# Human Muscle-Specific A-Kinase Anchoring Protein (mAKAP) Polymorphisms: Mechanistic Role in Cardiovascular Diseases

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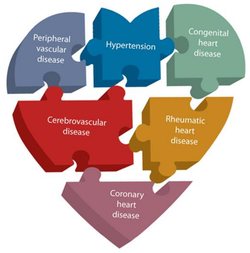
GRaSP- 2017

## BACKGROUND

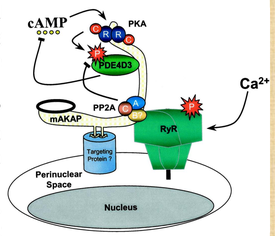
### CARDIOVASCULAR DISEASES AND SINGLE NUCLEOTIDE POLYMORPHISMS

- Cardiovascular diseases (CVDs) are the leading cause of deaths globally with **17.7 million deaths every year**, more than all forms of cancer combined.
- The National Human Genome Research Institute reported **5585 SNPs with association to CVDs** at $P < 10^{-5}$ (Kathiresan and Srivastava 2012).
- **Genetics** plays an incomparable role in the susceptibility of humans to CVDs.



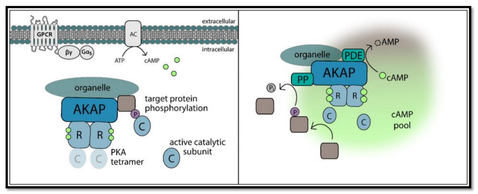

### mAKAP: A Kinase Anchoring Protein (AKAP)

- **AKAP**s, by definition, bind **protein kinase A (PKA)** along with other crucial proteins in the body (Wong and Scott, 2004).
- **mAKAP**, muscle-specific AKAP that is expressed in the heart, is a master scaffold for tight regulation of cAMP.
- By maintaining local cAMP pools, mAKAP organizes cardiac **cAMP/PKA signaling**.




Mol Pharmacol. 2002 Aug;62(2):193-9

### cAMP/PKA/AKAP TRIAD

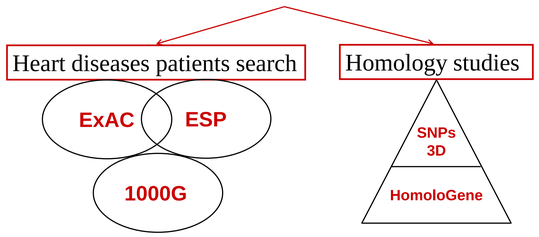



Cell Signal. 2015 Dec;27(12):2474-87

- Cyclic adenine monophosphate (**cAMP**) is a **universal intracellular second messenger** (Dema, Perets et al. 2015).
- cAMP **activates PKA** and modulates phosphorylation of PKA substrates; thus **controls basic heart functioning**.
- By binding to PKA, **AKAPs regulate cAMP/PKA signaling** where alterations in this signaling are implicated in CVDs.

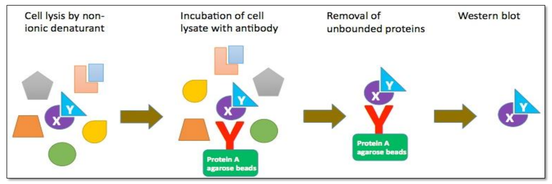
## METHODS

### COMPUTATIONAL STUDIES

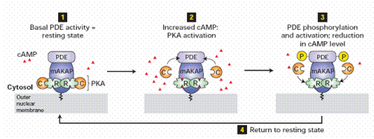



**ExAC**: Exome Aggregation Consortium } **Patients'**
**ESP**: Exome Sequencing Project } **Databases**
**1000G**: 1000 Genome Project – **Heathy people**
**SNPs3D and HomoloGene**: Softwares that assign molecular functional effects of SNPs based on the structure and sequence analysis

### IMMUNOPRECIPITATION



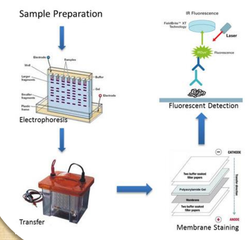

### cAMP LEVELS AND PKA ACTIVITY ASSAY




Trends Mol Med. 2006 Jul;12(7):317-23.

- Intracellular cAMP release was measured by Enzo kit.
- PKA activity was quantified by ELISA kit-based assay.

### WESTERN BLOTTING




- Western blotting was carried out as previously described (McConnell et al.,2009).
- Whole cell lysates and cytosolic fractions were resolved using SDS polyacrylamide gel electrophoresis based on their molecular weight as shown in the figure here.
- Image J (NIH) was used for the densitometric quantification of the western blots.

## RESULTS

### COMPUTATIONAL STUDIES DATA

| SNPs # | Amino acid positions | Nucleotide change | Amino acid change | Binding domain | Occurrence |
|---|---|---|---|---|---|
| 1 | 1653 | A/C | Serine (S) to Arginine (R) | PDE4D3 | Frequent in American, European, Canadian and Italian population |
| 2 | 2124 | A/G | Glutamic acid (E) to Glycine (G) | 3'-PKA RII | Exclusively frequent in African American population |

**Figure 1: Summary of computational studies.**
This table shows two mAKAP SNPs to be studied highlighting their position in the mAKAP protein and occurrence.

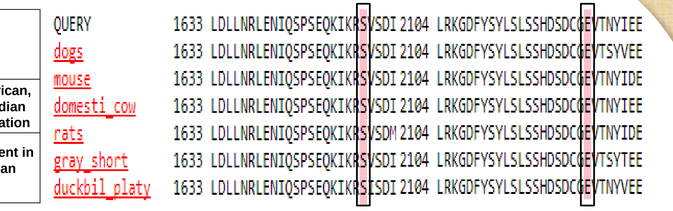
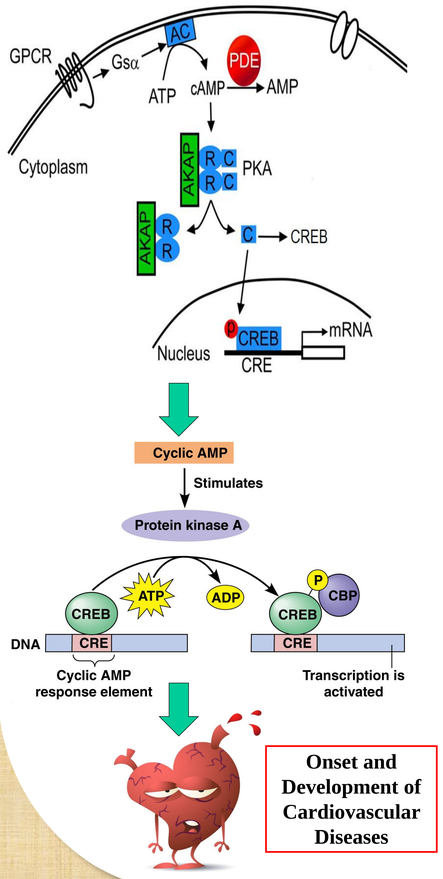



**Figure 2: The homology studies for human mAKAP Serine1653 and Glutamic acid2124.**
The amino acid residues (serine 1653 and glutamic acid 2124; highlighted) are evolutionarily conserved among different species. This shows that Serine and Glutamic acid residues are extremely crucial for mAKAP function.




### IMMUNOPRECIPITATION DATA

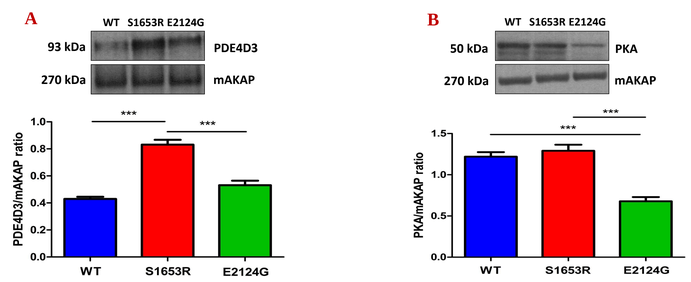



**Figure 3: Binding of PDE4D3 and PKA to WT and mutant mAKAP.**
**A**. Binding of PDE4D3 to mAKAP and **B**. Binding of PKA to mAKAP. Immunoprecipitation results showed that mAKAP polymorphisms changed binding of PDE4D3 and PKA to mAKAP differently.

### cAMP LEVELS and PKA ACTIVITY DATA

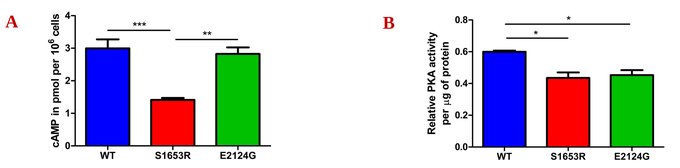



**Figure 4: Direct cAMP levels and PKA activity assay.**
**A.** Intracellular cAMP measured as pmol/$10^6$ cells. **B.** PKA activity normalized to per µg of protein. Each sample is performed in triplicate and four independent experiment were done for analyses.

### CREB PHOSPHORYLATION

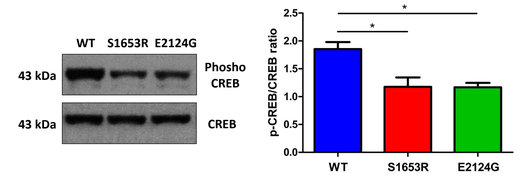
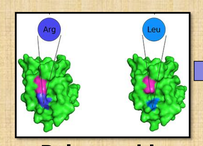



**Figure 5: Western blotting.**
Western blot analysis of PKA phosphorylation of CREB.
Left panel shows the bands with phospho-CREB and total CREB protein antibodies and right panel shows the corresponding quantifications graphs.

## OBJECTIVE

**To study the mechanistic role of mAKAP polymorphisms in increasing risk of cardiovascular diseases**

## SUMMARY OF RESULTS




Abrupt binding of PKA and PDE4D3 → Changes in intracellular cAMP release → Change in PKA activity → Higher PKA phosphorylation of CREB

## INNOVATION

- **This is the FIRST study to find out the relationship between the genetics of AKAPs and heart disease.**
- **Abstract based on this poster is *accepted* for the world's no. 1 heart conference (AHA) in Anaheim, California.**

## CONCLUSIONS

- ✓ **mAKAP polymorphisms may pre-dispose humans to CVDs by altering cAMP/PKA signaling in the heart.**
- ✓ **PKA-mAKAP interaction modulators can be developed as therapeutic target to augment current treatment of CVDs.**