UAEU

The cellular trafficking and targeting of ACE2

Presented by: Nesreen Fayyad Al Khofash

Supervisor : Prof. Bassam Ali

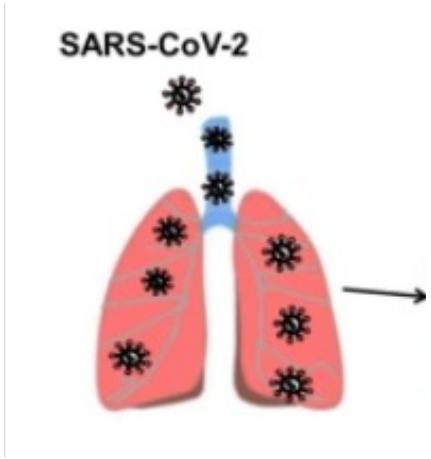
Department: Genetics and Genomics

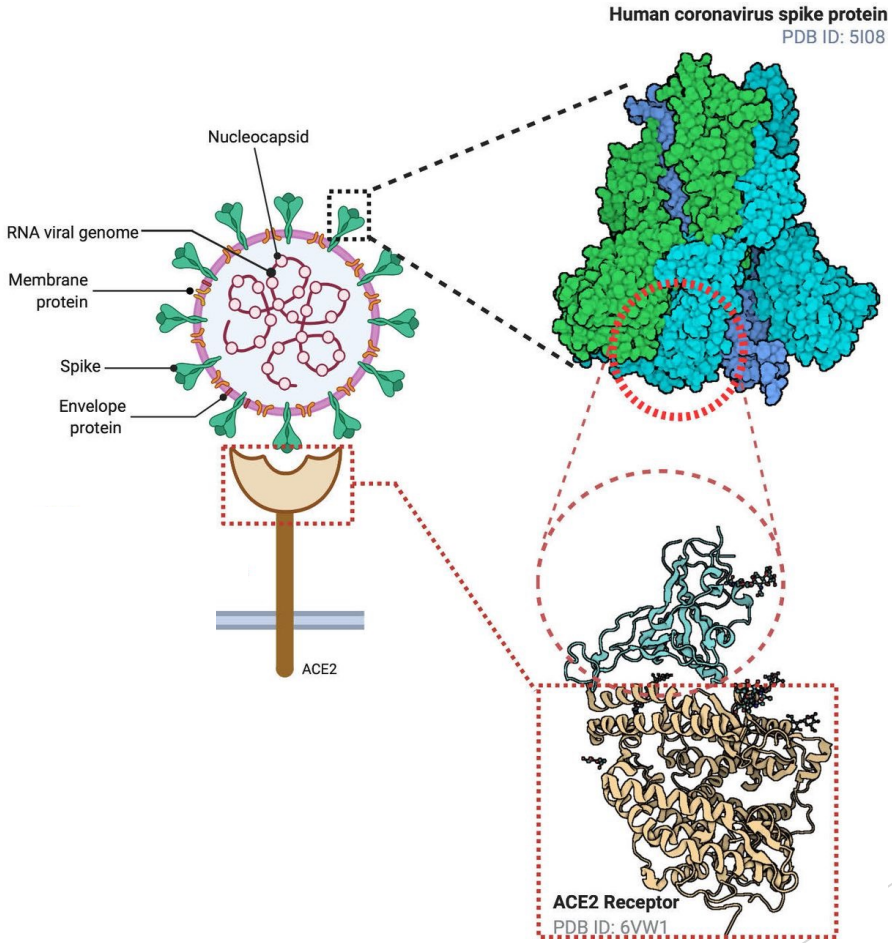
College: Medicine and Health Sciences.

Presentation index

- Introduction
- Significance of study and aims
- Study design
- Results
- Future plan

SARS-CoV-2 infection through ACE2





- COVID-19 caused by SARS-CoV-2 has been declared a pandemic in March 2020.
- This virus has been proven to be highly infectious and deadly in some cases, causing over 2.5 million deaths world-wide so far.
- **The spike S protein of SARS-CoV-2 mediates the viral attachment and entry into the host cell by binding to its target receptor, the (ACE2).**

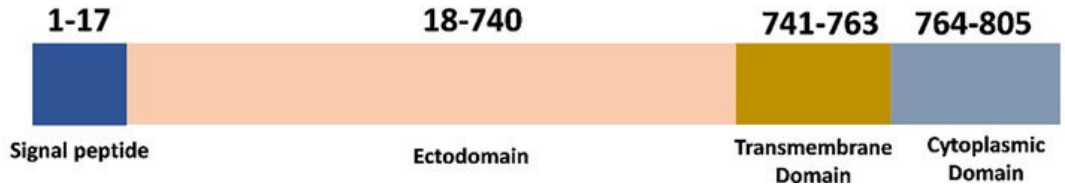
(Letko et al., 2020; Walls et al., 2020; Wan et al., 2020; Zhou et al., 2020).

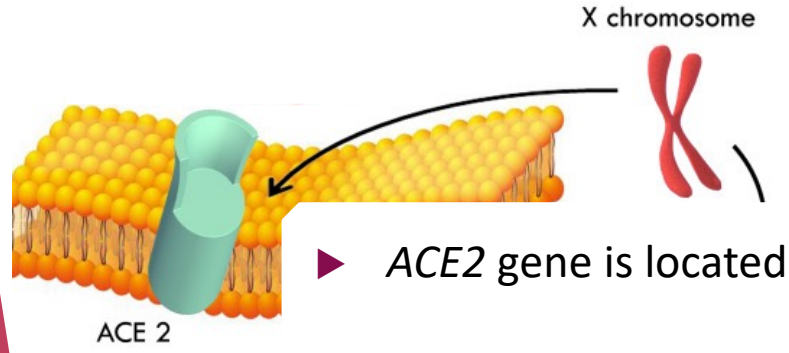
Angiotensin-Converting Enzyme2 (ACE2)

ACE2 is a member of the renin angiotensin system (RAS)

- Plays a role in blood pressure, fluid, electrolyte homeostasis.
- Facilitator of amino acid transport.
- SARS-CoV and SARS-CoV-2 receptor.

(Chung et al., 2020; Samavati and Uhal, 2020).



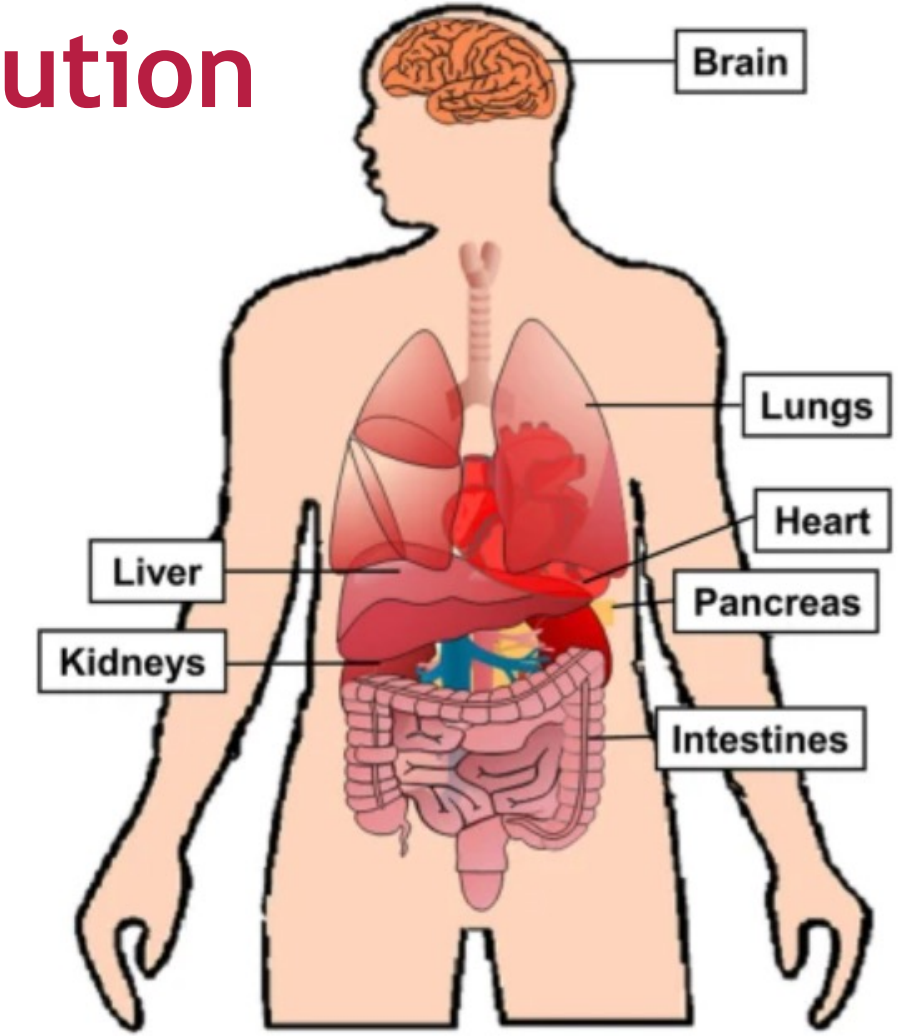


- *ACE2* gene is located on the X chromosome (Samavati and Uhal, 2020).

ACE2 tissue distribution

- RNA-seq and microarray studies showed that ACE2 is expressed in different cell types including in the lungs, cardiovascular system, gut, kidneys, central nervous system, and adipose tissue.

  (Chung et al., 2020).



ACE2 variants

- **Allele frequency (AF) analysis** of ACE2 coding variants between populations shows diversity of it's expression patterns in the different ethnic groups in the Asian population which might explain the different systemic response of SARS-CoV-2 in different populations under similar conditions (Cao et al., 2020).

- **Single nucleotide variation analysis** of human ACE2 shows:
  - Many variations that were mostly population-specific
  - Majority of ACE2 variations were located in the protein coding regions
  - Some were distributed in the spike protein-ACE2 contact region.

  (Fujikura and Uesaka, 2020).

Study hypothesis:

Reduced cell surface availability of ACE2 might affect the susceptibility and severity of SARS-CoV-2 infection.

Main Aim:

Evaluate the effects of the reported ACE2 missense variants on its trafficking, structure and function.

Significance of the study

- Several studies have investigated how ACE2 genetic variants affected the binding affinity to the spike protein but the effect of these variations on ACE2 cellular trafficking and localization is not tackled yet.

- It is predicted that reduced cell-surface availability of ACE2 might reduce the rate of SARS-CoV-2 infection → might explain some of the variability in the clinical severity of the disease observed among COVID-19 patients.

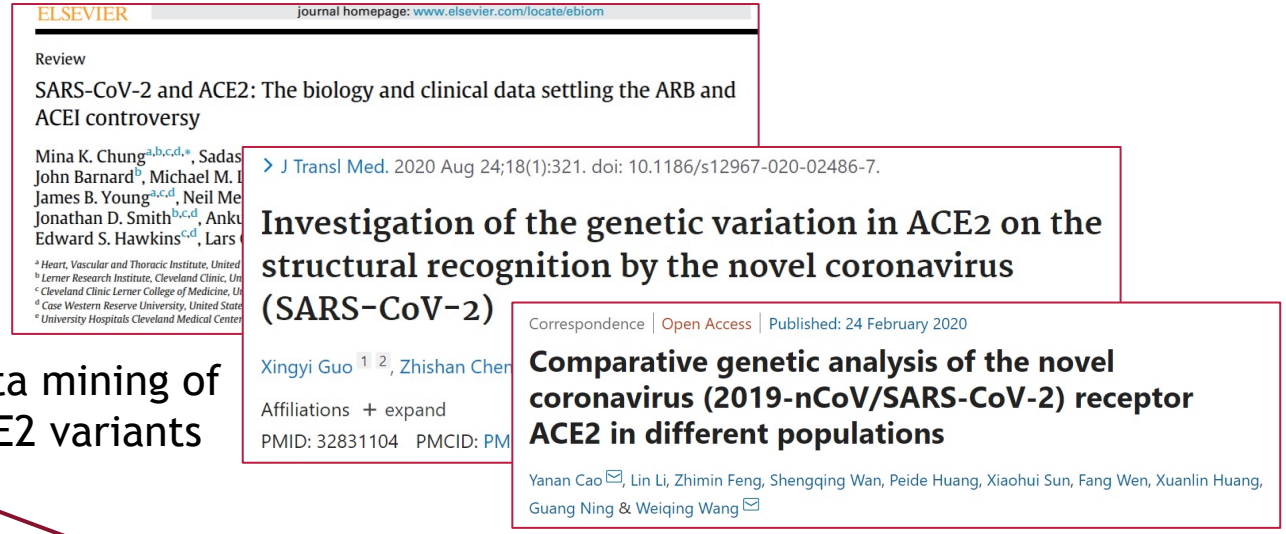
- Our finding might suggest that manipulating cell surface expression of this receptor could be a potent therapeutic target for COVID-19.

Our objectives include:

- 1. Generate the known ACE2 gene-coding missense variants reported in humans.
- 2. Elucidate the effect of the generated SNP variants on the receptor's expression, trafficking and subcellular localization
- 3. Perform functional analysis
- 4. Preform molecular dynamics (MD) simulation on the ACE2 receptor mutant forms that showed defective binding and retarded trafficking.

Study design

Data mining of ACE2 variants



ELSEVIER journal homepage: www.elsevier.com/locate/ebiom

Review

SARS-CoV-2 and ACE2: The biology and clinical data settling the ARB and ACEI controversy

Mina K. Chung[a,b,c,d,*], Sadas
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J Transl Med. 2020 Aug 24;18(1):321. doi: 10.1186/s12967-020-02486-7.

Investigation of the genetic variation in ACE2 on the structural recognition by the novel coronavirus (SARS-CoV-2)

Xingyi Guo [1 2], Zhishan Chen

Affiliations + expand

PMID: 32831104 PMCID: PM

Correspondence | Open Access | Published: 24 February 2020

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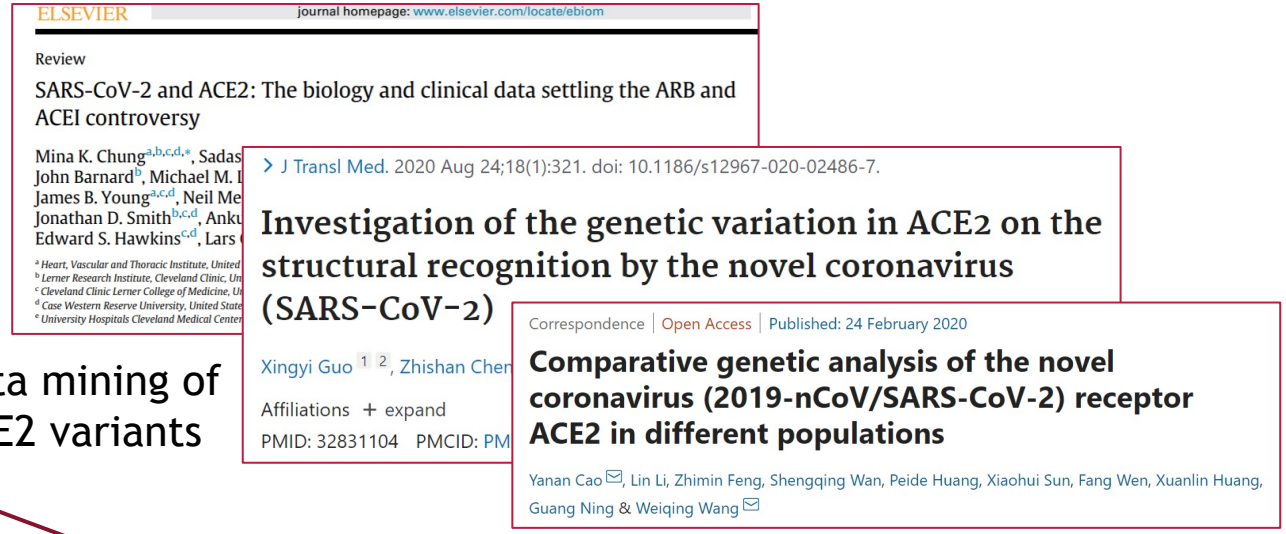

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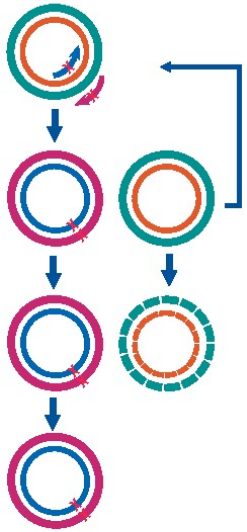
Ace2 mutants generation



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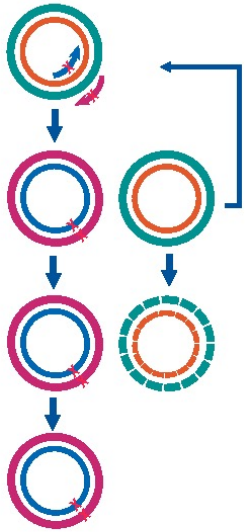
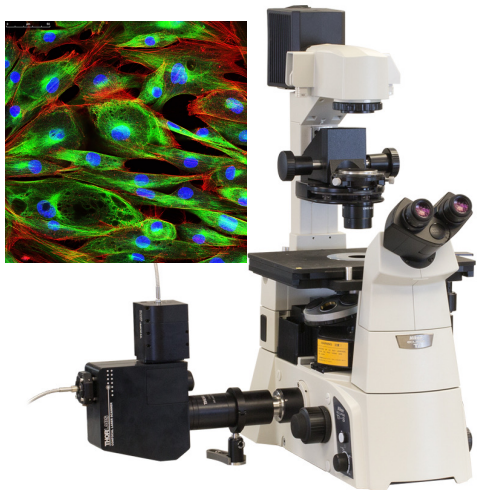


ACE2 trafficking and subcellular localization



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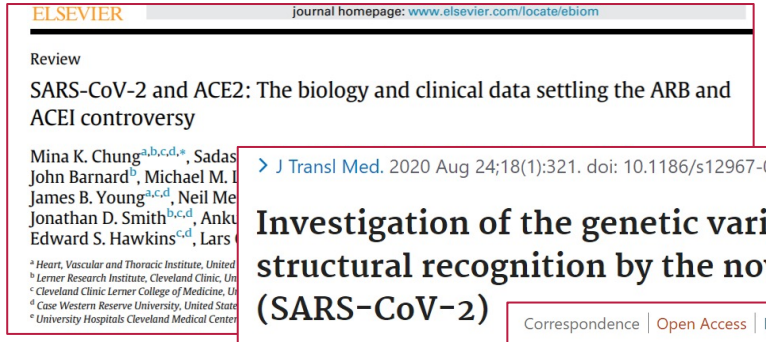
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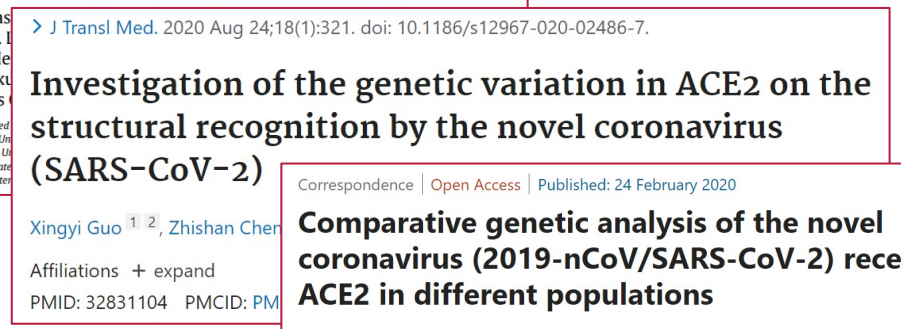
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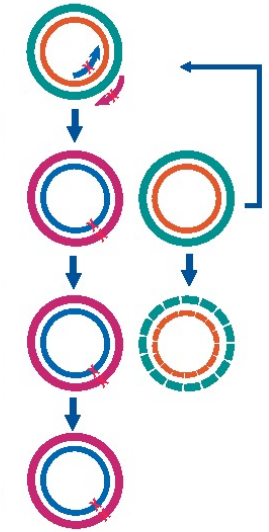
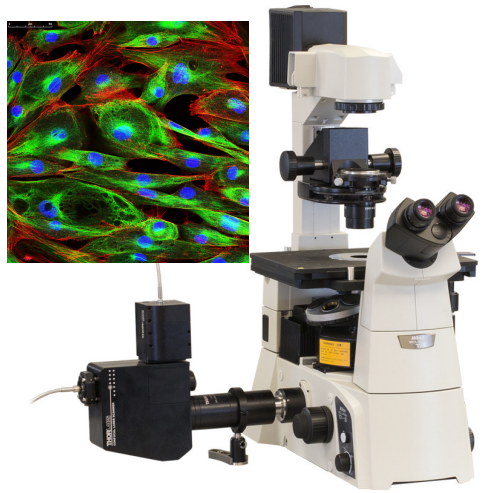
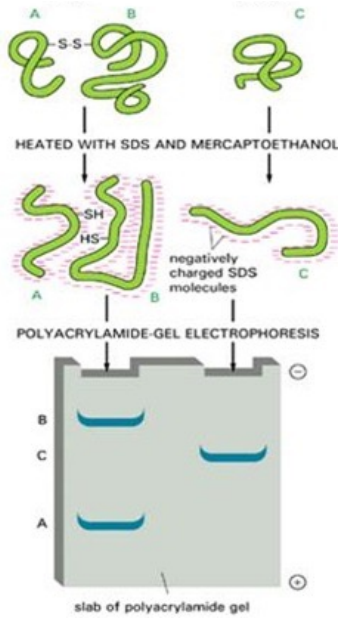


ACE2 trafficking and subcellular localization



Confirmatory tests



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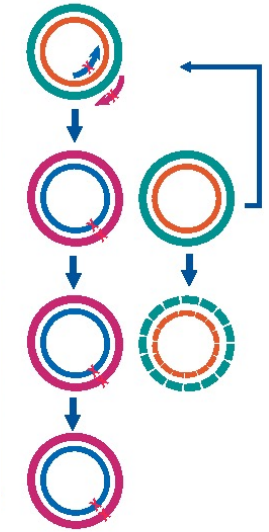

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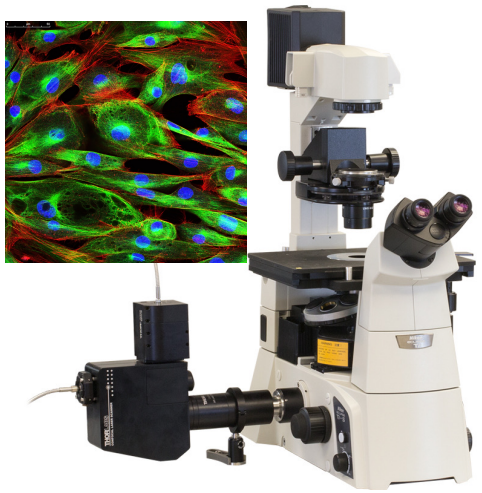

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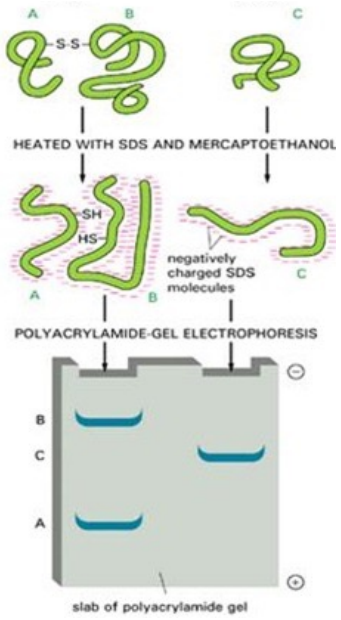
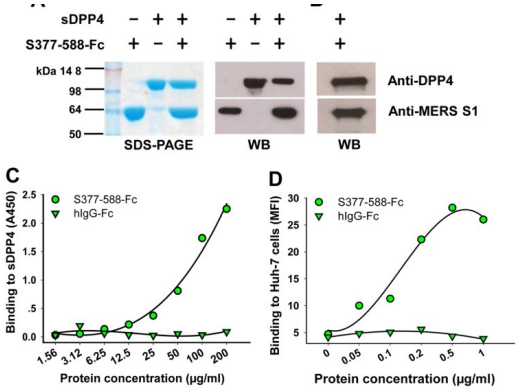
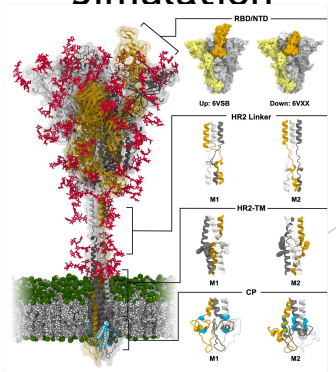


Functional analysis



Molecular Dynamics (MD) Simulation



Objective #1

- Generate the known ACE2 gene-coding missense variants reported in humans by site directed mutagenesis using expression vectors as templates.

Literature search of ACE2 variants

- At least 30 variants of the missense type among humans.

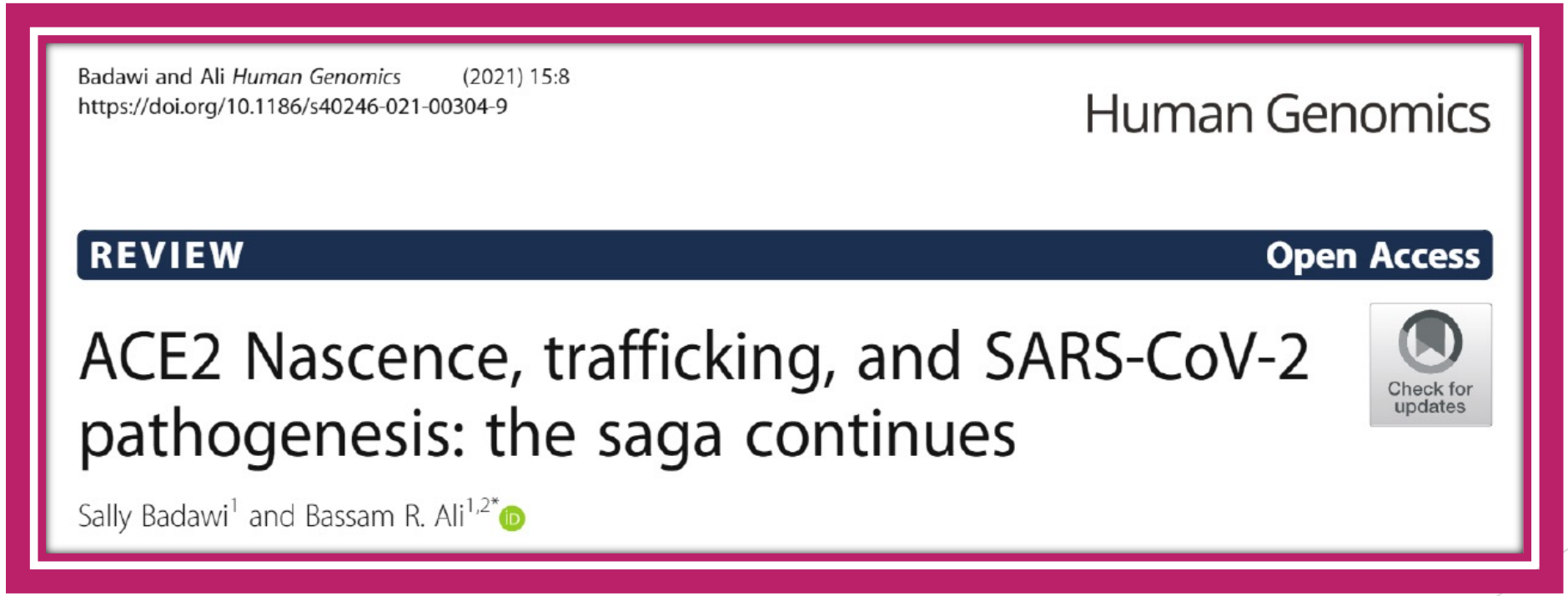
- The variants were largely extracted **from** a previously published comparative genetic analysis of the ACE2 receptor coding region in different populations (Cao et al., 2020).

- Guo and colleagues have shown that nine missense variants in ACE2 gene may cause disruption in the structure of ACE2 and alter the interaction occurring with the RBD of the S protein (Guo et al., 2020).

- **The implications of those variations on the cellular trafficking and targeting of ACE2 to the cell surface are not well established which is a major aim of this research.**

Literature search of ACE2 variants

A Review article by our lab published in 2021 ↓



Badawi and Ali *Human Genomics* (2021) 15:8
https://doi.org/10.1186/s40246-021-00304-9

Human Genomics

REVIEW Open Access

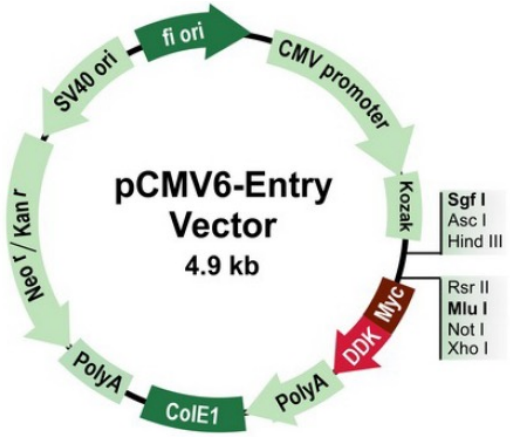
ACE2 Nascence, trafficking, and SARS-CoV-2 pathogenesis: the saga continues

Sally Badawi[1] and Bassam R. Ali[1,2*]

Check for updates

Site-directed-mutagenesis (SDM)

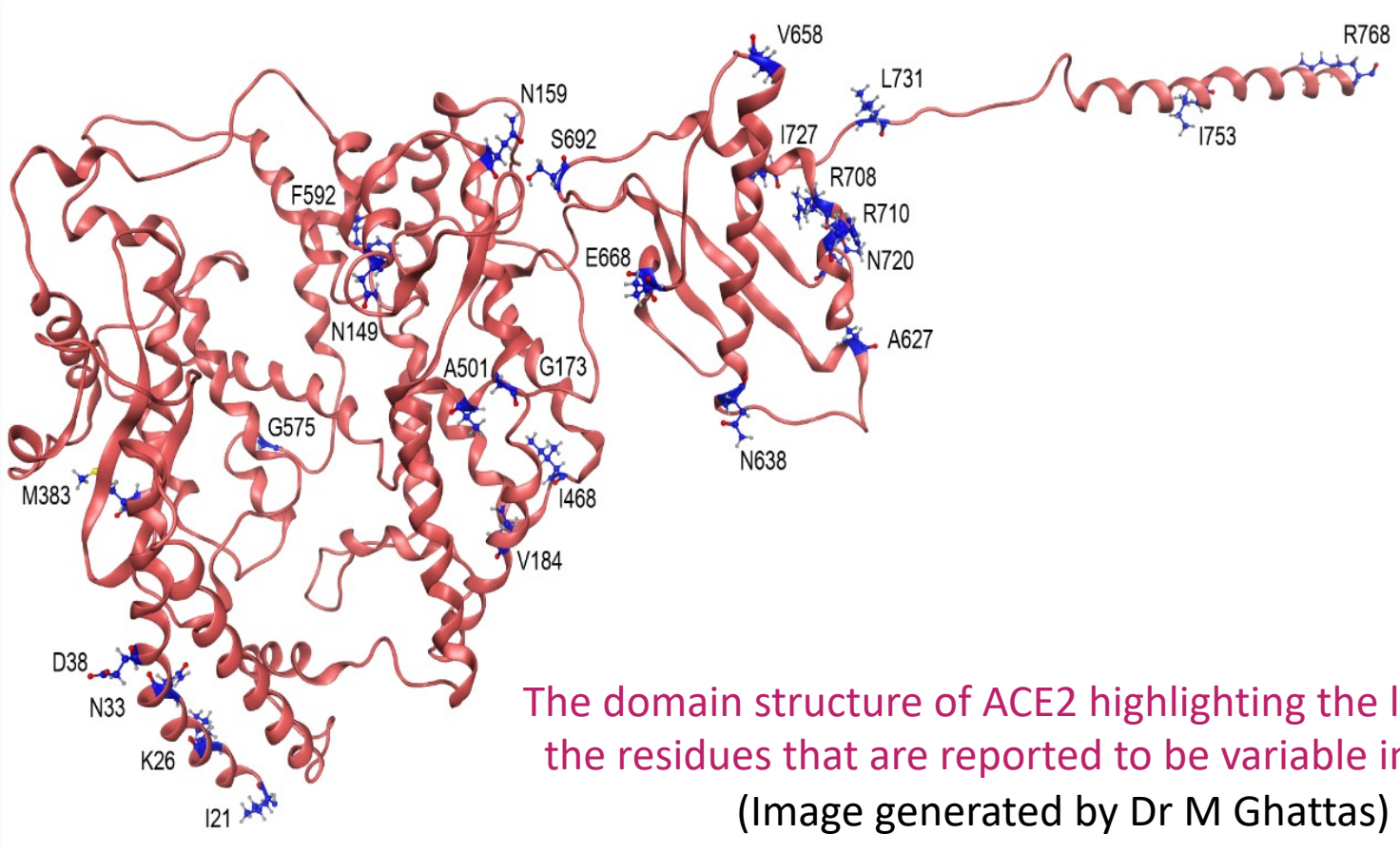
Variant (Protein)	Variant (DNA)
p.Val801Gly	c.2402T>G
p.Asp785Asn	c.2353G>A
p.Arg768Trp	c.2302C>T
p.Ile753Thr	c.2258T>C
p.Leu731Phe	c.2191C>T
p.Leu731Ile	c.2191C>A
p.Ile727Val	c.2179A>G
p.Asn720Asp	c.2158A>G
p.Arg710His	c.2129G>A
p.Arg708Trp	c.2122C>T
p.Ser692Pro	c.2074T>C
p.Glu668Lys	c.2002G>A
p.Val658Ile	c.1972G>A
p.Asn638Ser	c.1913A>G
p.Ala627Val	c.1880C>T
p.Phe592Leu	c.1774T>C
p.Gly575Val	c.1724G>T
p.Ala501Thr	c.1501G>A
p.Ile468Val	c.1402A>G
p.Met383Ile	c.1149G>A
p.Val184Ala	c.551T>C
p.Gly173Ser	c.517G>A
p.Asn159Ser	c.476A>G
p.Asn149Ser	c.446A>G
p.Asp38Glu	c.114C>G
p.Asn33Asp	c.97A>G
p.Lys26Arg	c.77A>G
p.Ile21Thr	c.62T>C
p.Ser19Pro	c.55T>C



Mammalian Expression Vector

- **C-Tag:** Myc-DDK
- **E. coli Selection:** Kanamycin
- **Cell Selection:** Neomycin

Structure of ACE2



The domain structure of ACE2 highlighting the locations of the residues that are reported to be variable in humans.

(Image generated by Dr M Ghattas)

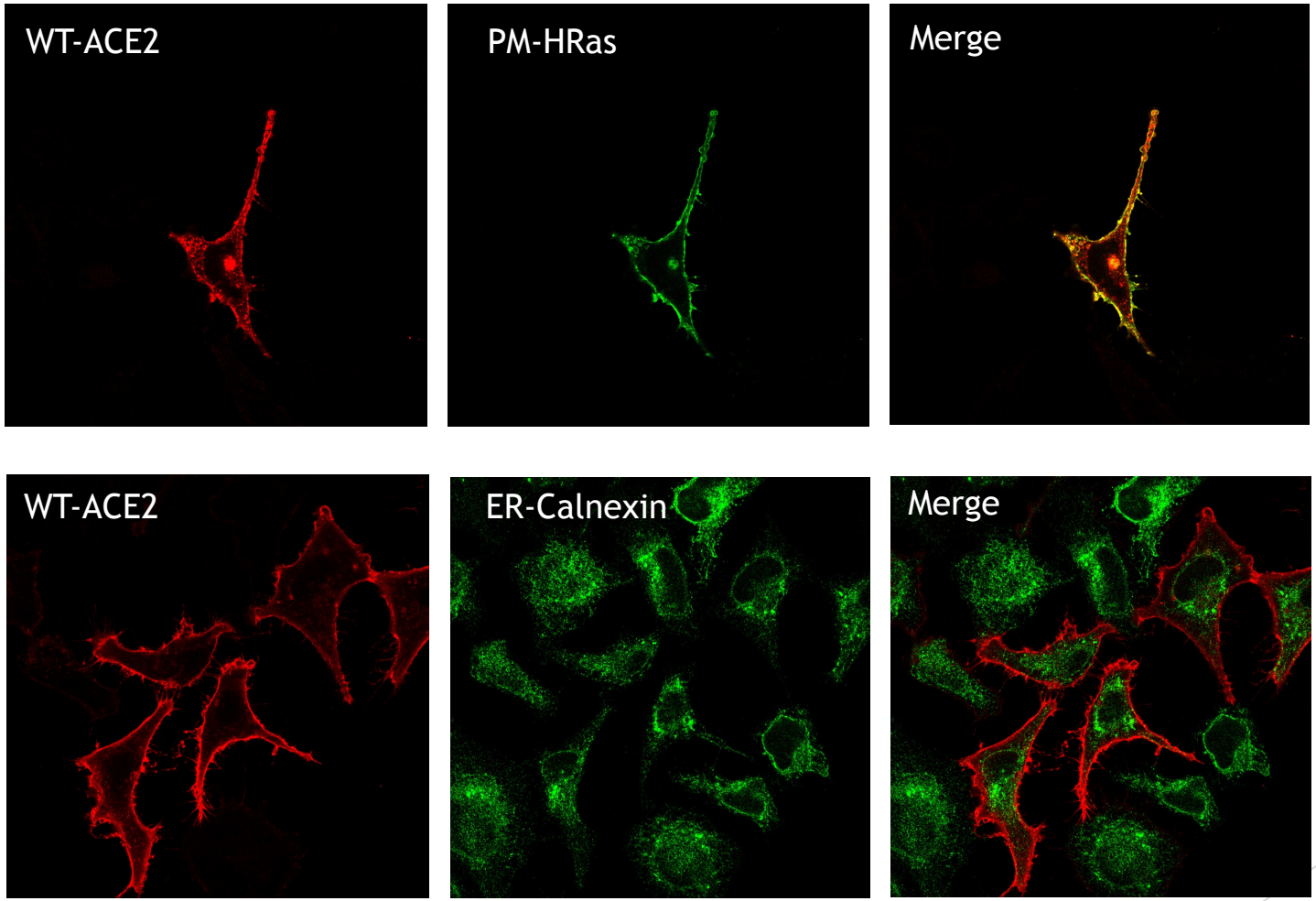
Objective #2

- Elucidate the effect of the generated variants on the receptor's expression, trafficking and subcellular localization.

Immmuno Fluoresce (IF)

Representative images

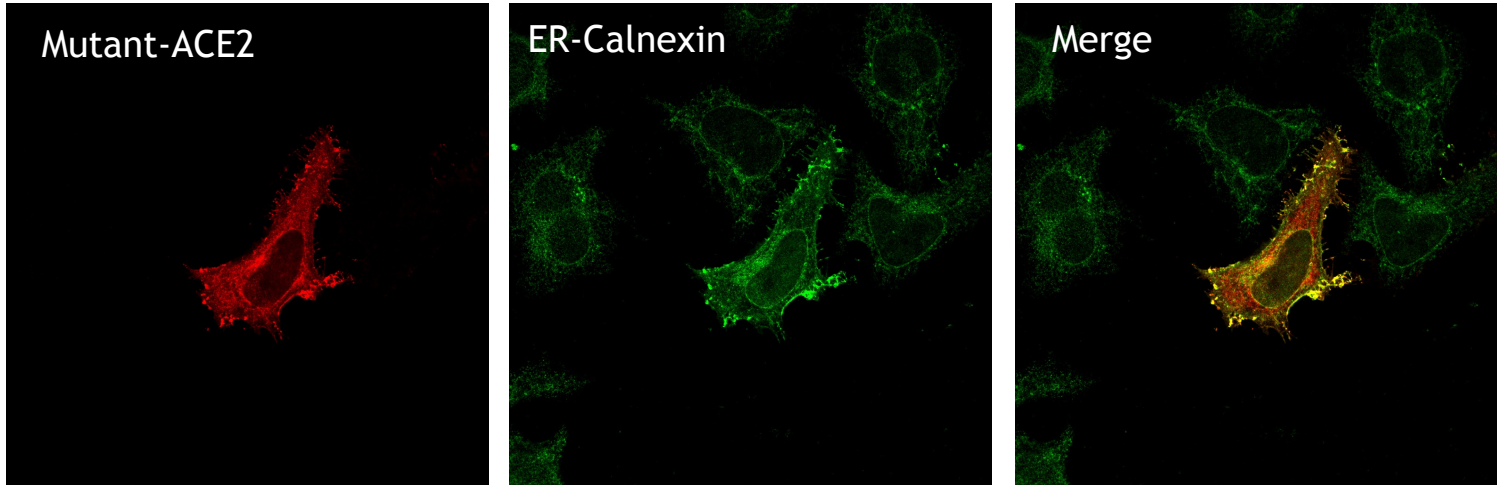
ACE2 Wilde type localization: Plasma membrane



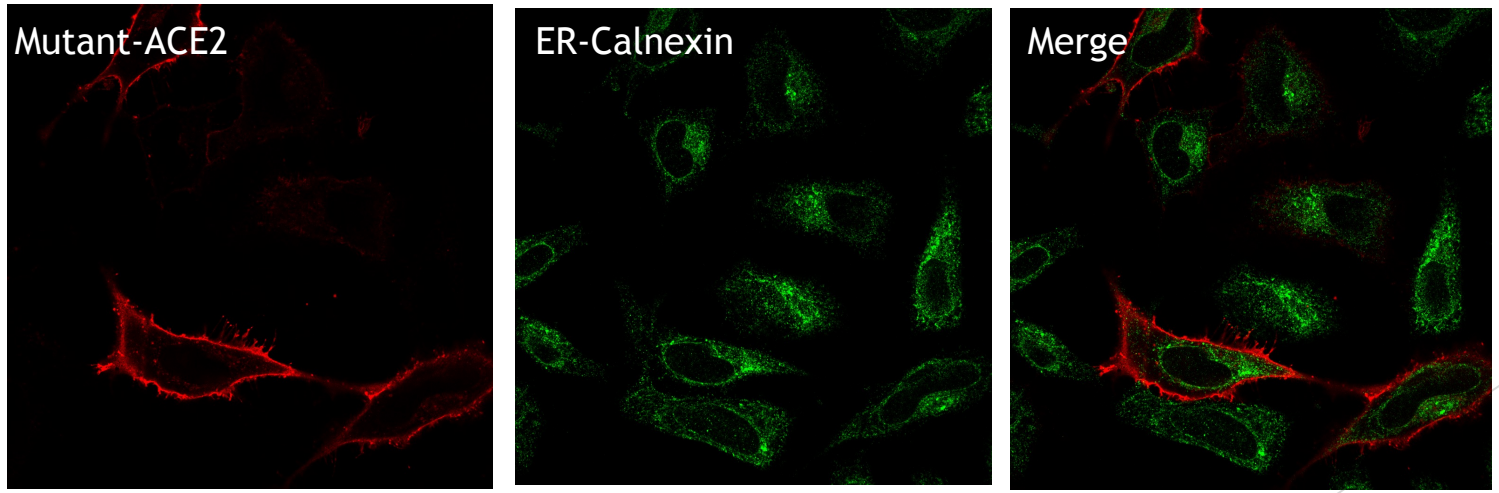
Immmuno Fluoresce (IF)

Representative images

ACE2 mutants localization: ER retained.



ACE2 mutants localization: Plasma membranal.



Plans for the near future

- Preform molecular dynamics (MD) simulation on the ACE2 receptor mutant forms that showed defective binding and retarded trafficking.

- To perform functional analysis on different mutated ACE2 receptors compared to WT-ACE2 including:
  - ACE2 activity
  - Binding capacity to the COV2-spike protein