine at codon 1,620 (T1620M; Fig. 1c) in the extracellular loop between S3 and S4 of domain IV. Studies with sodium-channel-specific toxins







Cardiac Sodium Channelopathies

Gain of Function (Increased I_{Na}, increased late I_{Na})

Long QT syndrome

SCN5A: cardiac sodium channel $Na_v1.5$

CAV3: caveolin 3

SCN4B: β4 subunit of $Na_v1.5$

SNTA1: α1-syntrophin

Loss of Functions (Decreased I_{Na})

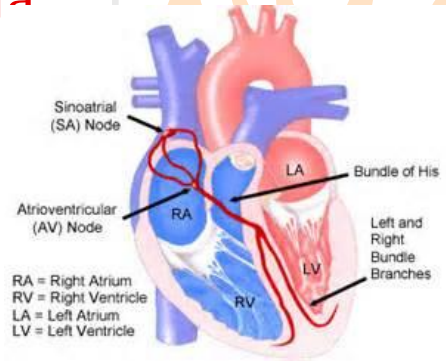
Brugada Syndrome (BrS)

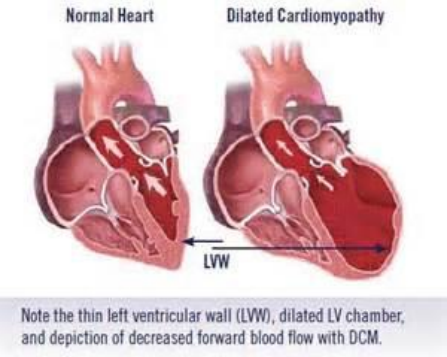
SCN5A: cardiac sodium channel $Na_v1.5$
MOG1: Nav1.5 regulatory protein
GPD1L: glycerol-3-phosphate dehydrogenase like peptide
SCN1B: β1 subunit of $Na_v1.5$
SCN3B: β1 subunit of $Na_v1.5$
SCN10A: sodium channel $Na_v1.8$

Cardiac Sodium Channelopathies

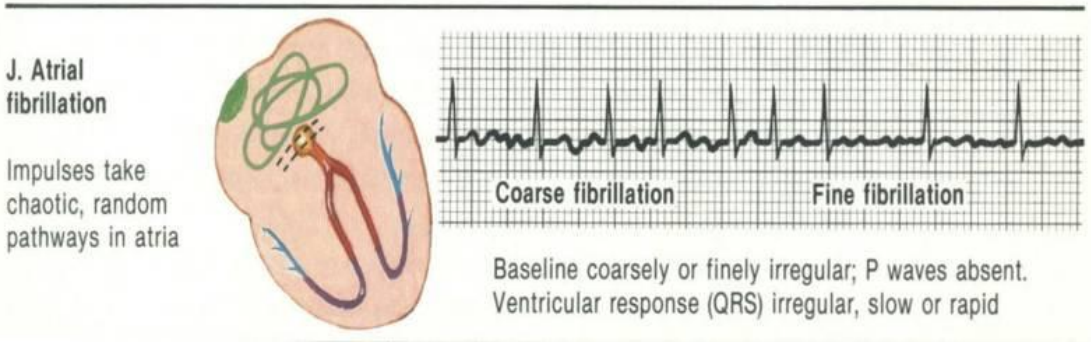
Loss of Functions (Decreased I_{Na})

- **Sick Sinus Syndrome (SSS)**
  - **SCN5A**
- **Idiopathic Ventricular Fibrillation (IVF)**
  - **SCN5A**
  - **SCN3B**
- **Cardiac Conduction Defects (CCC)**
  - **SCN5A**
- **Dilated Cardiomyopathy (DCM)**
  - **SCN5A**
- **Atrial Fibrillation**
  - ***SCN5A***
  - ***SCN1B***
  - ***SCN2B***
  - ***SCN3B***
  - ***SCN4B***





Note the thin left ventricular wall (LVW), dilated LV chamber, and depiction of decreased forward blood flow with DCM.



Cardiac Sodium Channelopathies

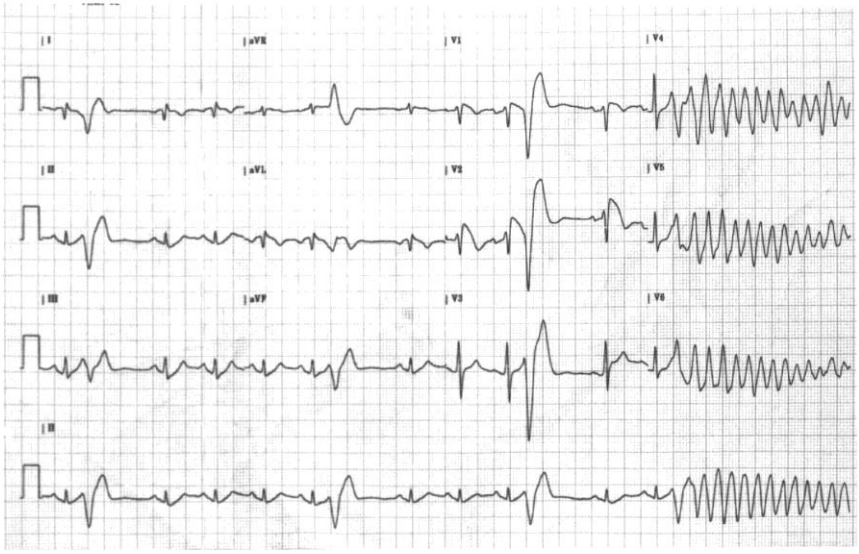
- **Genetic discoveries have revolutionized the diagnosis of cardiac sodium channelopathies**
- **Commercial genetic testing and counseling are available**
- **Gene-specific therapy was developed for LQT3 (sodium channel blockers)**

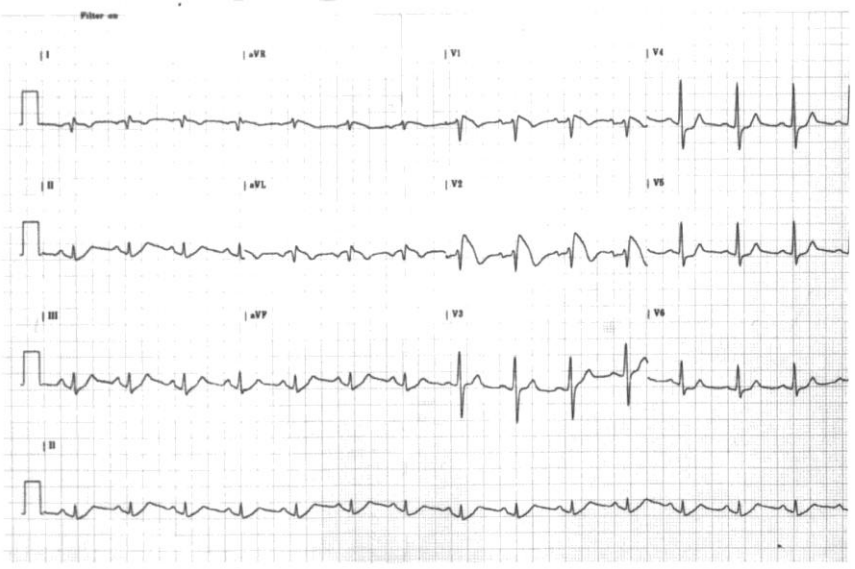
Key remaining Issue?

No effective drug therapy is available for other sodium channelopathies

Brugada Syndrome

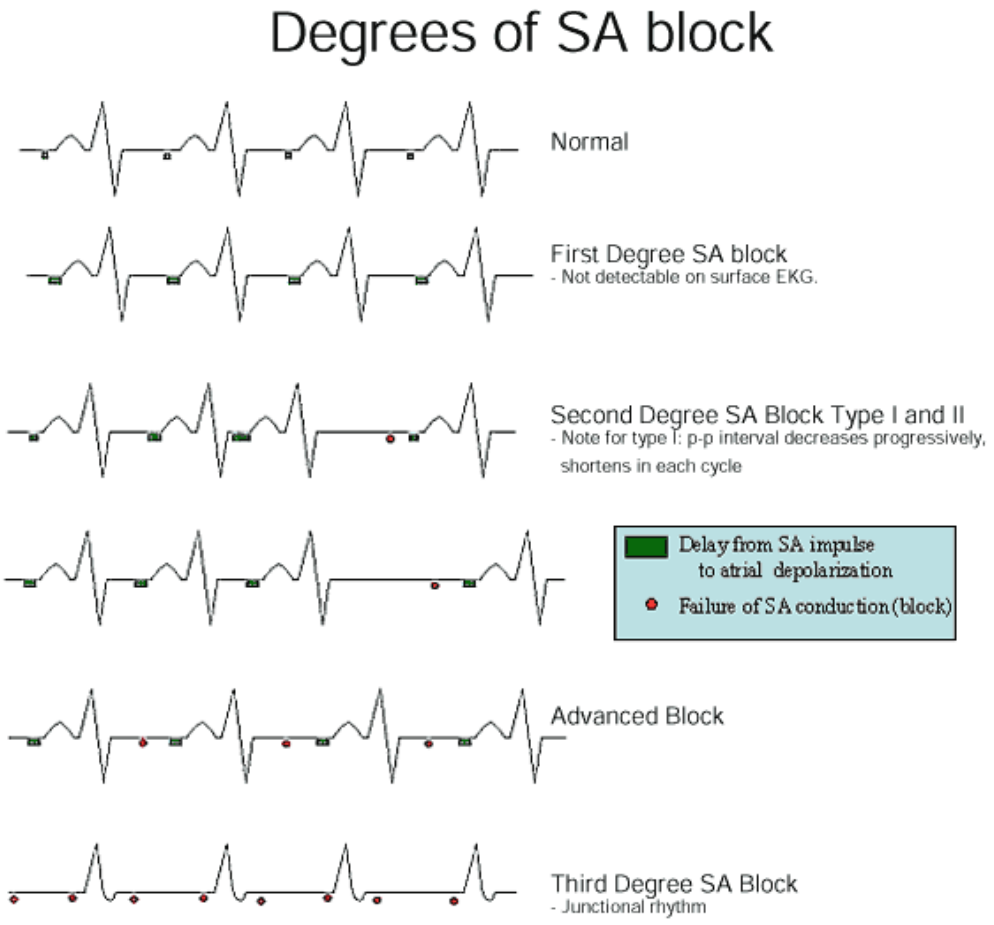
- Defective $Na_v1.5$ (Gene *SCN5A*);
- Most common, account for 15-30% BrS patients;
- Over 300 mutations have been identified;
- Loss-of-function mutations (reduced I_{Na})
- Implantation of a defibrillator (ICD)
- No medication available





Sick Sinus Syndrome

- Defective $Na_v1.5$ (Gene *SCN5A*);
- Loss-of-function mutations (reduced I_{Na})
- Implantation of a pace-maker
- No effective drug therapy

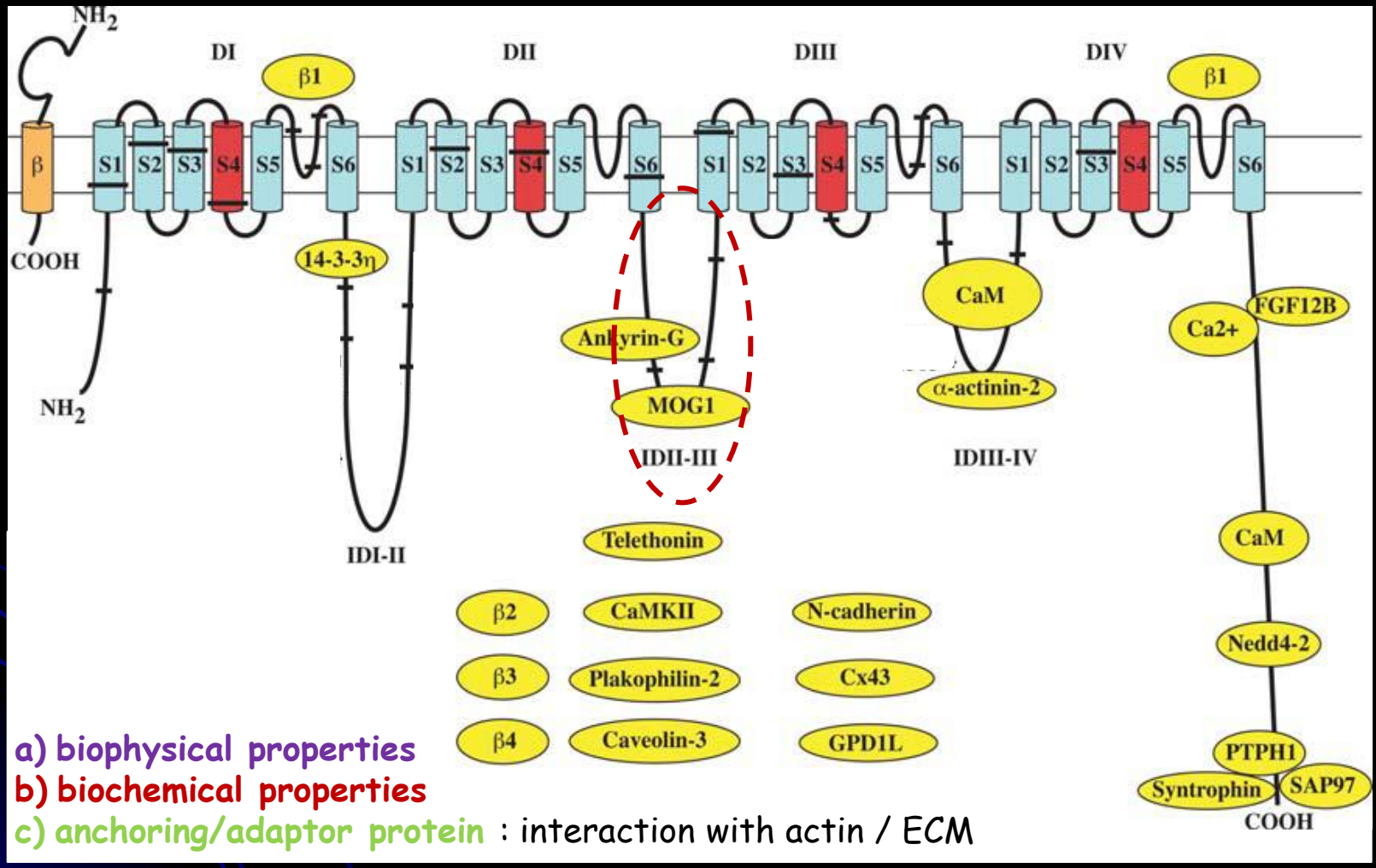


What to do next?

--Explore molecular mechanisms

--Try translational research

$Na_v1.5$ associated Proteins



a) biophysical properties
b) biochemical properties
c) anchoring/adaptor protein : interaction with actin / ECM

Rook MB *et al* Cardiovasc Res., 2012

MOG1 (Multicopy suppressor of *GSP1*)

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 15388–15393, December 1998
Cell Biology

A protein required for nuclear-protein import, Mog1p, directly interacts with GTP–Gsp1p, the *Saccharomyces cerevisiae* Ran homologue

MASAYA OKI AND TAKEHARU NISHIMOTO*

Department of Molecular Biology, Graduate School of Medical Science, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan

Mog1p (24 kD) : yeast ORF YJR074W

Suppressor of conditional alleles to *gsp1* (*S. cerevisiae* Ran homologue) : rescued temperature-sensitive growth defect

Ran : Ras family GTPase

- nucleo-cytoplasmic transport
- spatiotemporal organization of spindle

MOG1 (Multicopy suppressor of *GSP1*)

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 15388–15393, December 1998
Cell Biology

A protein required for nuclear-protein import, Mog1p, directly interacts with GTP–Gsp1p, the *Saccharomyces cerevisiae* Ran homologue

MASAYA OKI AND TAKEHARU NISHIMOTO*

Department of Molecular Biology, Graduate School of Medical Science, Kyushu University, Higashi-ku, Fukuoka 812-8582, Japan

Mog1p (24 kD) : yeast ORF YJR074W

Suppressor of conditional alleles to *gsp1* (*S. cerevisiae* Ran homologue) : rescued temperature-sensitive growth defect

Involved in nuclear-protein imports

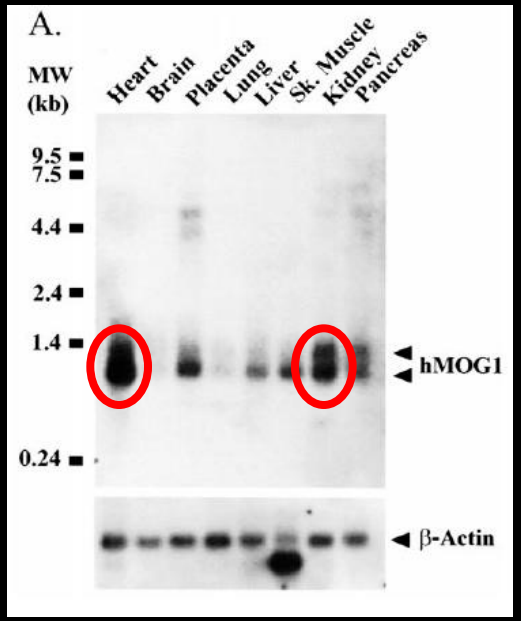
Mammalian MOG1 is a Guanine Nucleotide release factor for RAN

Human Mog1 : 2 splice variants encoded by *MOG1* at 17p13.1

hMOG1a (20 kD) : 29% identity, 47% similarity with scMOG1

hMOG1b (16 kD) : 30% identity, 49% similarity with scMOG1

mRNA expressed in a variety of tissues



Mammalian MOG1 is a Guanine Nucleotide release factor for RAN

Human Mog1 : 2 splice variants encoded by *MOG1* at 17p13.1

hMOG1a (20 kD) : 29% identity, 47% similarity with scMOG1

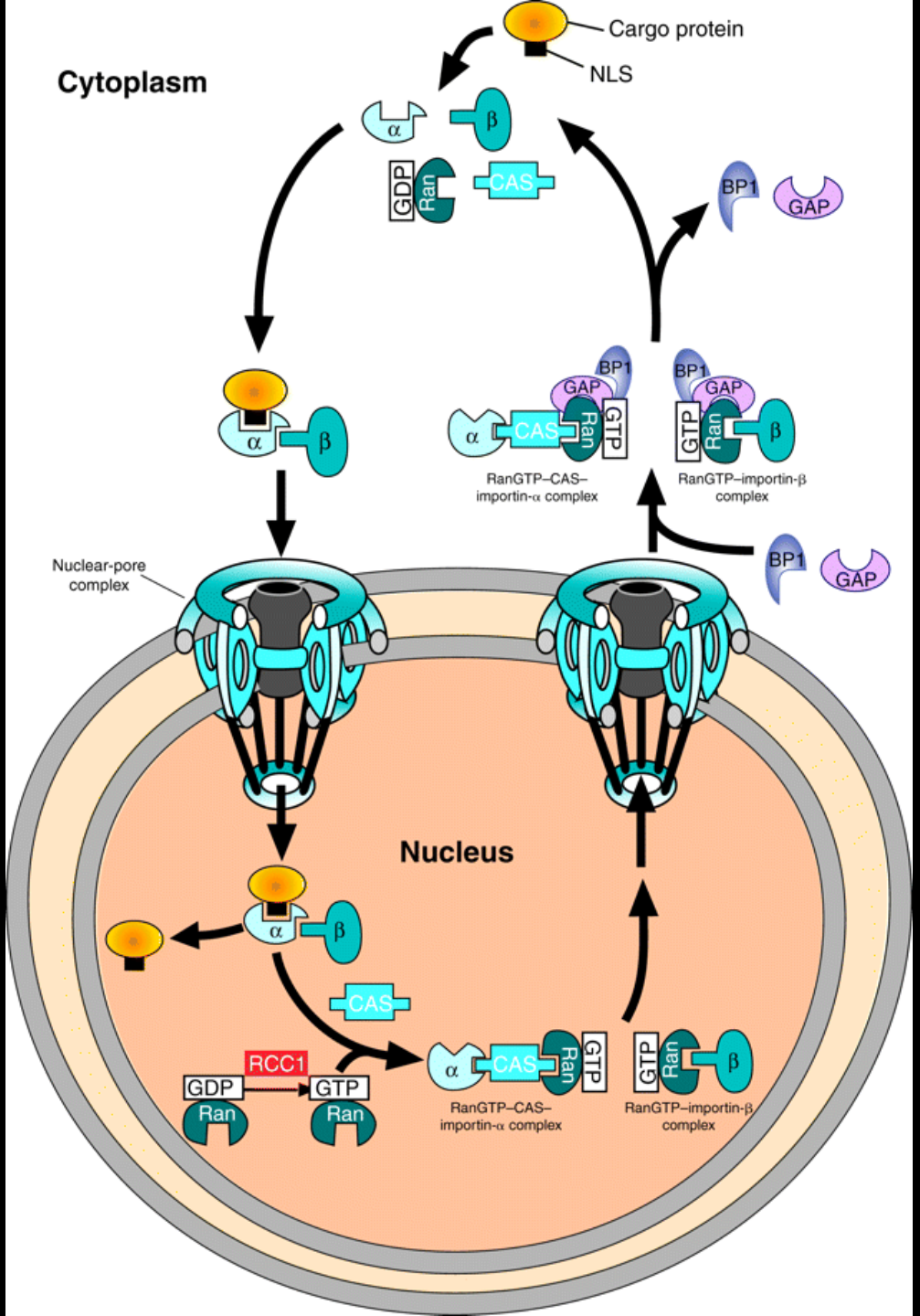
hMOG1b (16 kD) : 30% identity, 49% similarity with scMOG1

mRNA expressed in a variety of tissues

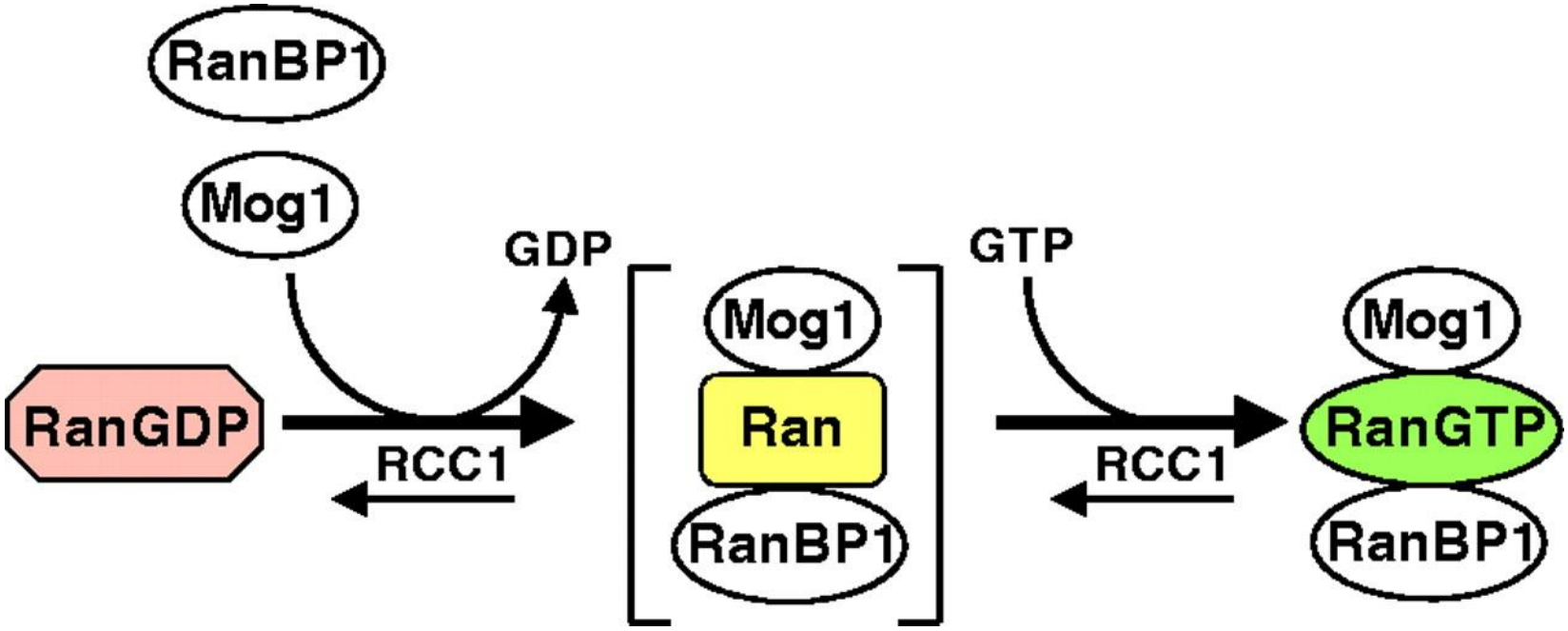
Binds to Ran-GTP : regulates nuclear Ran-GTP

Evolutionarily conserved Ran binding protein that could play a role in regulating nuclear protein trafficking by shuttling between cytoplasm and nucleus

MOG1 Regulates the Ran Cycle of Nucleocytoplasmic Transport



A model for the action of Mog1-related proteins and RanBP1 on guanine nucleotide exchange on Ran



When RanBP1 is present, Mog1 destabilises GDP binding to Ran to form a transient, nucleotide-free complex. RanBP1 promotes GTP loading by stabilising the GTP-conformation. RCC1 catalyses guanine nucleotide exchange by increasing the rate at which equilibrium is acheived. Thus, Mog1 and RanBP1 together alter the equilibrium of the reaction to favour the formation of Ran-GTP.

Nicolás F J et al. J Cell Sci 2001;114:3013-3023

Journal of Cell Science
jcs.biologists.org

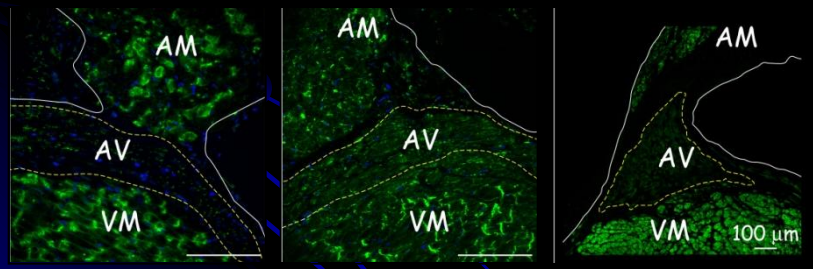
Part 1

MOG1 plays a novel physiological role in mammalian cells

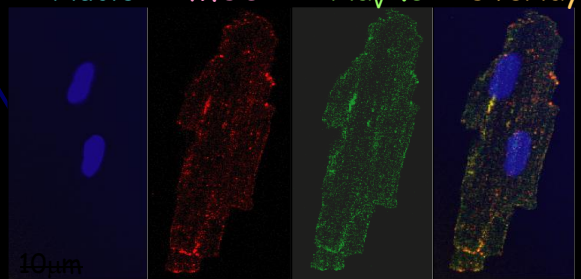
MOG1 interacts with $Na_V1.5$

- Novel accessory protein for $Na_V1.5$ (yeast 2-hybrid screening)
- Interacts with $Na_V1.5$ *in vitro/ in vivo* (GST pull down/ Co-IP)
- Mouse heart : atrial/ ventricular tissues
- Mouse cardiomyocytes : intercalated discs
- Double staining : co-localized with $Na_V1.5$

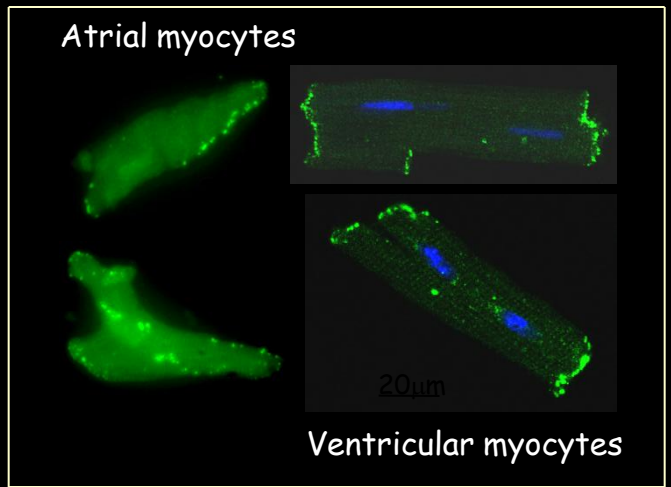
Cx43 MOG1 $Na_V1.5$



Nuclei MOG1 $Na_V1.5$ Overlay

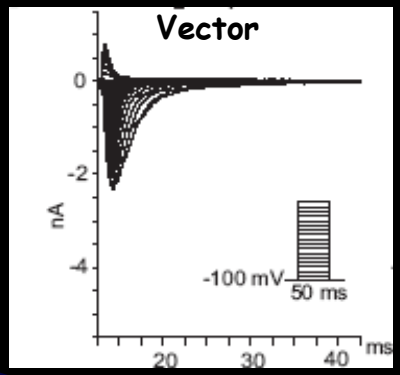


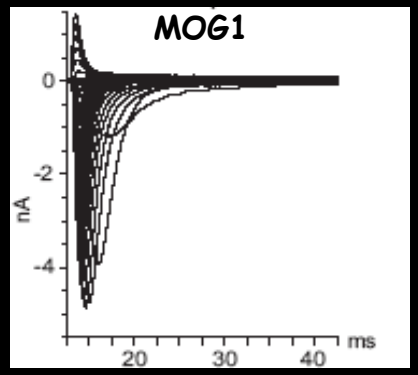
Wu *et al.* J. Biol. Chem., 2008

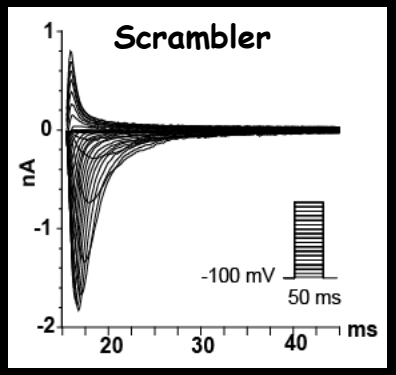


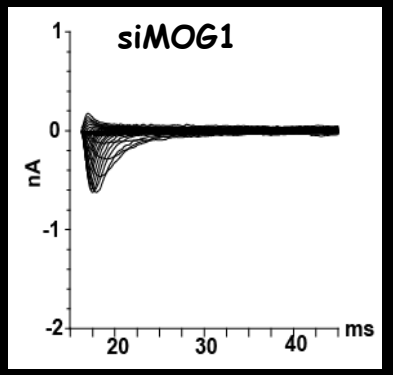
MOG1 modulates Sodium Current (I_{Na}) in HEK293/ $Na_V1.5$ stable cells

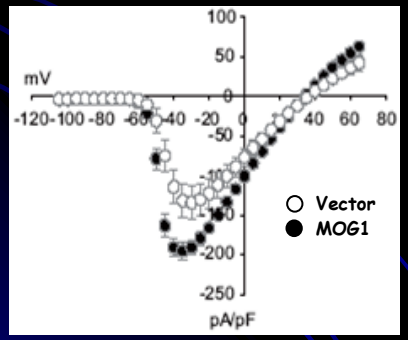
Sodium Currents in Whole Cell Patches

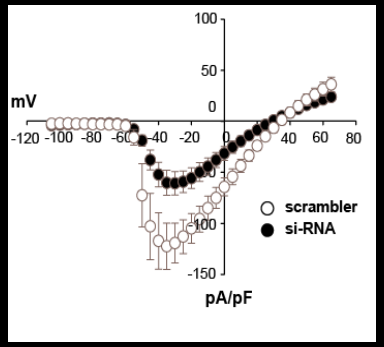








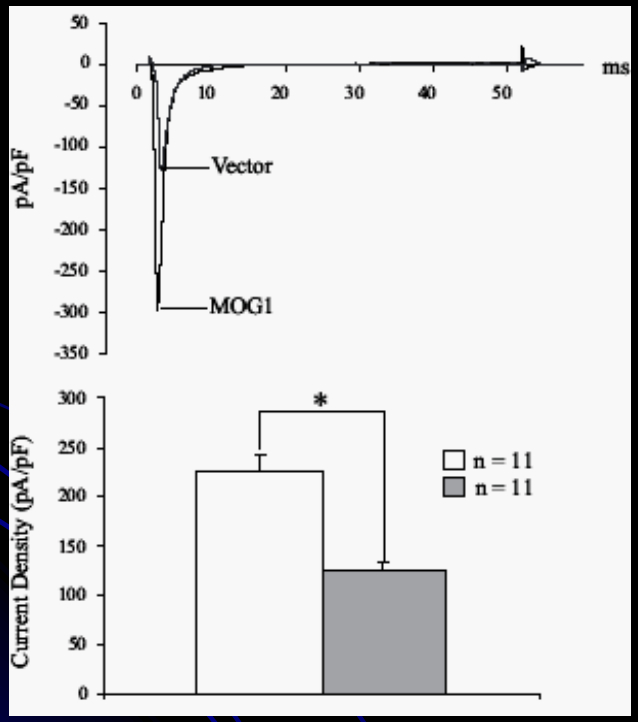




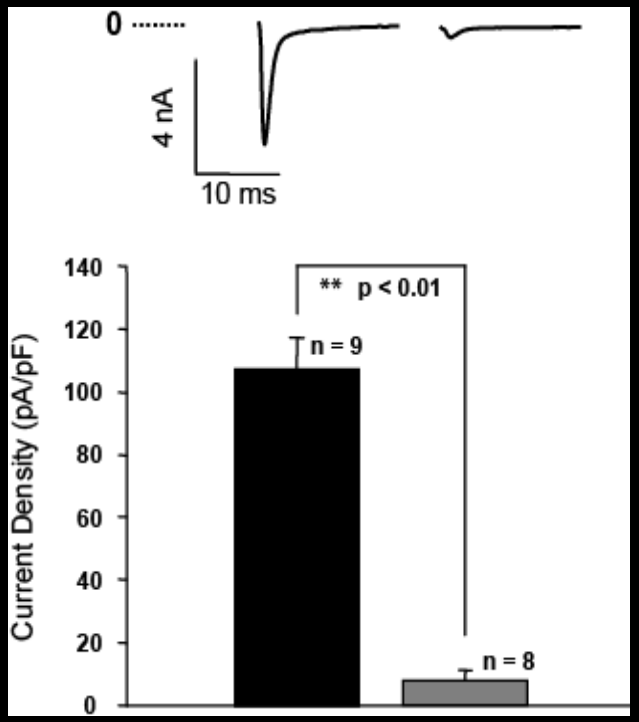
Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 modulates Sodium Current (I_{Na}) in mouse neonatal cardiomyocytes

Sodium Currents in Whole Cell Patches



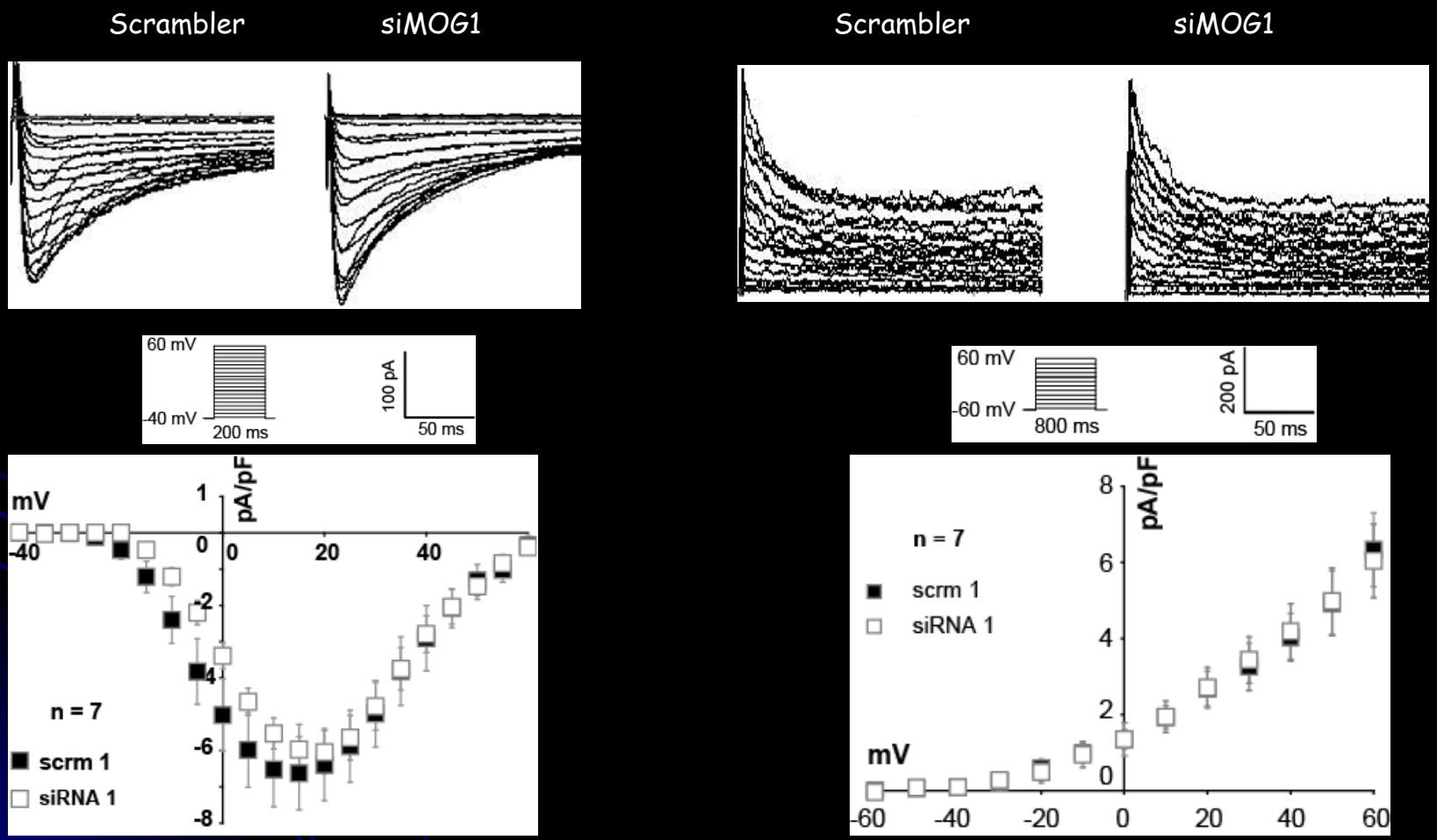
MOG1 Vector



Scrambler siMOG1

Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 does not modulate inward L-type Calcium Current (I_{CaL}) or transient outward Potassium Current (I_{To}) in mouse neonatal cardiomyocytes



Effect of MOG1 is specific for I_{Na}

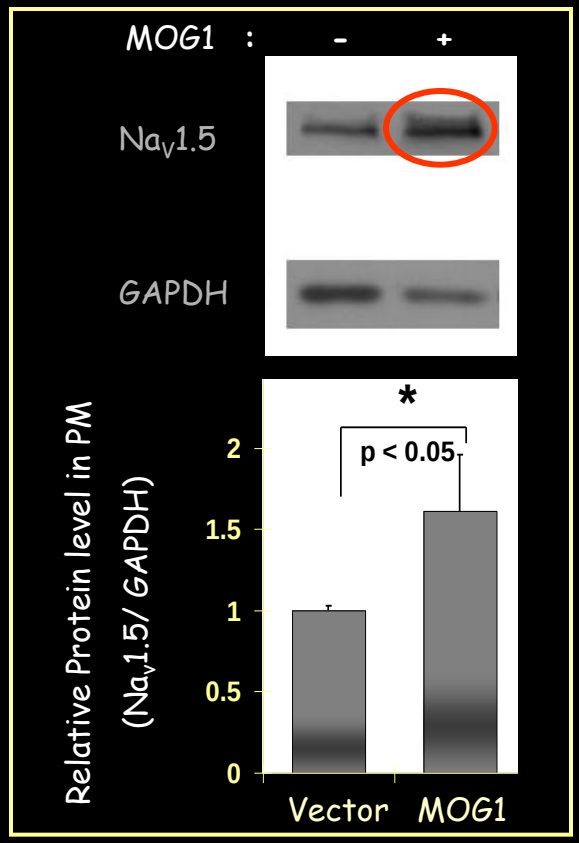
Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 increases Sodium channels on PM

How does MOG1 induce increased sodium current?

- ~~Increasing mRNA/ protein expression~~
- ~~Altering channel open probability/ unitary conductance~~
- ✓ Increasing number of available channels on PM

Relative Protein Expression on PM



Increase in PM sodium channels

Wu *et al.* J. Biol. Chem., 2008

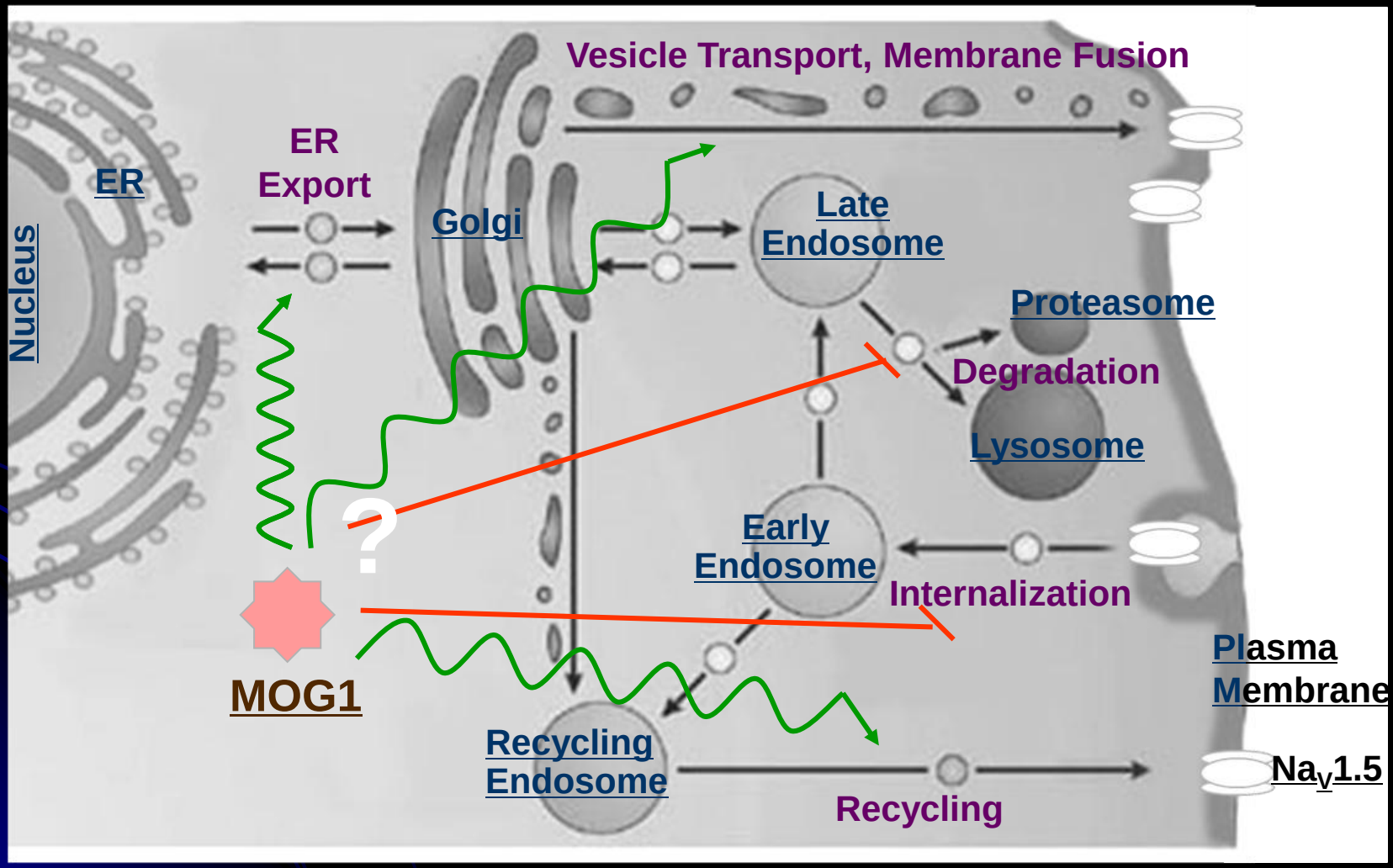
Summary : Part1

- MOG1 : novel accessory protein for $Na_V1.5$
- Expressed in atrial / ventricular tissues : co-localized with $Na_V1.5$
- Increase/ decrease in MOG1 expression : increase/ decrease in Sodium Current
- No effect on Late Sodium Current/ L-type Calcium Current/ transient outward Potassium Current
- Modulates sodium current by controlling the PM $Na_V1.5$ expression

Part 2

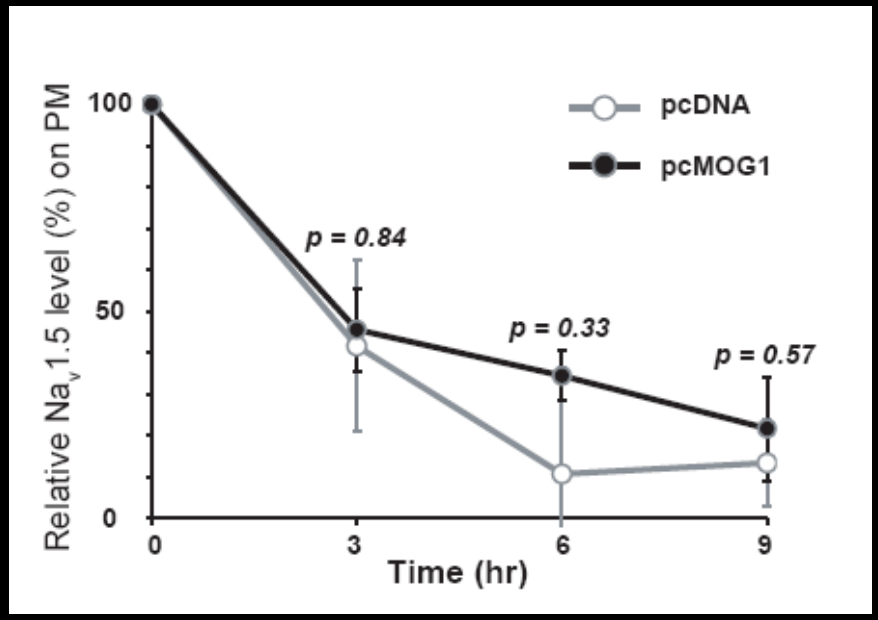
How can MOG1 enhance PM $Na_v1.5$ expression ?

Steps of $Na_v1.5$ from synthesis to PM



MOG1 does not increase membrane stability of $Na_v1.5$

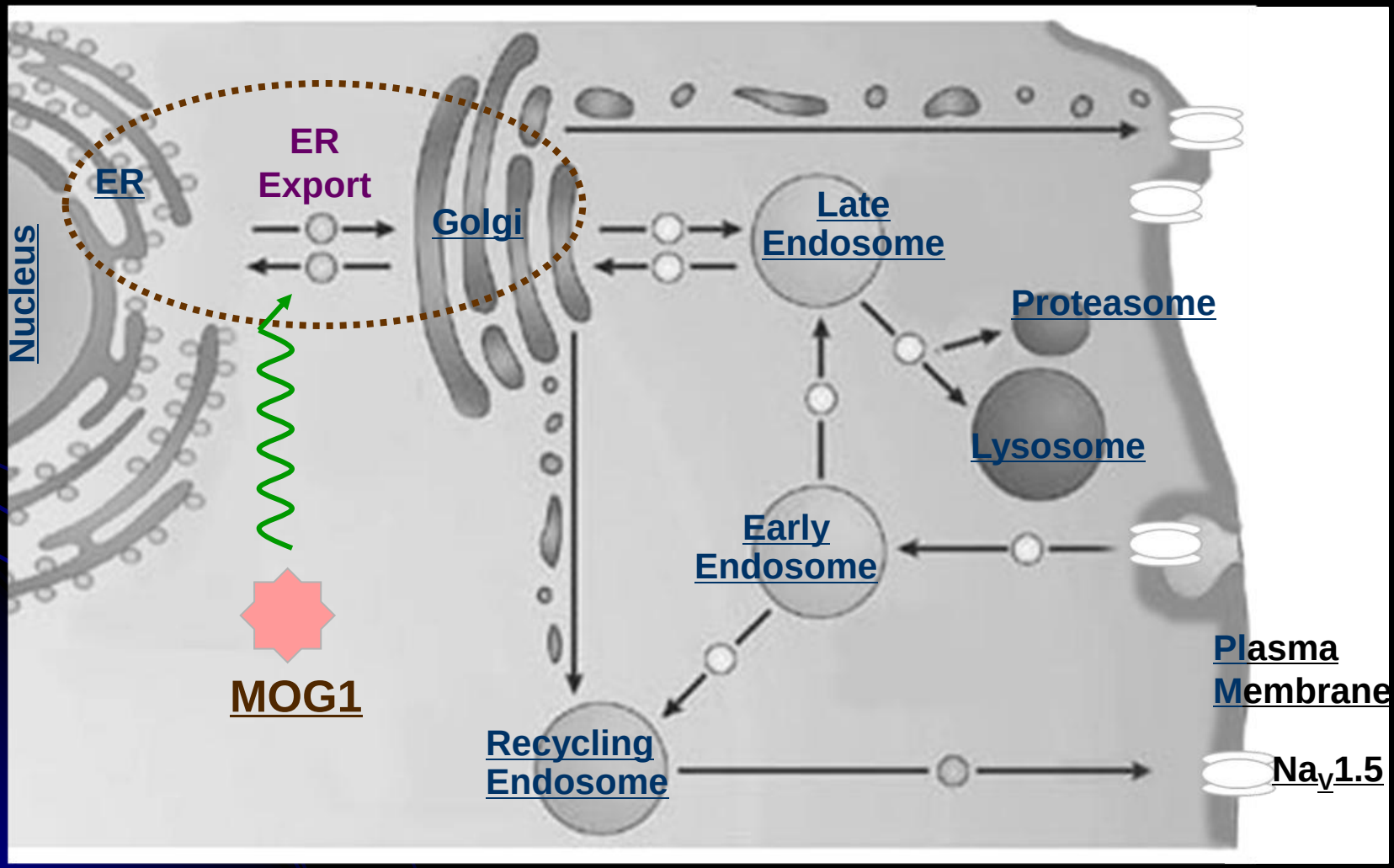
- tsA-201 cells : $Na_v1.5$ and MOG1/ vector
- PM stability assay : biotin/avidin
- 3 time points : 3, 6, 9h



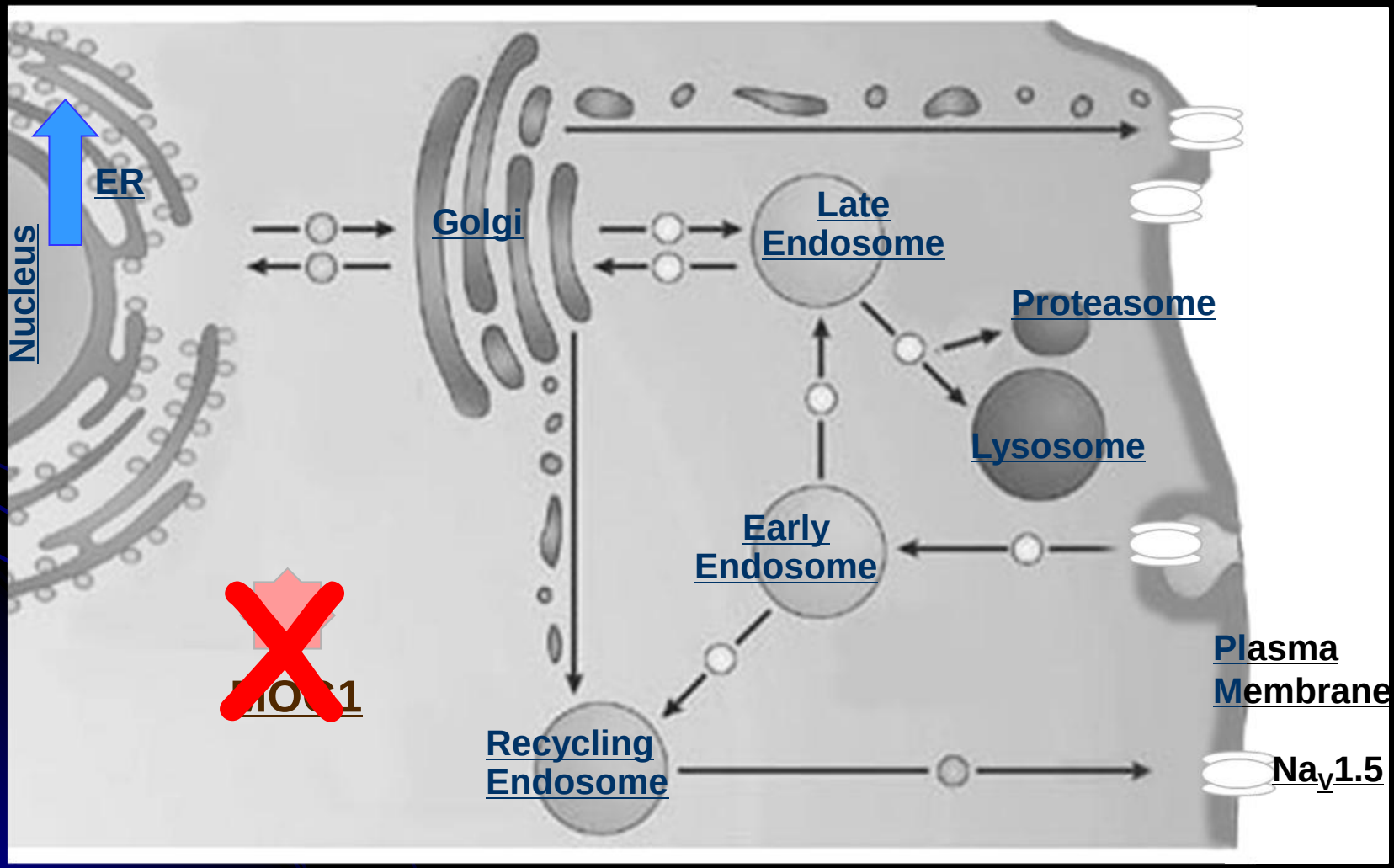
MOG1 does not affect stability of $Na_v1.5$ on PM

Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

Steps of $Na_v1.5$ from synthesis to PM

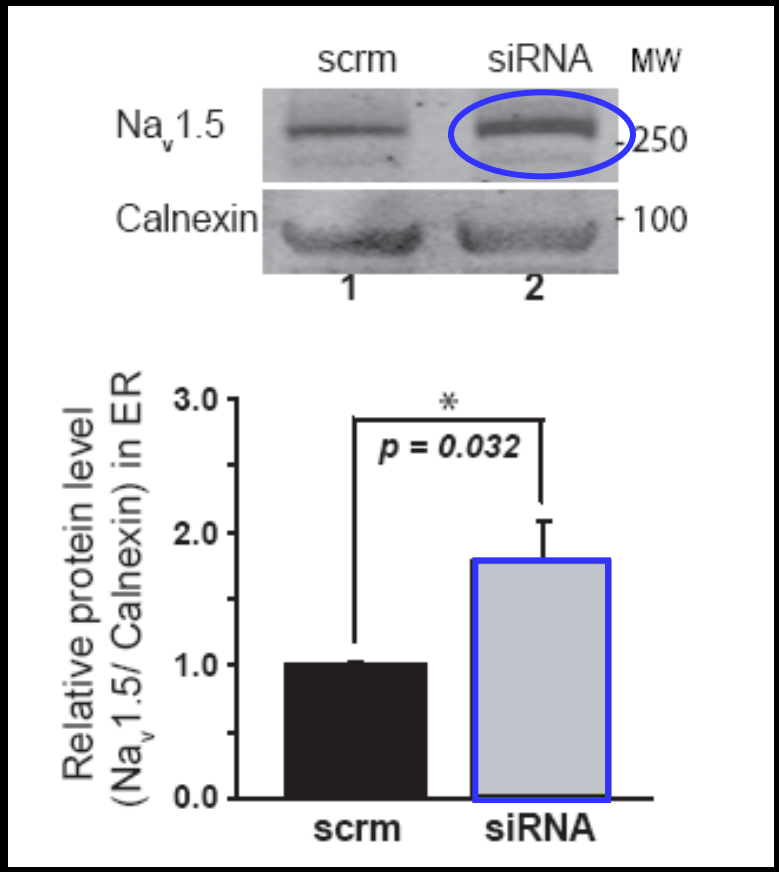


Steps of $Na_v1.5$ from synthesis to PM



MOG1 knock down increases $Na_v1.5$ accumulation into RER fraction in HEK/$Na_v1.5$ stable cells

- HEK293/ $Na_v1.5$ stable cells : siMOG1 / scrambler
- Knockdown of MOG1 expression increased $Na_v1.5$ expression in RER fraction compared to control cells

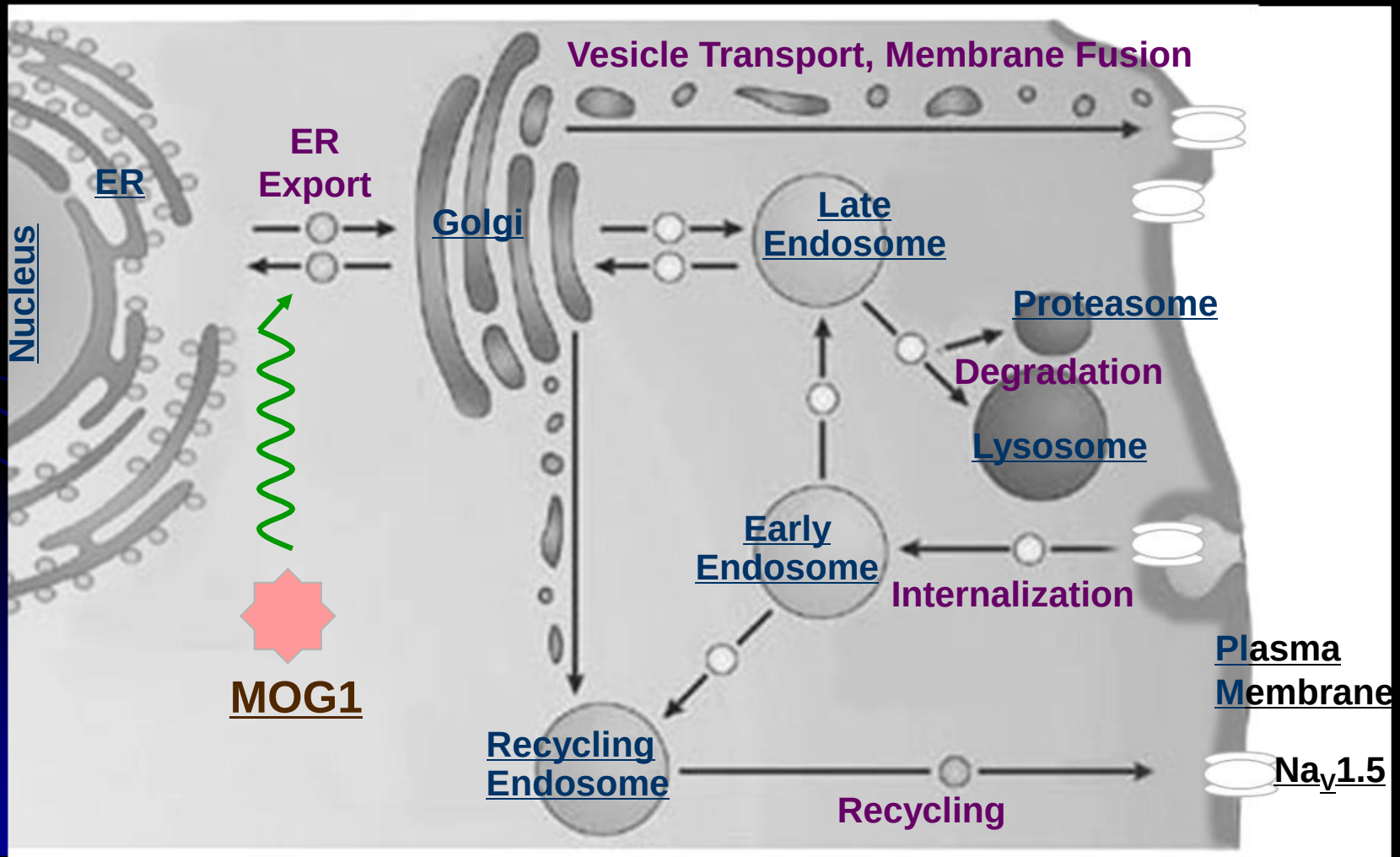


MOG1 affects ER export of $Na_v1.5$

Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

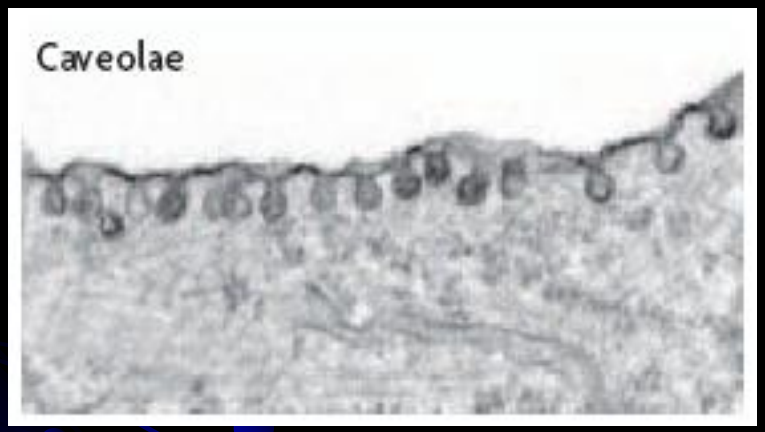
Summary : Part2

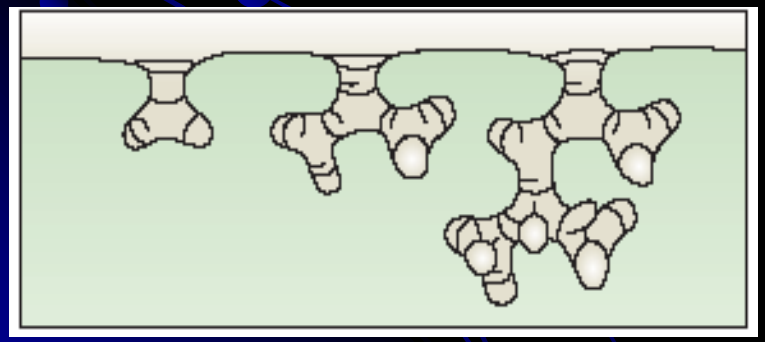
- MOG1 does NOT affect $Na_v1.5$ stability on PM
- MOG1 regulates ER export of $Na_v1.5$

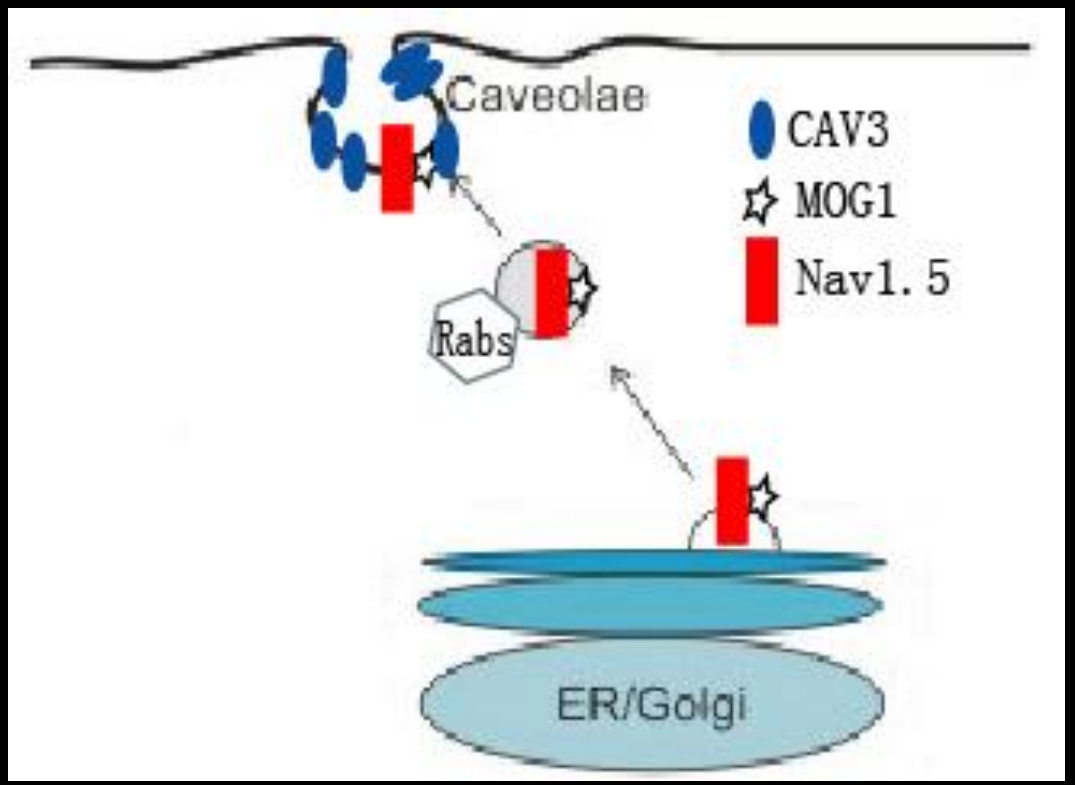


Part 3

MOG1 Is Required for Correct Targeting of Nav1.5 to Caveolae

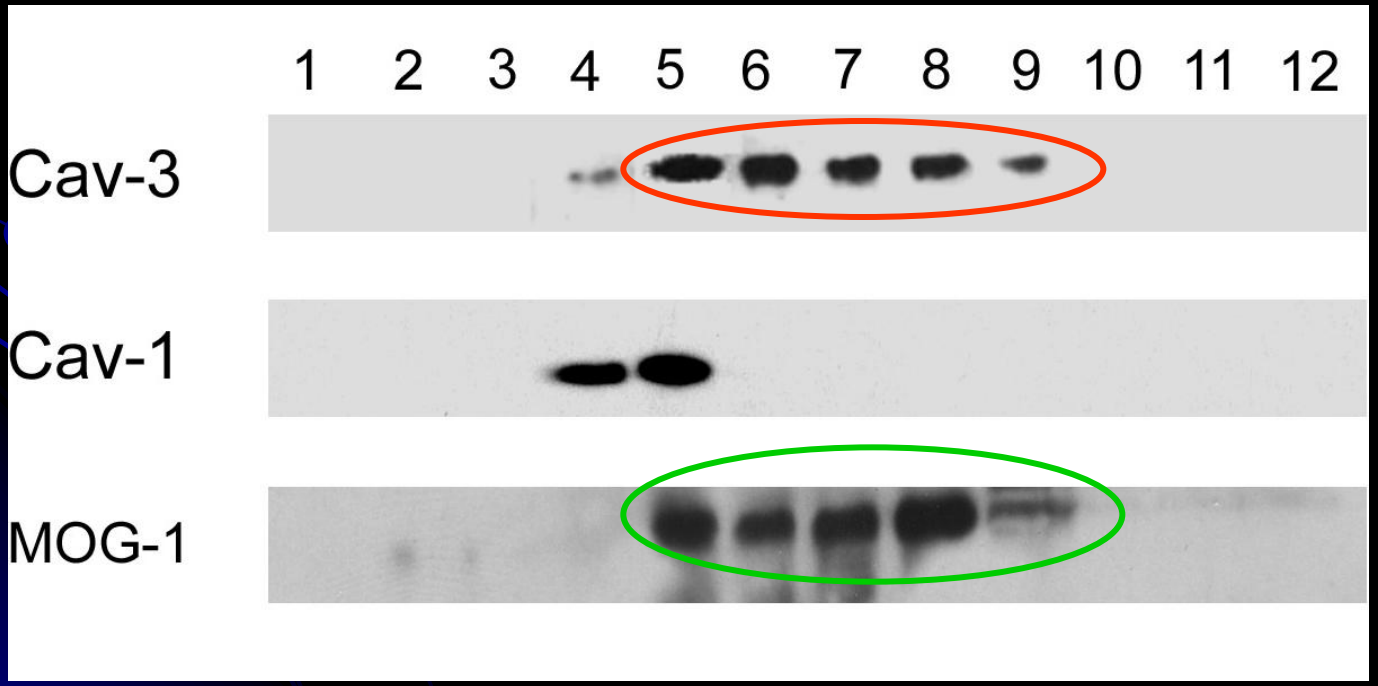






MOG1 is also present in Caveolar fraction

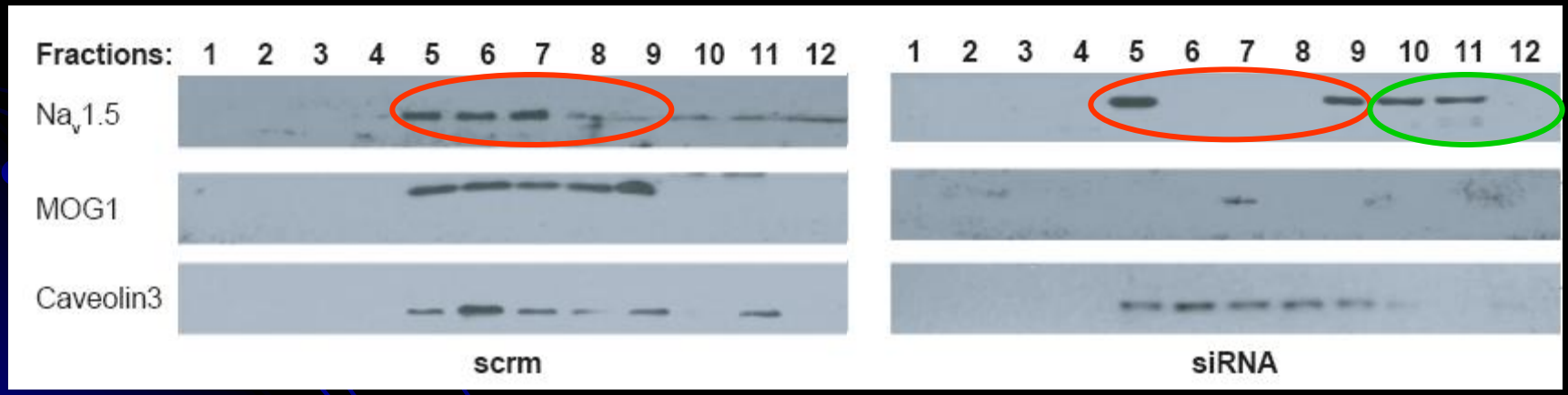
- Co-loclaizaiton of MOG1 with caveolin-3 in rat ventricular cardiomyocytes
- Detergent-free sucrose gradient (5-45%) : 12 fractions (top - bottom)
- MOG1 co-localized with cavolin-3-rich cavveolar fractions



MOG1 knock down alters $Na_v1.5$ distribution in Caveolin microdomains

MOG1 knockdown : redistribution of $Na_v1.5$ from caveolar fractions 6-8 to non-caveolar fractions

Caveolar $Na_v1.5$ content : 84.19±8.90 % to 52.03±9.63 % $p<0.05$ (n=3)

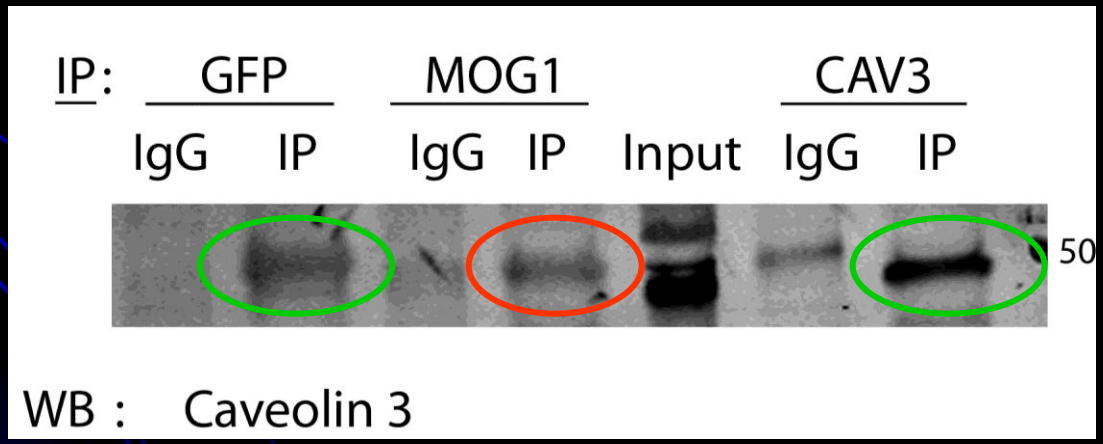


MOG1 knockdown disrupts caveolar localization of $Na_v1.5$

Chakrabarti *et al.* Circ Arrhythm Electrophysiol, 2013

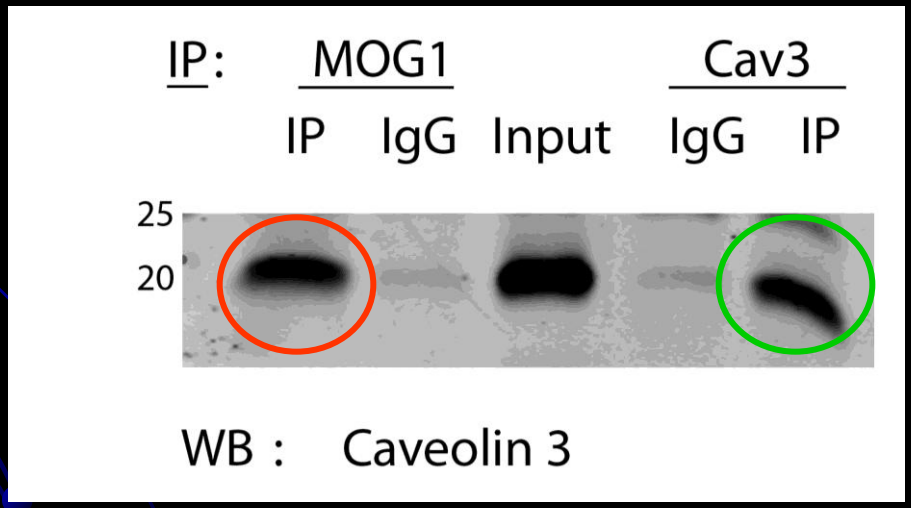
MOG1 can immuno-precipitate Caveolin 3

- HEK293 cells / $Na_v1.5$/ Cav3-GFP/ MOG1
- Immuno-precipitation with anti-MOG1 (anti-GFP, anti-Cav3 : +ve control)
- Western blot with anti-Cav3
- MOG1 can immuno-precipitate cavolin-3 in heterologous cell system



MOG1 can immuno-precipitate Caveolin 3

- Mouse heart extract
- Immuno-precipitation with anti-*MOG1* (anti-Cav3 : +ve control)
- Western blot with anti-Cav3
- *MOG1* can immuno-precipitate cavolin-3 *in vivo*



Summary : Part 3

- MOG1 co-localizes with $Na_v1.5$ in caveolae
- Knockdown of MOG1 leads to re-distribution of $Na_v1.5$ from Cav3-rich fractions to other areas
- MOG1 forms a complex with Cav3 in the mouse heart and in HEK293 cells

Part 4
Translation Research

Can MOG1 enhance PM expressions / restore lost sodium currents of trafficking-deficient mutant $Na_v1.5$ channels ?

Rationale

MOG1 increases PM $Na_v1.5$ expression

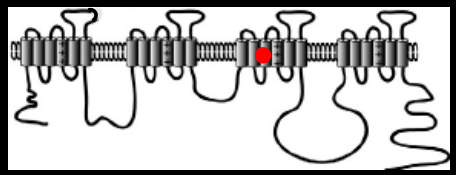
Various cardiac arrhythmic syndromes associated with $Na_v1.5$ trafficking mutants with less/no channels on PM (R282H, P336L, D1275N, G1743R, R1432G, G1740R, M1766L …)

For Brugada Syndrome patients implantation of an ICD (implantable cardioverter defibrillator), for Sick Sinus Syndrome patients implantation of pacemakers : painful shocks/ initiate rhythm disturbances

MOG1 rescues disrupted PM expression of p.D1275N mutant in tsA201 cells

p.D1275N

- Associated with Sick Sinus Syndrome (SSS), Atrial Fibrilation (AF), Dialated Cardiomyopathy (DCM); atrial arrhythmias, intra-cardiac conduction defects, strokes
- Located at DIII of $Na_v1.5$ (S3)



Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 rescues disrupted PM expression of p.D1275N mutant in tsA201 cells

p.D1275N

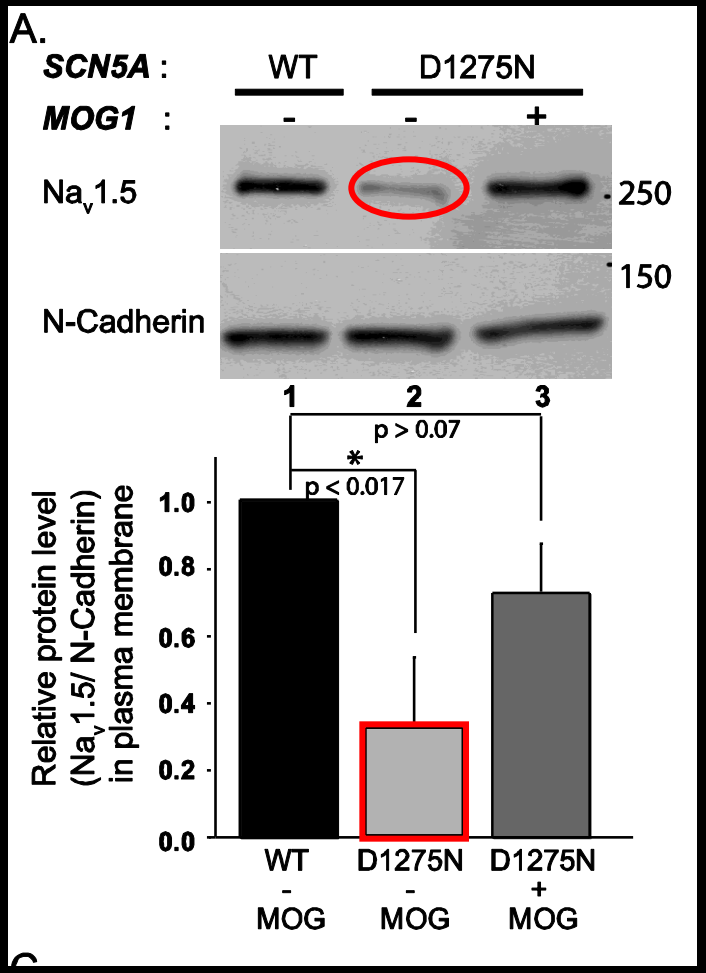
- Associated with Sick Sinus Syndrome (SSS), Atrial Fibrilation (AF), Dialated Cardiomyopathy (DCM); atrial arrhythmias, intra-cardiac conduction defects, strokes
- Located at DIII of $Na_v1.5$ (S3)
- Reduced I_{Na} : blocking cell surface localization
- Altered channel kinetics

Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 rescues disrupted PM expression of p.D1275N mutant in tsA201 cells

Observations

- PM expression of p.D1275N was markedly low compared to WT as reported

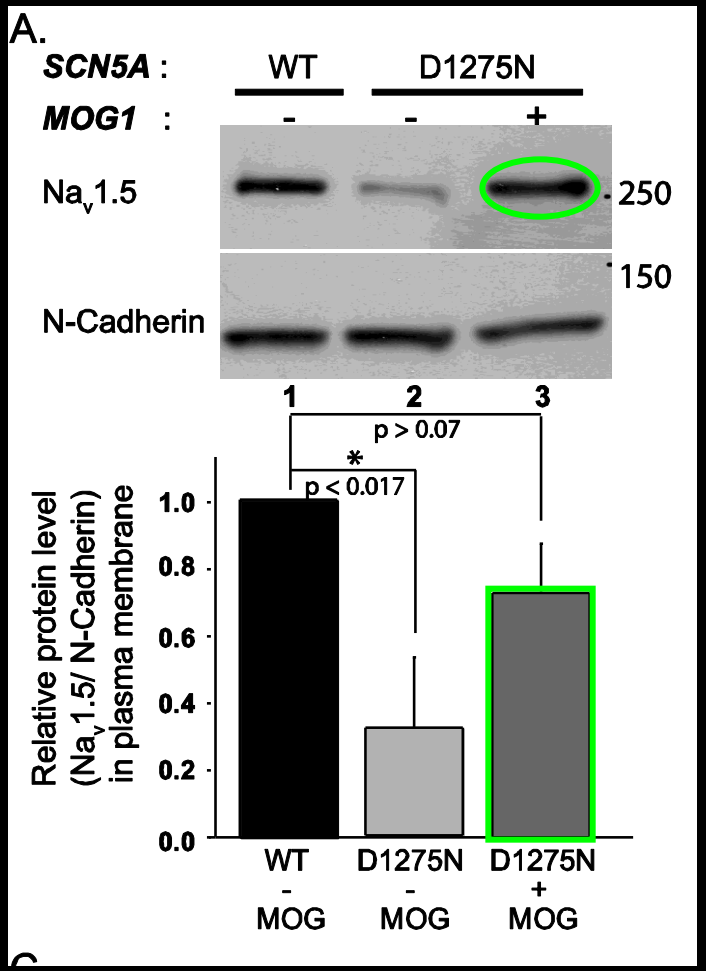


Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 rescues disrupted PM expression of p.D1275N mutant in tsA201 cells

Observations

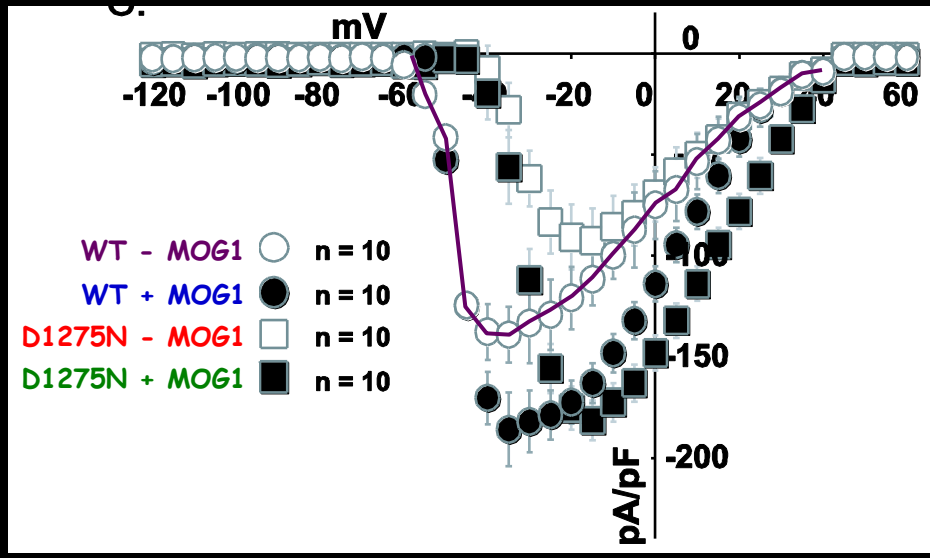
- PM expression of p.D1275N was markedly low compared to WT as reported
- Over-expression of *MOG1* enhanced PM p.D1275N expression significantly



Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 rescues reduced I_{Na} of p.D1275N mutant in tsA201 cells

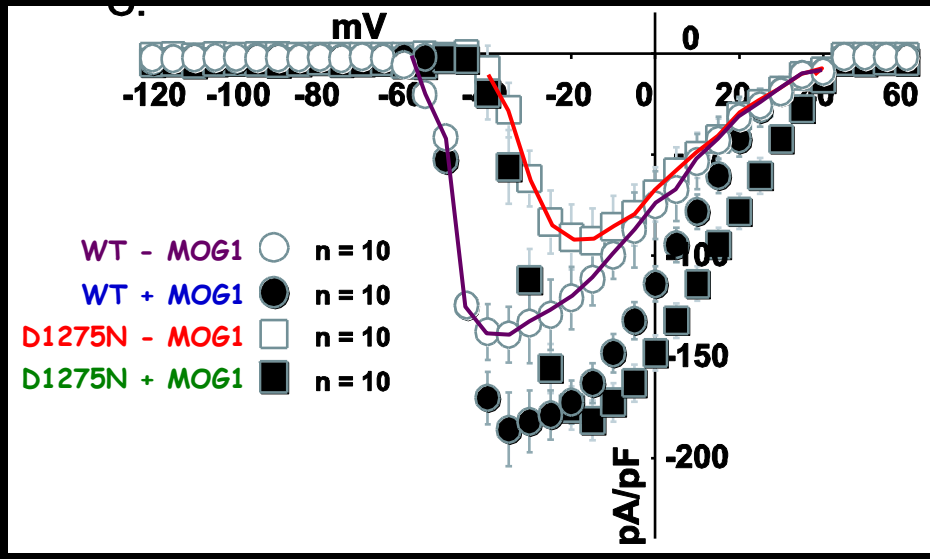
- D1275N mutation drastically reduced the I_{Na} densities



Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 rescues reduced I_{Na} of p.D1275N mutant in tsA201 cells

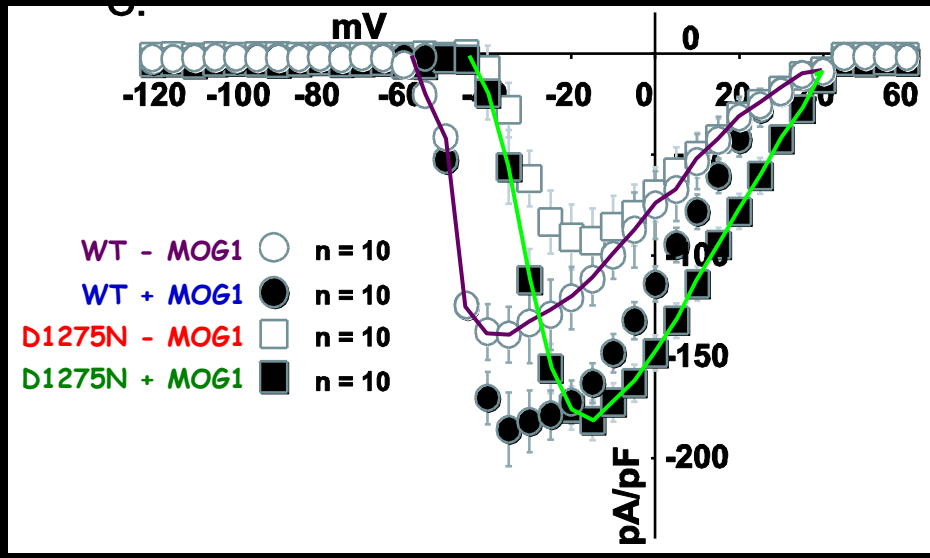
- D1275N mutation drastically reduced the I_{Na} densities



Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 rescues reduced I_{Na} of p.D1275N mutant in tsA201 cells

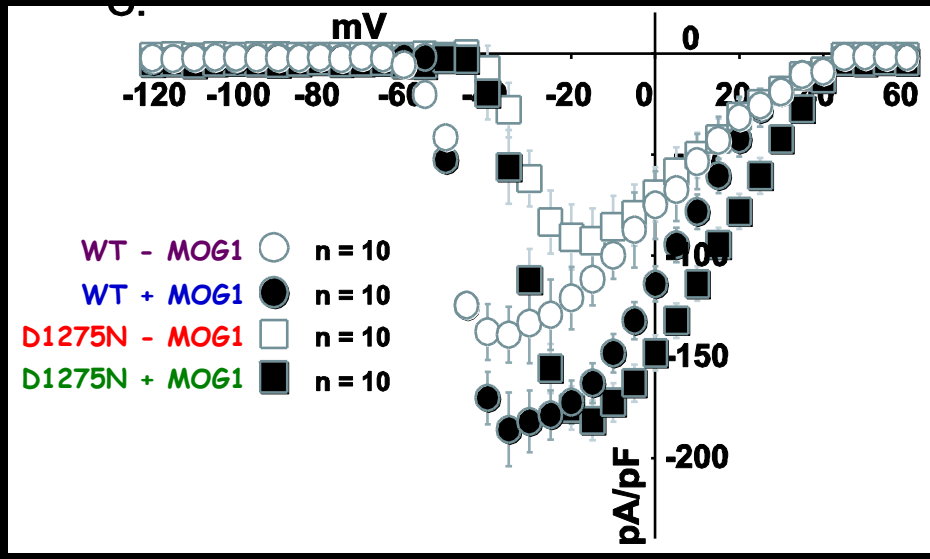
- D1275N mutation drastically reduced the I_{Na} densities
- *MOG1* fully rescued this defect

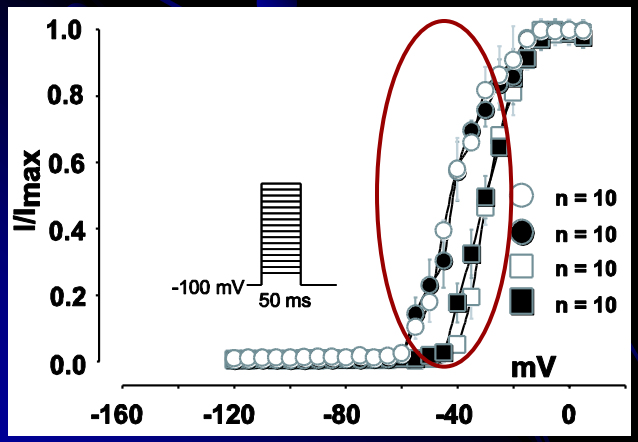


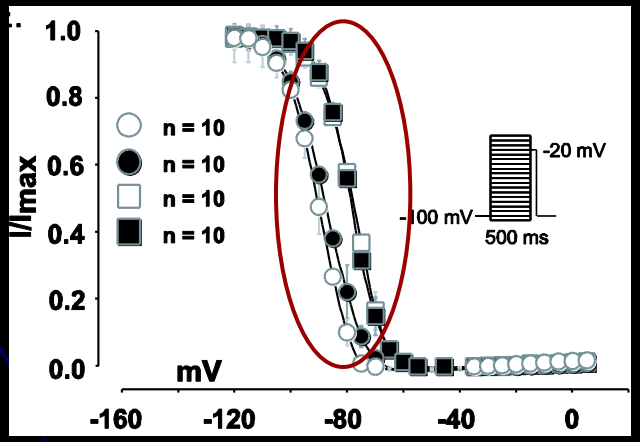
Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

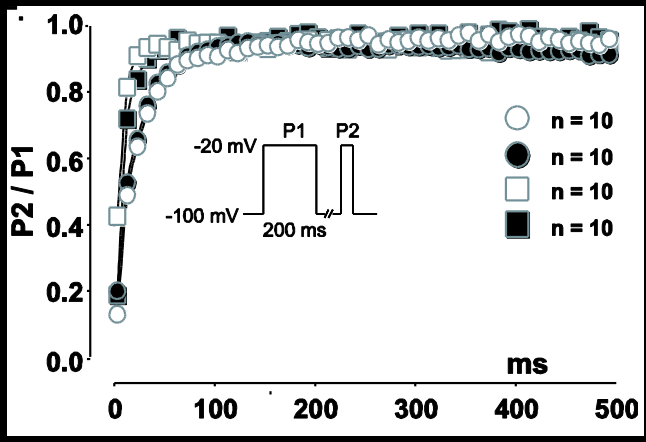
MOG1 rescues reduced I_{Na} of p.D1275N mutant in tsA201 cells

- D1275N mutation drastically reduced the I_{Na} densities
- *MOG1* fully rescued this defect
- D1275N shifted activation/ inactivation curves to more +ve potentials
- *MOG1* did not alter channel kinetics







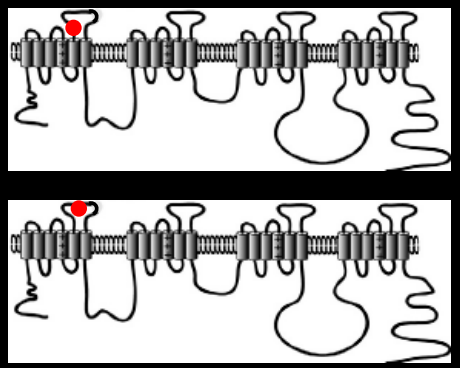


Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 rescues disrupted PM expression of p.P336L, p.R282H mutants in tsA201 cells

p.R282H, p.336L

- Associated with Brugada Syndrome
- Located at DI of $Na_v1.5$

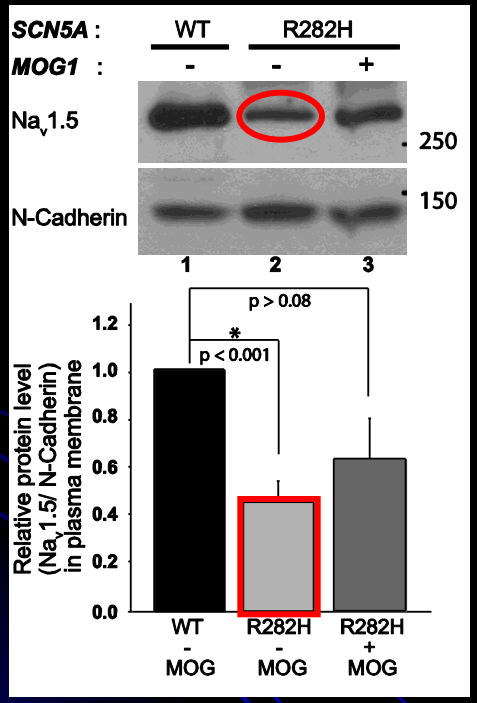


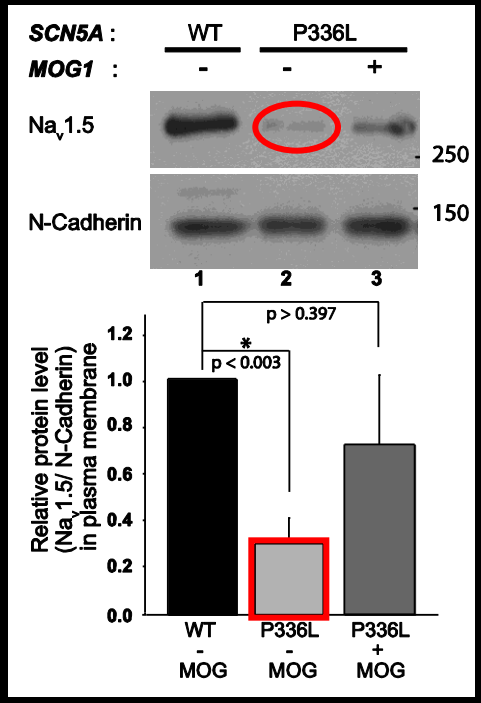
MOG1 rescues disrupted PM expression of p.P336L, p.R282H mutants in tsA201 cells

p.R282H, p.336L

- Associated with Brugada Syndrome
- Located at DI of $Na_v1.5$
- Severe reduction in I_{Na} : defective trafficking of p.R282H
- Similar reduction in I_{Na} for p.P336L : mechanism unknown
- No alteration in channel kinetics

MOG1 rescues disrupted PM expression of p.P336L, p.R282H mutants in tsA201 cells

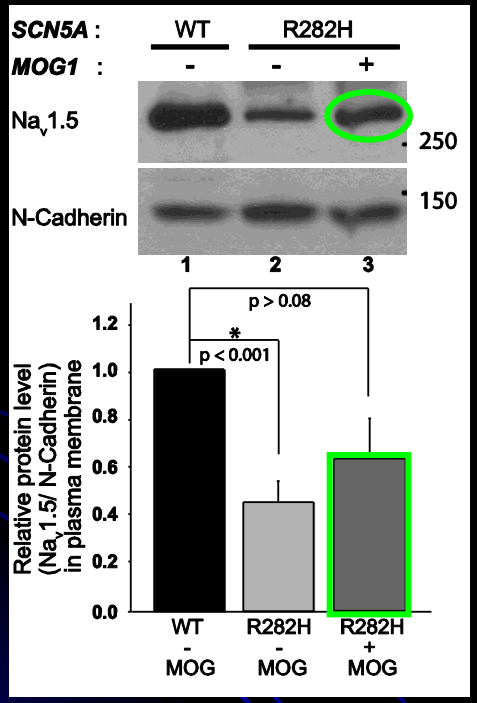


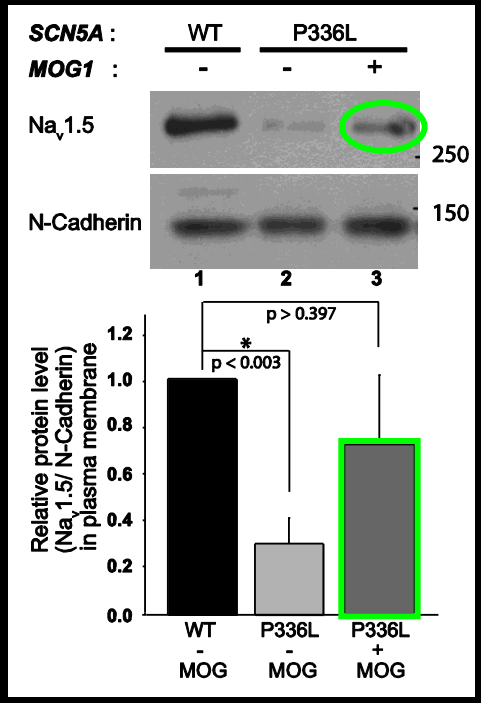


Observations

- PM expressions of mutant channels were considerably low compared to WT

MOG1 rescues disrupted PM expression of p.P336L, p.R282H mutants in tsA201 cells



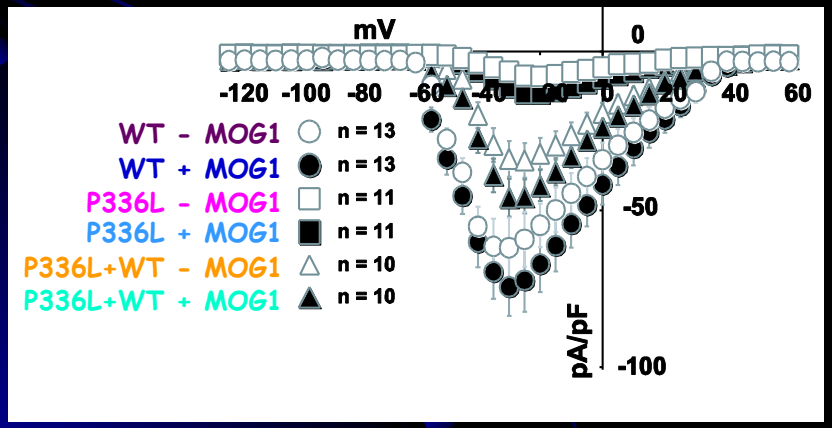


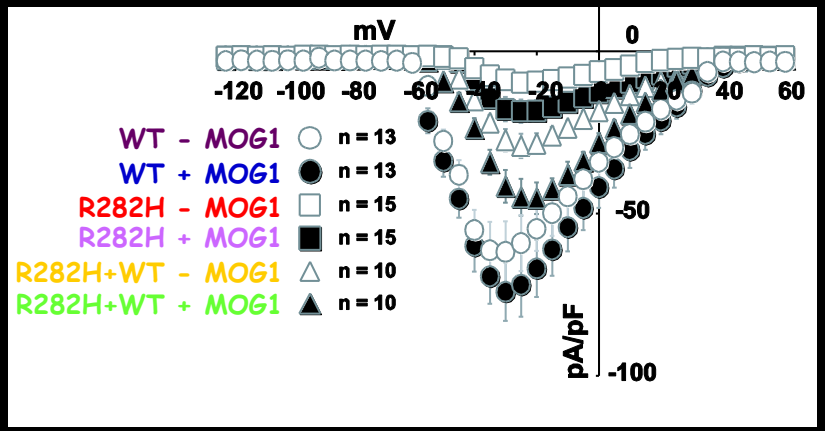
Observations

- PM expressions of mutant channels were considerably low compared to WT
- Over-expression of *MOG1* significantly enhanced PM expression of mutant channels

Effects of MOG1 on I_{Na} of BrS mutations

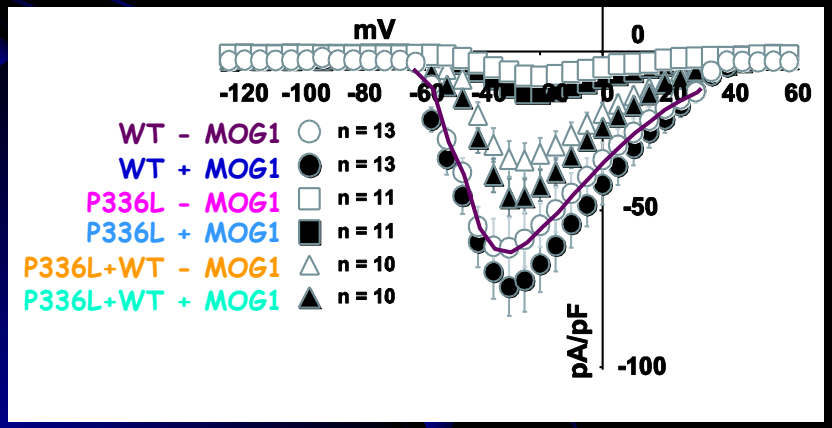
- p.P336L, p.R282H reduced the peak I_{Na} density by 83-87%

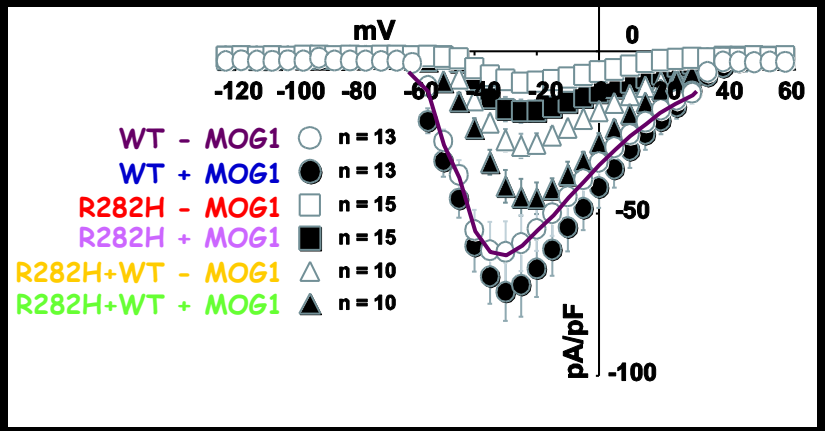




Effects of MOG1 on I_{Na} of BrS mutations

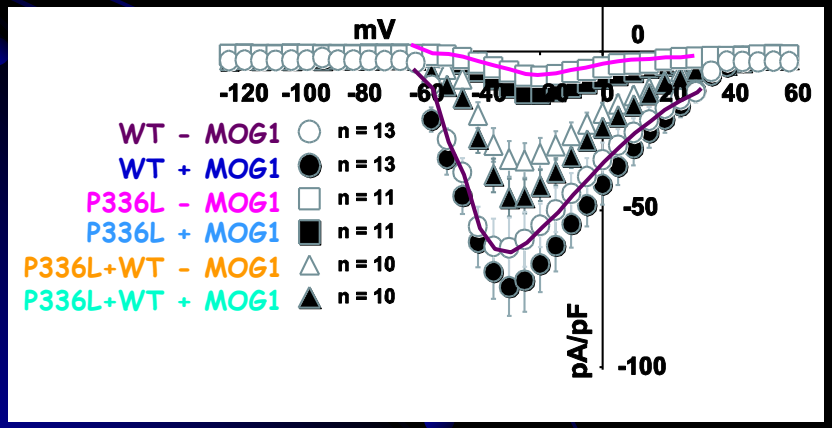
- p.P336L, p.R282H reduced the peak I_{Na} density by 83-87%

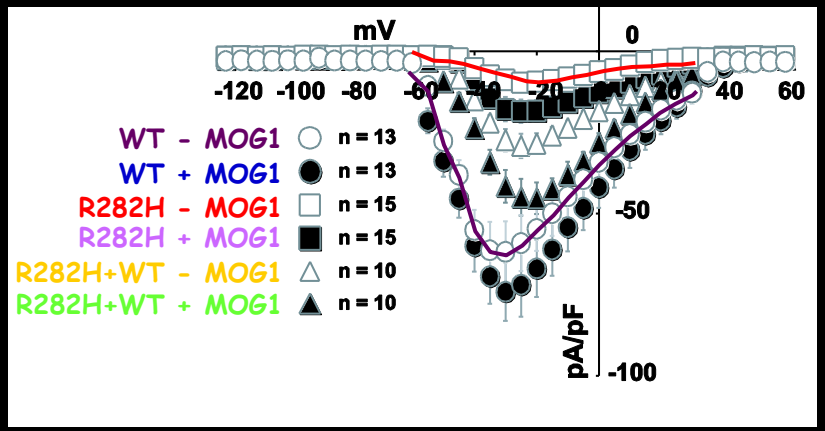




Effects of MOG1 on I_{Na} of BrS mutations

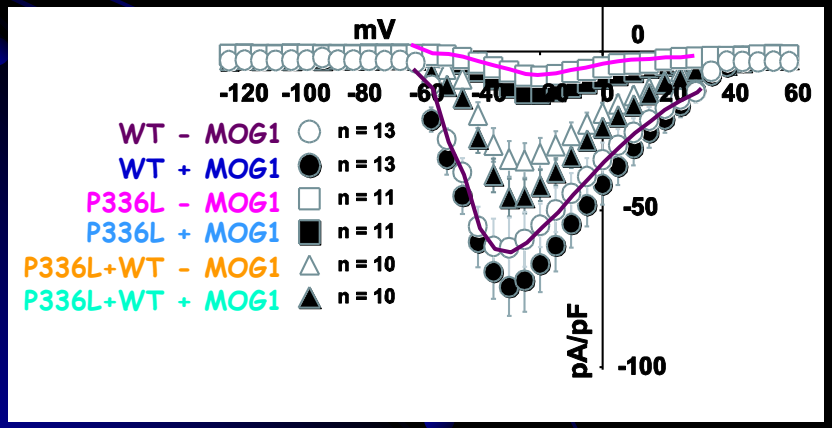
- p.P336L, p.R282H reduced the peak I_{Na} density by 83-87%

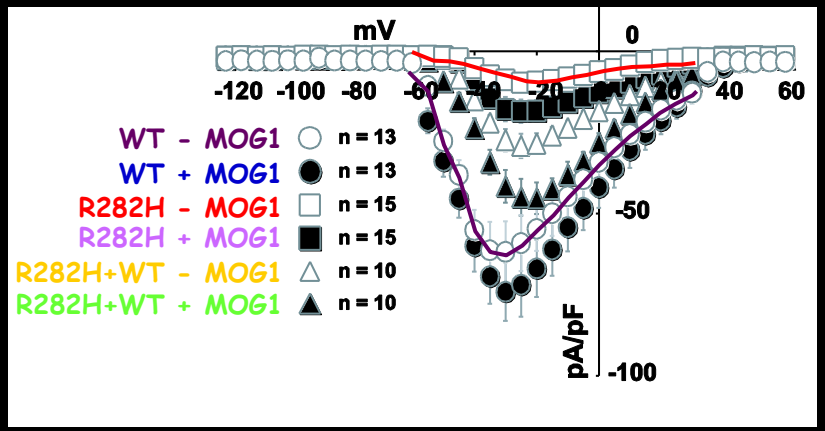




Effects of MOG1 on I_{Na} of BrS mutations

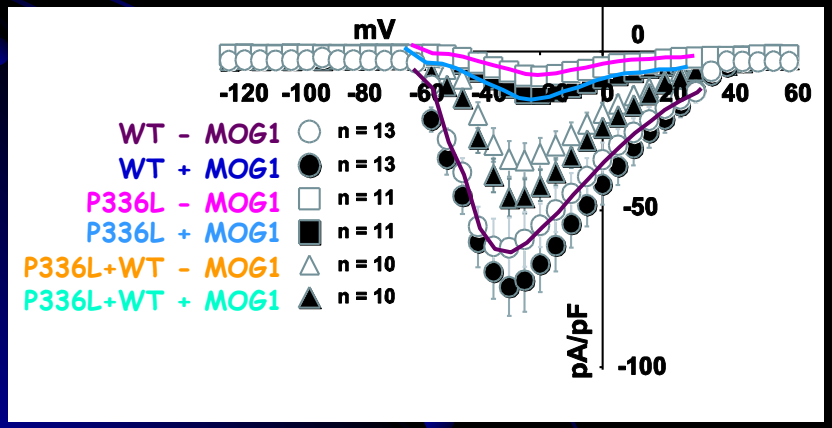
- p.P336L, p.R282H reduced the peak I_{Na} density by 83-87%
- MOG1 increased the peak current density of mutant channels by ~2-fold

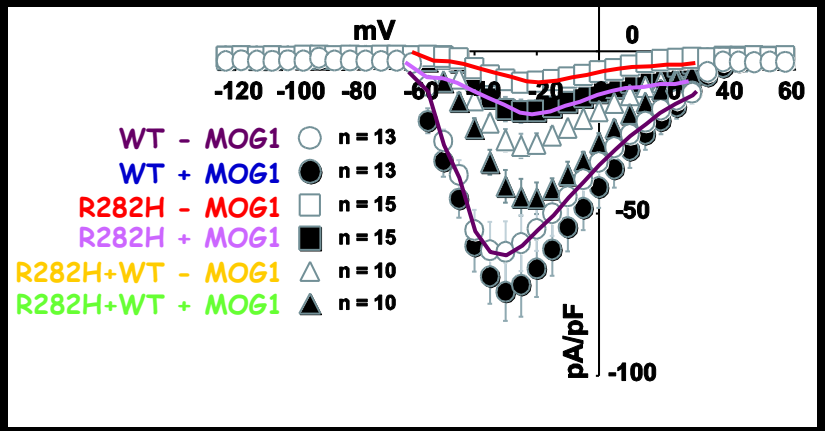




Effects of MOG1 on I_{Na} of BrS mutations

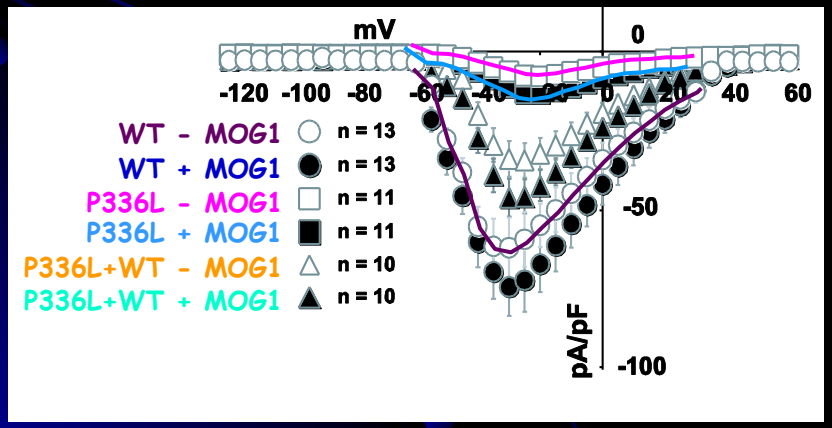
- p.P336L, p.R282H reduced the peak I_{Na} density by 83-87%
- MOG1 increased the peak current density of mutant channels by ~2-fold

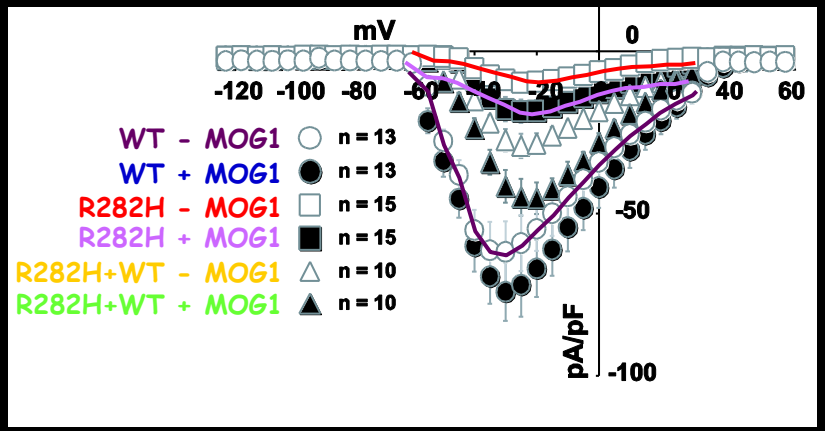




Effects of MOG1 on I_{Na} of BrS mutations

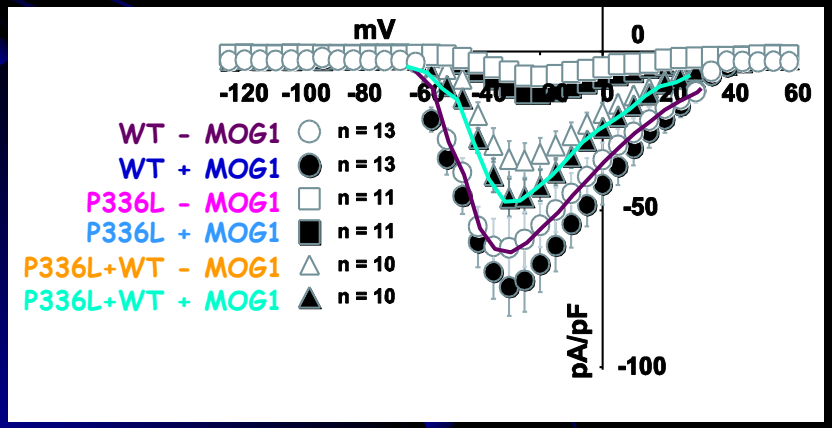
- p.P336L, p.R282H reduced the peak I_{Na} density by 83-87%
- MOG1 increased the peak current density of mutant channels by ~2-fold
- In BrS patients mutation P336L is heterozygous
- After mimicing *in vivo* heterozygous condition in tsA201 cells, over-expression of MOG1 increased peak I_{Na} density to 72-75% level of homozygous WT channels for both mutations

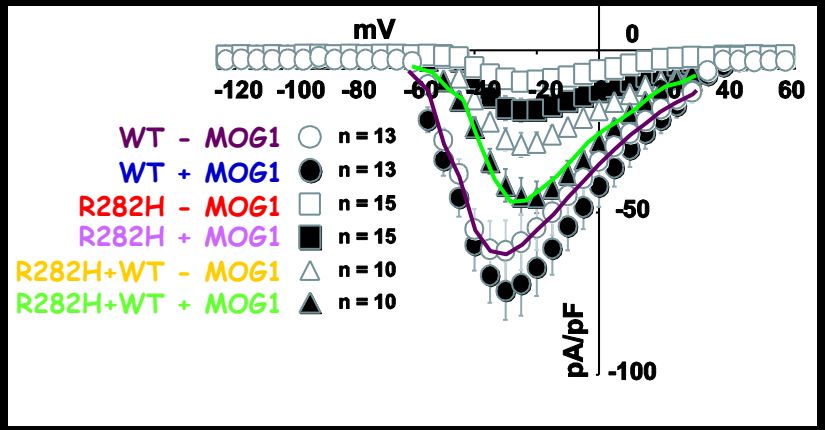




Effects of MOG1 on I_{Na} of BrS mutations

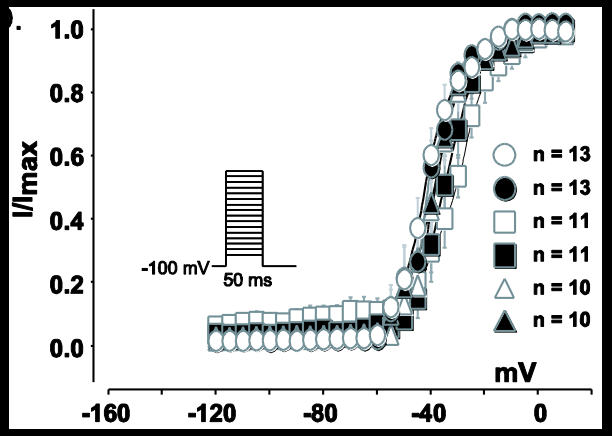
- p.P336L, p.R282H reduced the peak I_{Na} density by 83-87%
- MOG1 increased the peak current density of mutant channels by ~2-fold
- In BrS patients mutation P336L is heterozygous
- After mimicing *in vivo* heterozygous condition in tsA201 cells, over-expression of MOG1 increased peak I_{Na} density to 72-75% level of homozygous WT channels for both mutations

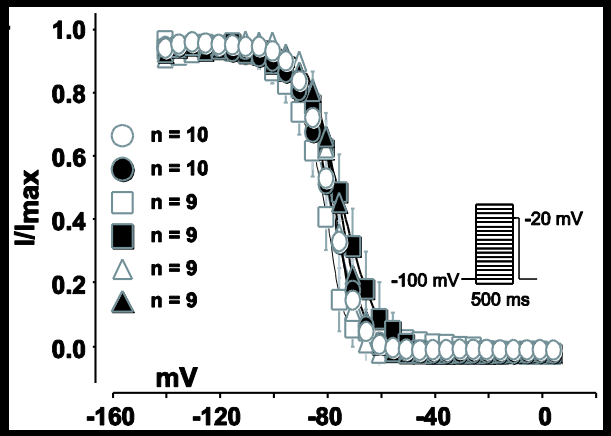


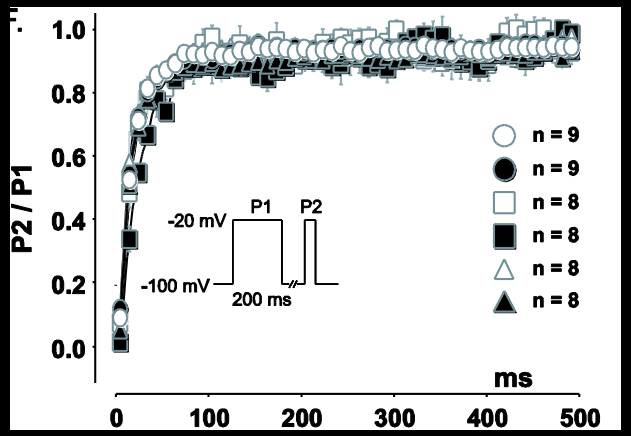


MOG1 does not alter channel kinetics of p.P336L, p.R282H mutants

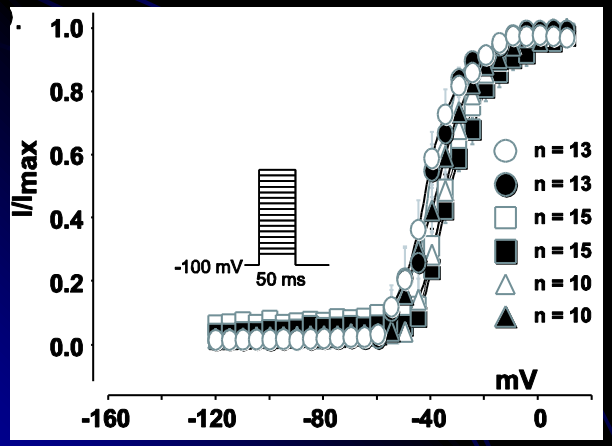
p.P336L

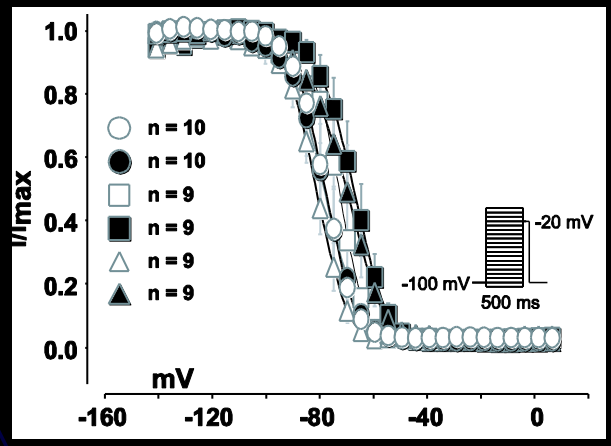


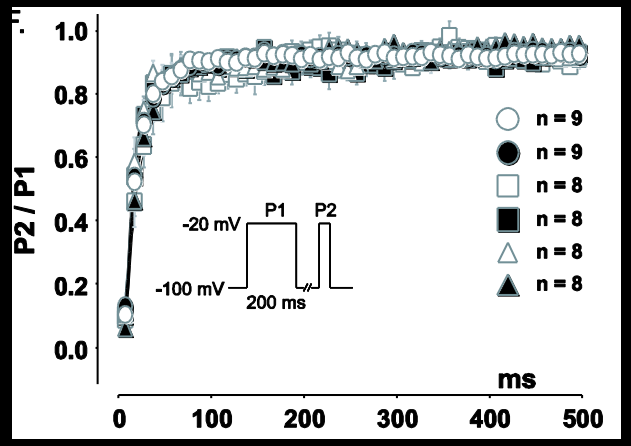




p.R282H



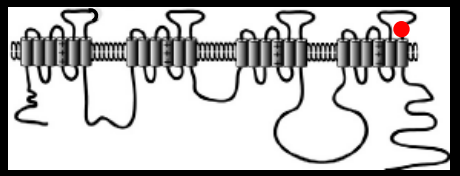




MOG1 rescues disrupted PM expression of p.G1743R mutant in tsA201 cells

p.G1743R

- Associated with Brugada Syndrome
- Located at DIV of $Na_v1.5$ (pore : S5-S6)



Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 rescues disrupted PM expression of p.G1743R mutant in tsA201 cells

p.G1743R

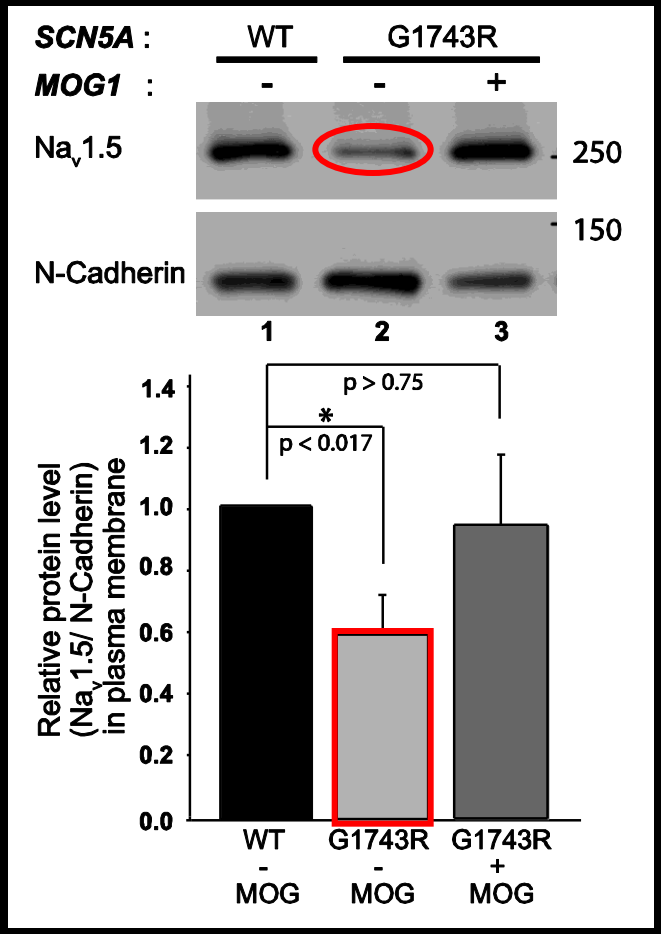
- Associated with Brugada Syndrome
- Located at DIV of $Na_v1.5$ (pore : S5-S6)
- Negligible I_{Na} (HEK293 cells) : complete loss of PM expression/ cytoplasmic retention
- High concentration quinidine/ mexiletine restored I_{Na} to <16% of WT I_{Na}
- Channel blockers : potential side-effects : safety concern

Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 rescues disrupted PM expression of p.G1743R mutant in tsA201 cells

Observations

- PM expression of p.G1743R was significantly low compared to WT

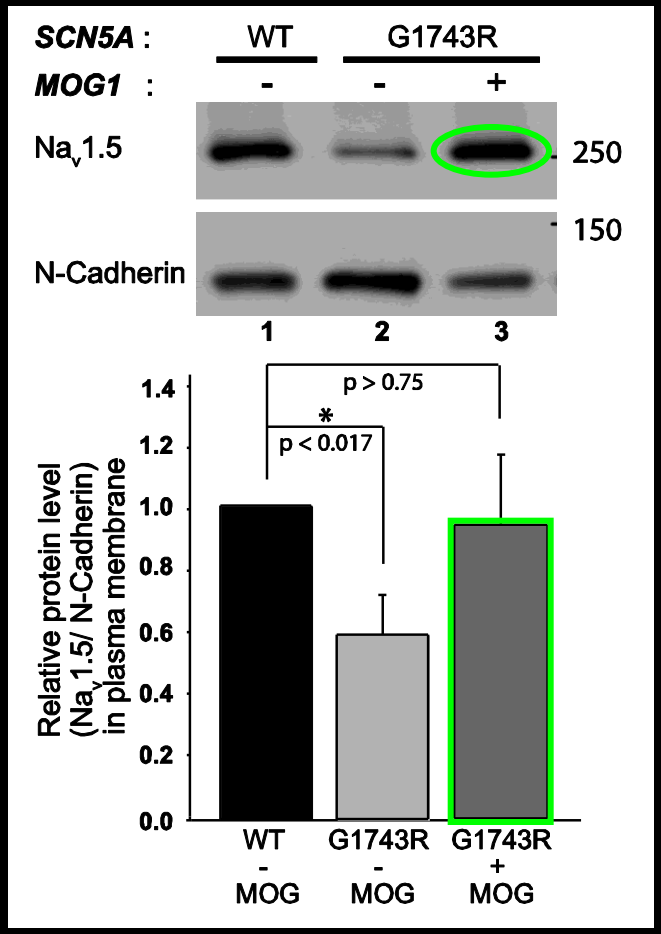


Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 rescues disrupted PM expression of p.G1743R mutant in tsA201 cells

Observations

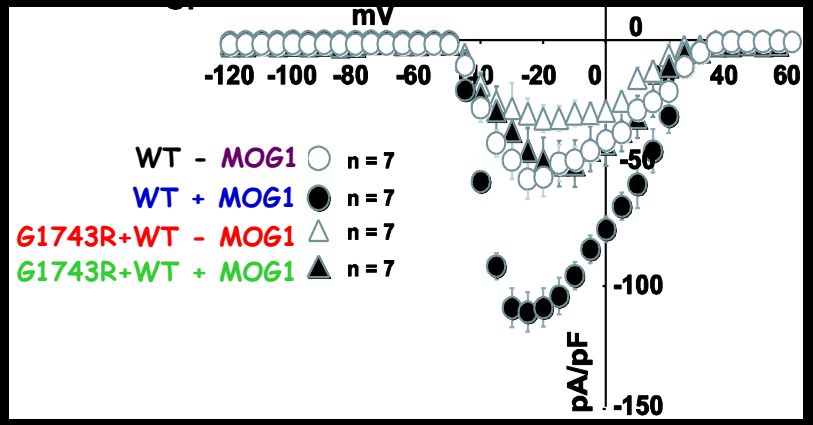
- PM expression of p.G1743R was significantly low compared to WT
- Over-expression of *MOG1* completely restored defective PM p.G1743R expression



Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 fails to rescue reduced I_{Na} of p.G1743R mutant in tsA201 cells

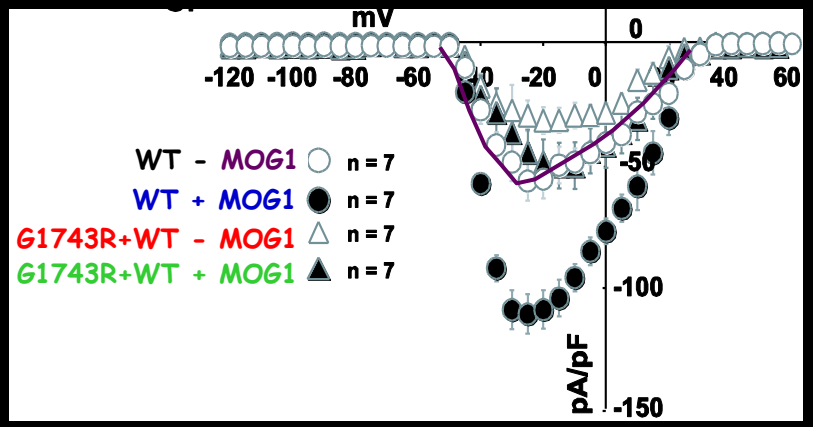
- G1743R channels have no I_{Na}
- MOG1 over-expression failed to rescue I_{Na}
- In heterozygous condition, MOG1 over-expression increases peak I_{Na} density to that of homozygous WT : the highest level reported



Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 fails to rescue reduced I_{Na} of p.G1743R mutant in tsA201 cells

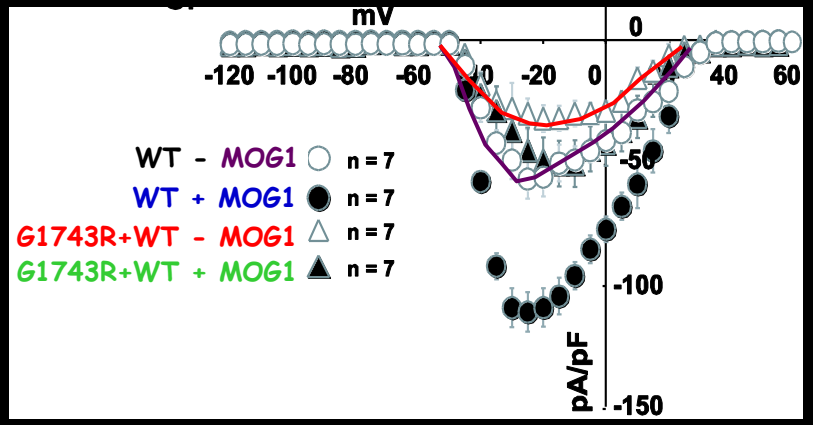
- G1743R channels have no I_{Na}
- MOG1 over-expression failed to rescue I_{Na}
- In heterozygous condition, MOG1 over-expression increases peak I_{Na} density to that of homozygous WT : the highest level reported



Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 fails to rescue reduced I_{Na} of p.G1743R mutant in tsA201 cells

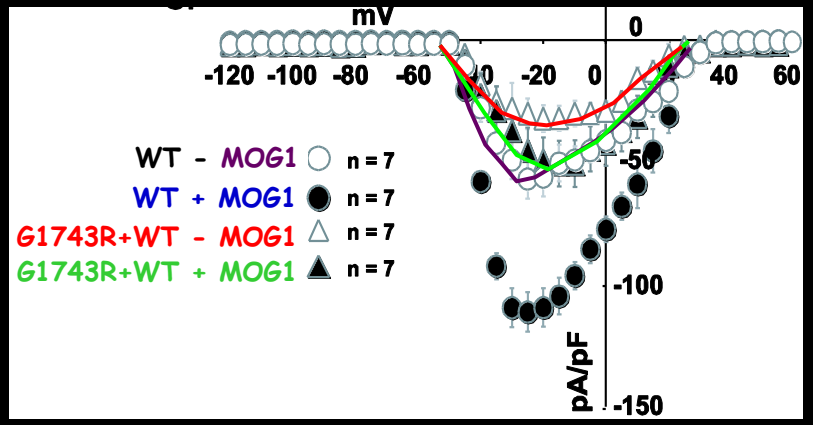
- G1743R channels have no I_{Na}
- MOG1 over-expression failed to rescue I_{Na}
- In heterozygous condition, MOG1 over-expression increases peak I_{Na} density to that of homozygous WT : the highest level reported



Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

MOG1 fails to rescue reduced I_{Na} of p.G1743R mutant in tsA201 cells

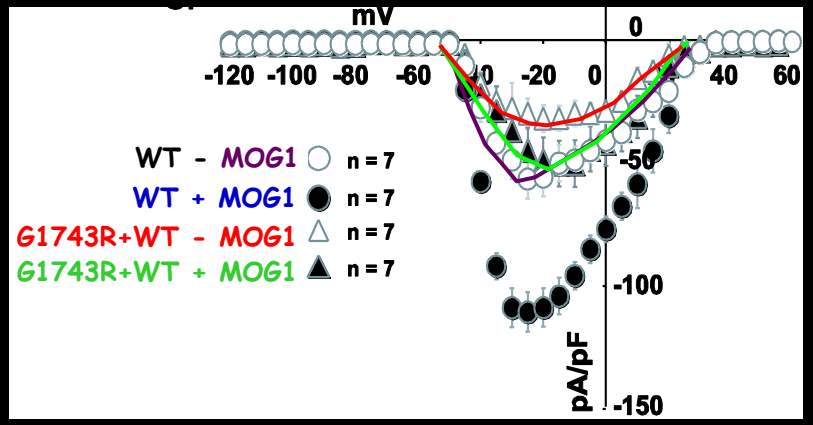
- G1743R channels have no I_{Na}
- MOG1 over-expression failed to rescue I_{Na}
- In heterozygous condition, MOG1 over-expression increases peak I_{Na} density to that of homozygous WT : the highest level reported

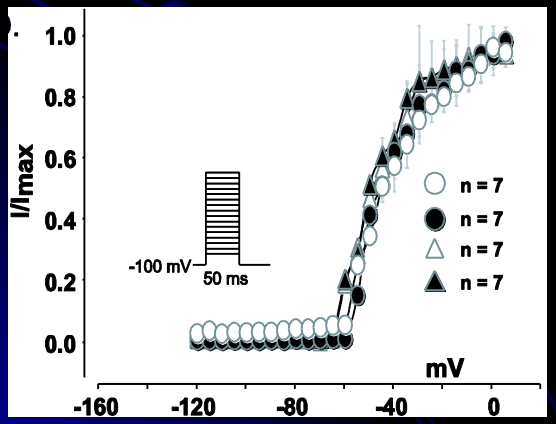


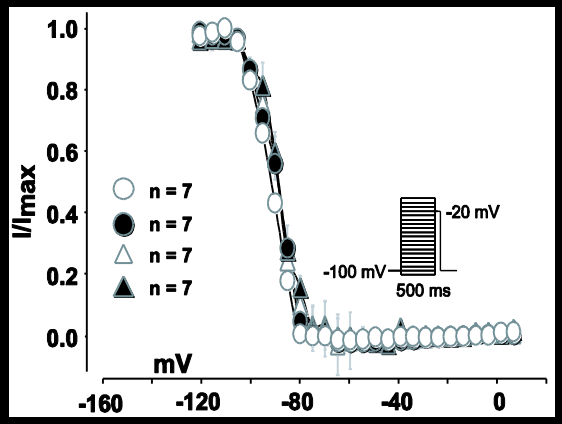
Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

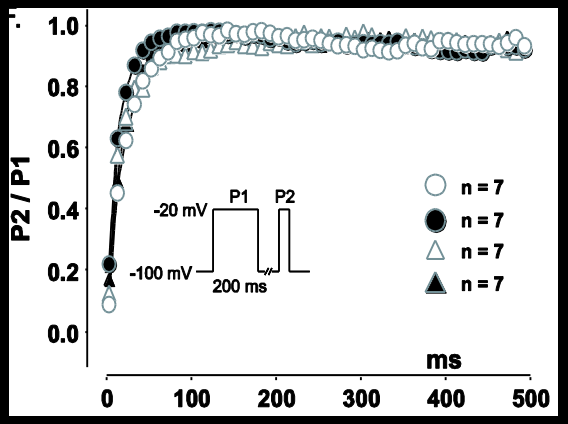
MOG1 fails to rescue reduced I_{Na} of p.G1743R mutant in tsA201 cells

- G1743R channels have no I_{Na}
- MOG1 over-expression failed to rescue I_{Na}
- In heterozygous condition, MOG1 over-expression increases peak I_{Na} density to that of homozygous WT : the highest level reported
- MOG1 does not affect channel kinetics



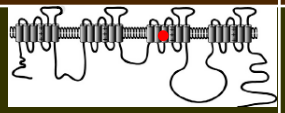
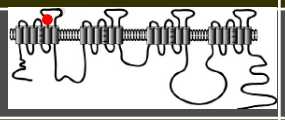
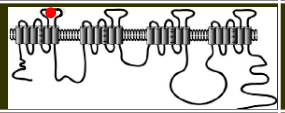
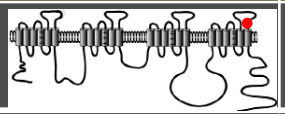






Chakrabarti *et al* Circulation Arrhythmia & Electrophysiol., 2013

Summary Table for MOG1 mediated restorations sodium currents of trafficking-deficient mutant $Na_v1.5$ channels

Mutation	Disease	Mutation position	Mutant I_{Na}	Altered Kinetics	I_{Na} Rescue	
					mut $Na_v1.5$	mut + Wt $Na_v1.5$
D1275N	SSS,AF, DCM,CCD	DIII 	~ 60%	yes	100%	-
R282H	BrS	DI 	~ 13%	no	~ 2 fold	~ 75%
P336L	BrS	DI 	~ 17%	no	~ 2 fold	~ 73%
G1743R	BrS	DIV 	negligible	n/a	no	100%

Summary : Part 4

MOG1 can rescue impaired PM expression of $Na_v1.5$ trafficking mutants identified in patients with BrS/ DCM/ SSS/ atrial arrhythmias

MOG1 can restore peak I_{Na} to 75-100% of WT channels under condition mimicking heterozygous Na channel state : potential therapeutic tool for loss-of-function mutations/ reduced $Na_v1.5$ expression

MOG1 does NOT alter kinetic properties of WT/ mutant channel : tool to distinguish if a loss-of-function mutation in $Na_v1.5$ is due to impaired trafficking/ impairment of gating

MOG1 therapy : more advantageous for patients having $Na_v1.5$ mutations with complete loss of gating - rescued current would have WT properties

Acknowledgement

MOG1 Rescues Defective Trafficking of Nav1.5 Mutations in Brugada Syndrome and Sick Sinus Syndrome

Chakrabarti S, Wu X, Yang Z, Wu L, Yong SL, Zhang C, Hu K, Wang QK, Chen Q.

Dr. Thomas Zimmer, Friedrich-Schiller Universität, Jena, Germany

Dr. J. C. Makielski, Univ. of Wisconsin

Drs. S. Prasad and S. Karnik at Cleveland Clinic

Dr. Charles Antzelevitch at Masonic Medical Research Laboratory

Dr. A. H. Corbett, Emory Univ

Funding

-NIH R01 HL094498

-American Heart Association Scientist Development grant (0630193N to Q.C.)

-973 program 2013CB531101