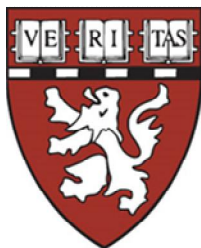


# Autism Pathways Analysis: A Functional Framework and Clues for Further Investigation

Martha Herbert PhD MD

Ya Wen PhD

July 2016



# Report on pathway network analyses in autism, based on open-access paper



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RESEARCH ARTICLE

## Pathway Network Analyses for Autism Reveal Multisystem Involvement, Major Overlaps with Other Diseases and Convergence upon MAPK and Calcium Signaling

Ya Wen , Mohamad J. Alshikho, Martha R. Herbert 

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# Challenges in ASD research

Autism spectrum disorders (ASDs) has:

- many etiologies
- heterogeneous phenotypes (and many comorbidities)
- many levels of issues in each person
  - with components of these levels heterogeneous across individuals



Many comorbidities

Heterogeneous phenotypes

Common behaviors

Many etiologies

Could there be one or few common mechanisms that could explain all of these aspects?

# Figuring out the functional network is a challenge

- Hundreds of genes have been associated with ASDs. The number keeps going up.
- These genes are involved in a lot of biological activities and multiple systems.
- It is no longer “one gene, one disease”. It is “one disease, many genes” and “one gene, multiple functions”.
- To make a biological sense of this large list of genes is a challenge.



What has been found and catalogued



The reality – but how do we see this starting from lists of genes?



# Our objective: To understand the functional network (a functional “city map”) of the ASD associated genes

Genes encode for gene products



Gene products interact with each other...



...and function in pathways



A biological pathway is a series of actions among molecules in a cell that leads to a change in a cell.

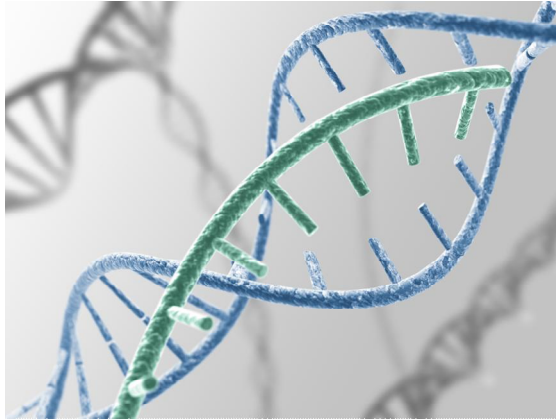
Pathway interactions assemble the network



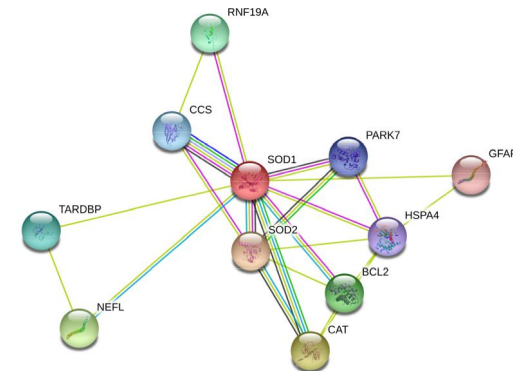
Network: a system of molecular interactions and reactions

# Our objective: To understand the functional network (a functional "city map") of the ASD associated genes

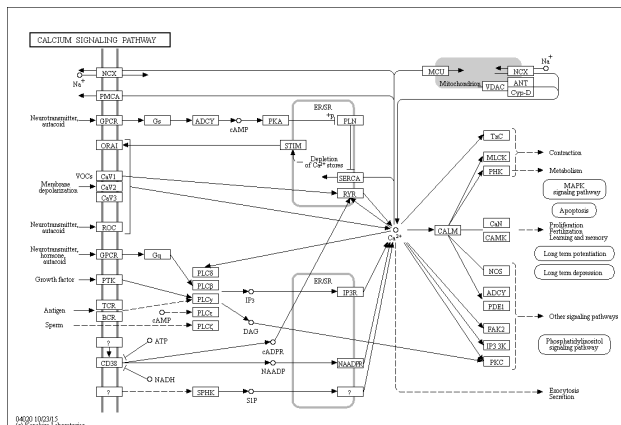
Genes encode gene products



Gene products interact with each other...

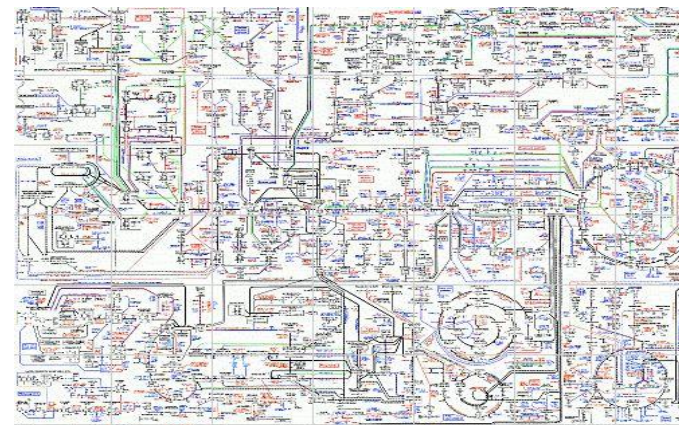


...and function in pathways



A pathway map

Pathway interactions assemble the network



A network map

# Previous studies that aim to discern potential important pathways to ASDs

- By developing protein interaction networks (Sakai Y et al., 2011, Cristino AS et al., 2014)
- By developing gene coexpression networks (Parikshak NN et al., 2013)
- By identifying specific affected pathways from ASD genetic databases (Skafidas E et al., 2014).

However, there are very few studies that have systematically investigated pathways or pathway networks that may link to ASDs, and to our knowledge there are no prior studies looking at pathway-pathway interactions at the functional level in ASDs.

In order to identify possible important pathways for ASDs, we used SFARI genes and KEGG Pathways →



# ASD gene database we used: SFARI Gene-Human Module

SFARI GENE Home  
A Modular Database for Autism Research

ABOUT NEWS TIMELINE HELP

HUMAN GENE CNV ANIMAL MODEL PIN GENE SCORING

Latest News → [Study to encourage participation of the autism research community, May 15, 2012](#)

EXPLORE HUMAN GENES

CLICK ON ANY CHROMOSOME NUMBER TO GET A LIST OF CANDIDATE GENES ON THAT CHROMOSOME.

Human Gene Module  
An integrated catalogue of human genetic studies related to autism

HUMAN HOME ABOUT CURATION EVIDENCE STATISTICS

Studies of Autism Spectrum Disorders (ASD) have uncovered many potential candidate genes. The Human Gene module of SFARI Gene serves as a comprehensive, up-to-date reference for all known human genes associated with autism spectrum disorders (ASD). The content of the Human Gene module originates entirely from manual curation of published scientific literature by our expert researchers. [Read More!](#)

EXPLORE HUMAN GENES

Welcome

The Human Gene Module of SFARI Gene is a publicly available, manually curated, web-based, searchable database of human genes with links to ASD.

Latest Statistics (Updated March, 2016)

|  |
|--|
| # of Genes: 806                              |
| # of Curated References: 1339                |
| # of Additional Non-curated References: 1289 |

CNV ANIMAL MODEL PIN GENE SCORING

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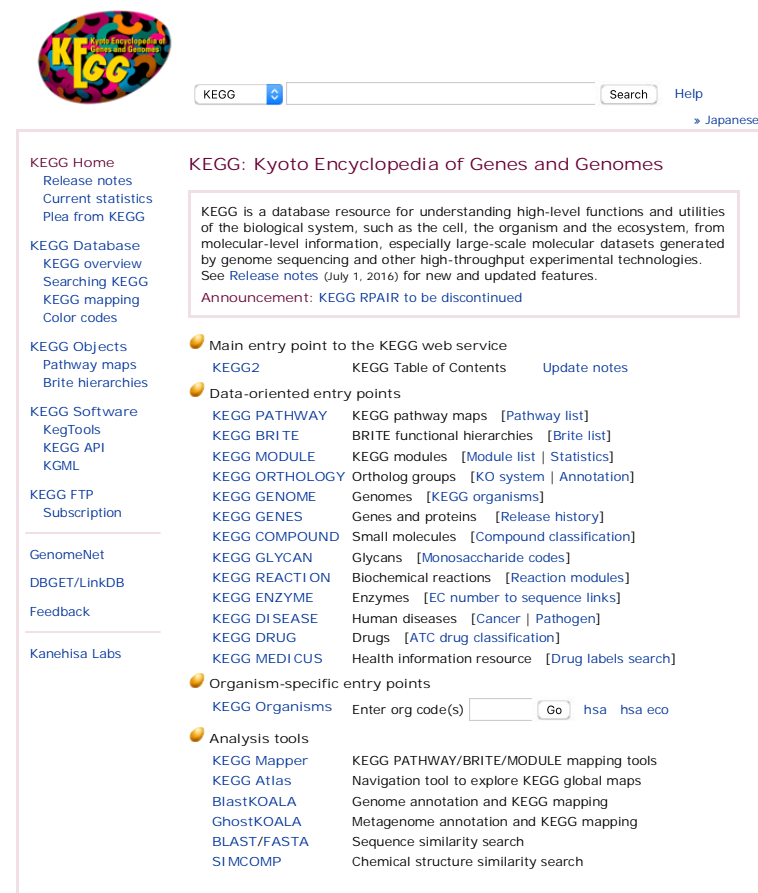


# Introducing the KEGG pathway database

KEGG: Kyoto Encyclopedia of Genes and Genomes, is a popular pathway search database (with open access) highly used by biologists.

KEGG PATHWAY is a collection of manually drawn pathway maps representing our knowledge on the molecular interaction and reaction networks for:

1. Metabolism
2. Genetic Information Processing
3. Environmental Information Processing
4. Cellular Processes
5. Organismal Systems
6. Human Diseases



The screenshot shows the KEGG website homepage. At the top left is the KEGG logo, a colorful globe with the letters 'KEGG' in the center. To the right of the logo is a search bar with the text 'KEGG' and a search button. Further right are links for 'Help' and '> Japanese'. Below the search bar is a navigation menu with categories: 'KEGG Home' (Release notes, Current statistics, Plea from KEGG), 'KEGG Database' (KEGG overview, Searching KEGG, KEGG mapping, Color codes), 'KEGG Objects' (Pathway maps, Brite hierarchies), 'KEGG Software' (KegTools, KEGG API, KGML), 'KEGG FTP' (Subscription), 'GenomeNet', 'DBGET/LinkDB', 'Feedback', and 'Kanehisa Labs'. The main content area is titled 'KEGG: Kyoto Encyclopedia of Genes and Genomes' and contains a description: 'KEGG is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies. See Release notes (July 1, 2016) for new and updated features. Announcement: KEGG RPAIR to be discontinued'. Below this is a list of entry points: 'Main entry point to the KEGG web service' (KEGG2, KEGG Table of Contents, Update notes), 'Data-oriented entry points' (KEGG PATHWAY, KEGG BRITE, KEGG MODULE, KEGG ORTHOLOGY, KEGG GENOME, KEGG GENES, KEGG COMPOUND, KEGG GLYCAN, KEGG REACTION, KEGG ENZYME, KEGG DISEASE, KEGG DRUG, KEGG MEDICUS), 'Organism-specific entry points' (KEGG Organisms), and 'Analysis tools' (KEGG Mapper, KEGG Atlas, BlastKOALA, GhostKOALA, BLAST/FASTA, SIMCOMP).

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Website: <http://www.genome.jp/kegg/>

## Methods: combine information from several existing and well established databases: SFARI and KEGG Pathways

ASD genes recorded by SFARI (Simons Foundation Autism Research Initiative) in SFARI Gene database.



Investigate how they function in groups using enrichment analysis with KEGG pathways. This evaluates the overlap of SFARI genes with KEGG pathways, and finds the statistically significant / over-represented pathways (meaning pathways that are relatively more important to ASDs)



Identify pathway-pathway interactions in reference to KEGG Pathway maps



Generate a network using pathway-pathway interactions information



Systems network analysis to gain insights into the underlying molecular bases of ASDs

# Enriched pathways and their grouping

Enrichment analysis using SFARI genes (human module) VS KEGG Pathway:

- Final yield is a list of 40 pathways
- Most significant pathway: calcium signaling pathway

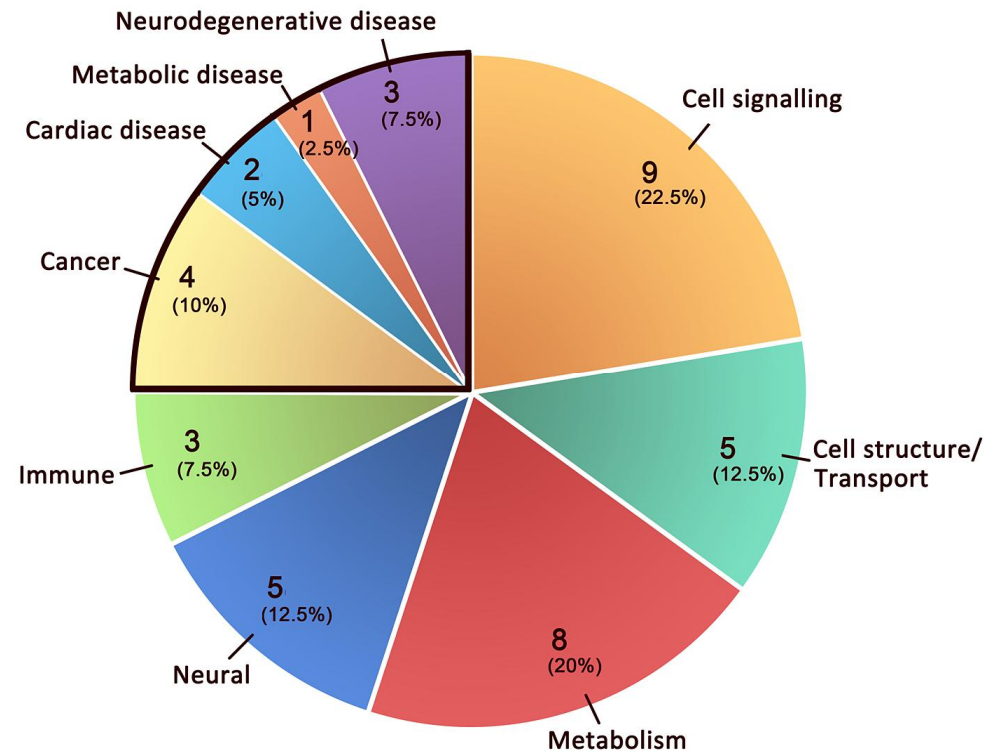
Pathway grouping:

Disease pathways (inside the black outline, 25%)

Functional pathways (75%)

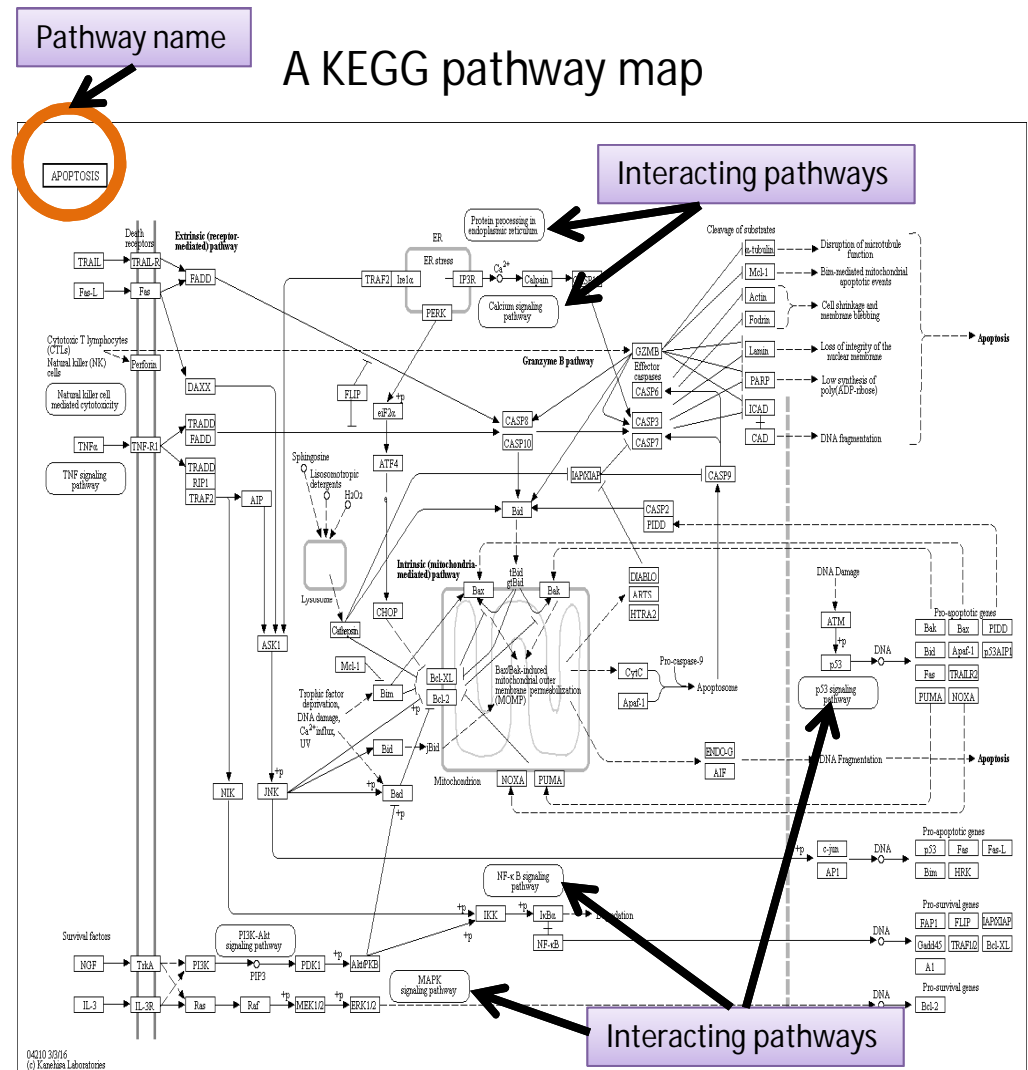
Yield includes diverse categories

Neural does not predominate



# Identify pathway-pathway interactions using KEGG pathway maps

- Pathway-pathway interactions are represented in KEGG in each pathway map by displaying the name of an interacting pathway in that map.
- For example, if one pathway appears at the certain location in the map of another pathway, this suggests that these two pathways were interacting in relation to the activities or functions represented in that part of the map, or that one was involved in a certain reaction or process of another.
- Bear in mind that pathways can be at a variety of levels, not just genetic





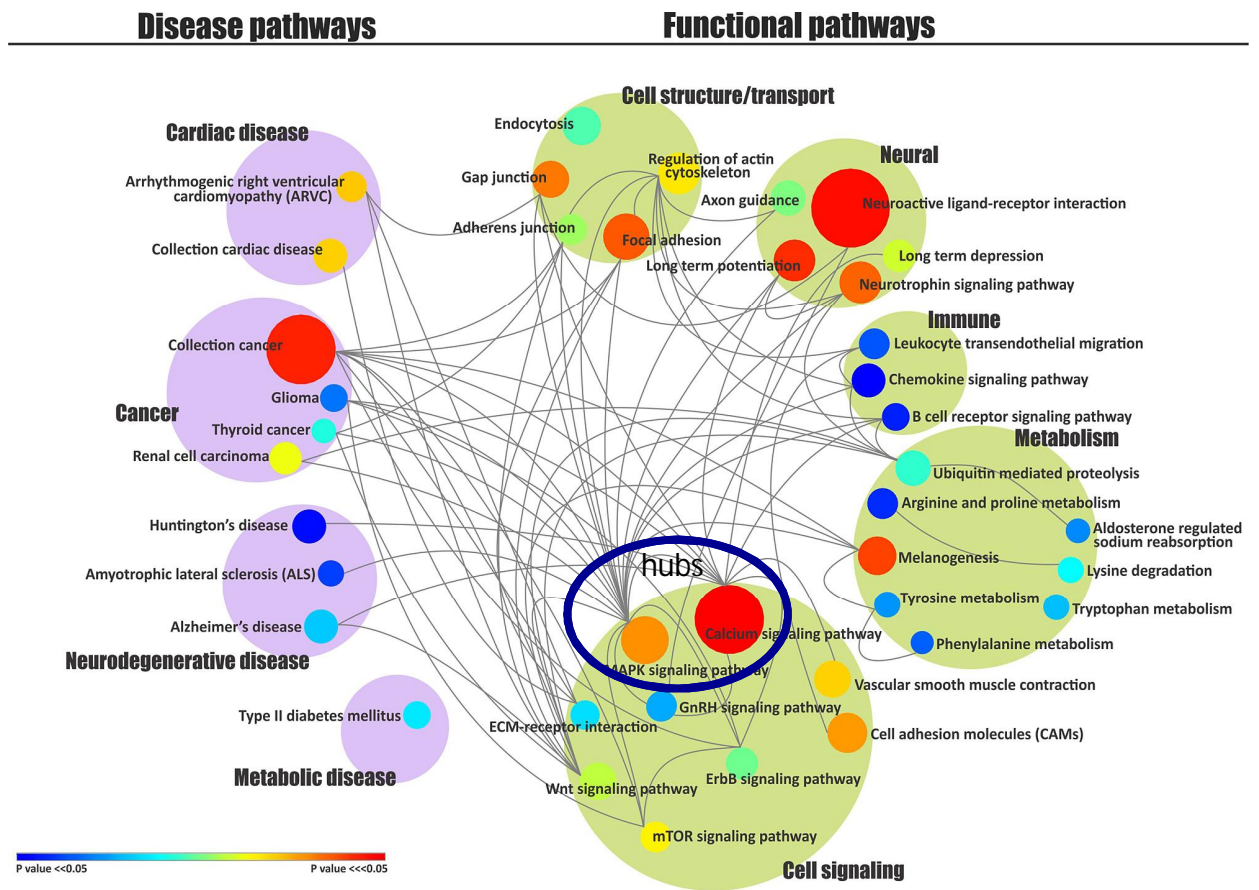
# Generate the pathway network and find the hubs

Identify hub pathways – pathways that interact with the most number of other pathways in the network: MAPK signaling pathway and calcium signaling pathway (interacts with 20/40, and 12/40 pathways respectively, in the network).

Imaging hub pathways are main streets in cities.

This figure also shows the interactions between functional and disease pathways.

## ASD pathway network



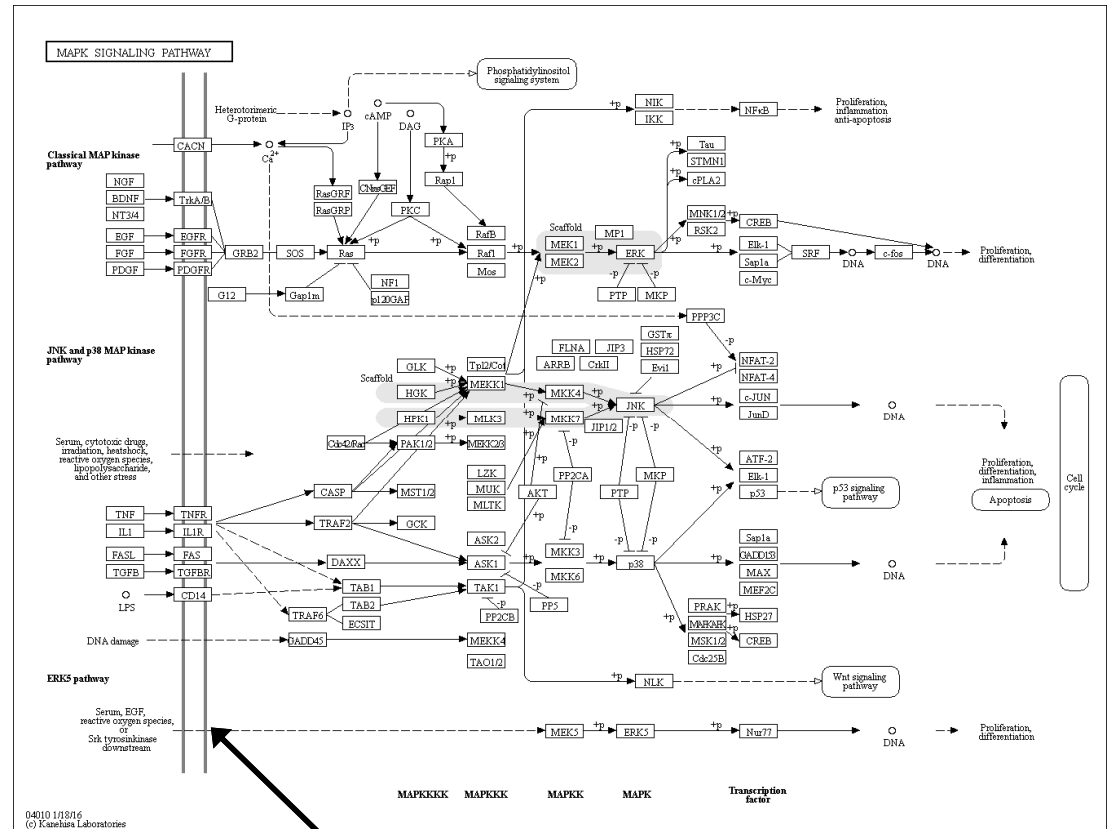
# MAPK signaling pathway

The most interactive pathway in the network:

MAPK signaling pathway is a chain of proteins in the cell that communicates a signal from a receptor on the surface of the cell to the DNA in the nucleus of the cell.

It is involved in a variety of fundamental cellular processes and has a diverse range of relationships to functions and diseases:

- Defects in this pathway may lead to cancer.
- It plays roles in neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS).



cell membrane

# MAPK signaling, cancer and ASDs

Relationships:

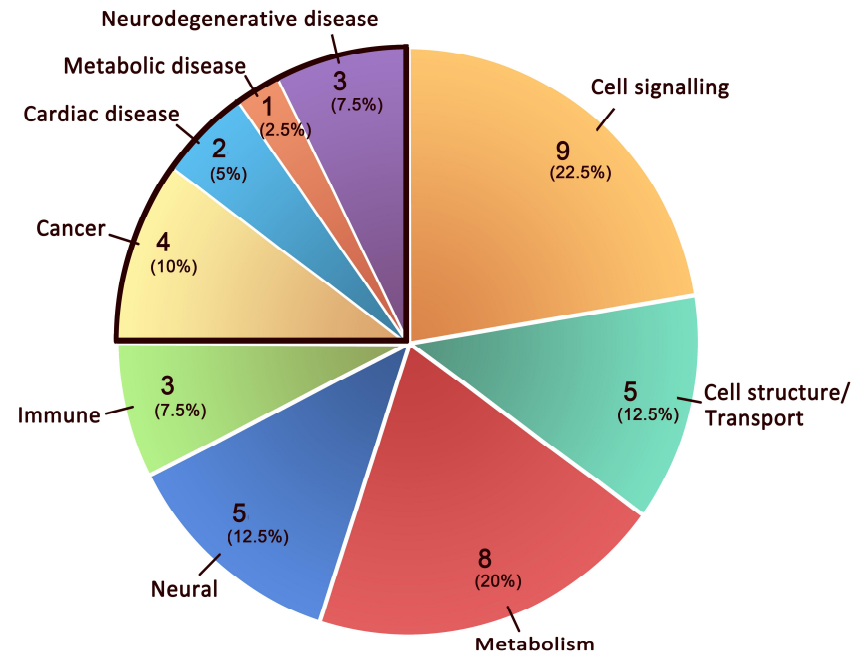
MAPK signaling & cancer

MAPK signaling & ASDs

MAPK signaling & metabolism

Cancer & metabolism

ASDs & metabolism



Hypothesis:

ASDs and Cancer overlap at MAPK signaling mediated Metabolic processes

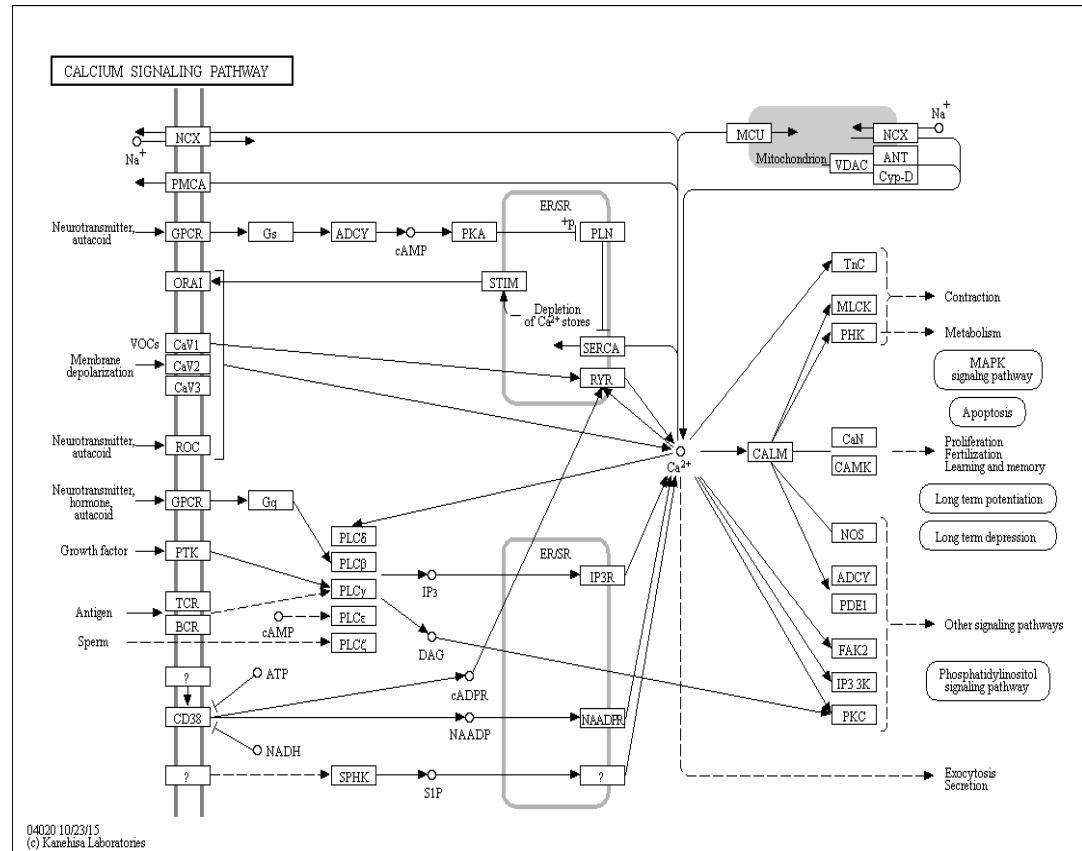
# Calcium signaling pathway

Calcium signaling pathway is:

- 1) the most statistically significant pathway,
- 2) the second most interactive pathway in the network.

Calcium ions ( $\text{Ca}^{2+}$ ) impact nearly every aspect of cellular life. Calcium signaling involves in a variety of intracellular activities. For example:

- calcium signaling contributes to dysregulation of excitation/inhibition balance, and can contribute to problems ranging from irritability to seizures
- It is vital for heart function





# Calcium signaling, cardiac diseases and ASDs

Relationships:

Calcium signaling & heart function

Calcium signaling & neural system function

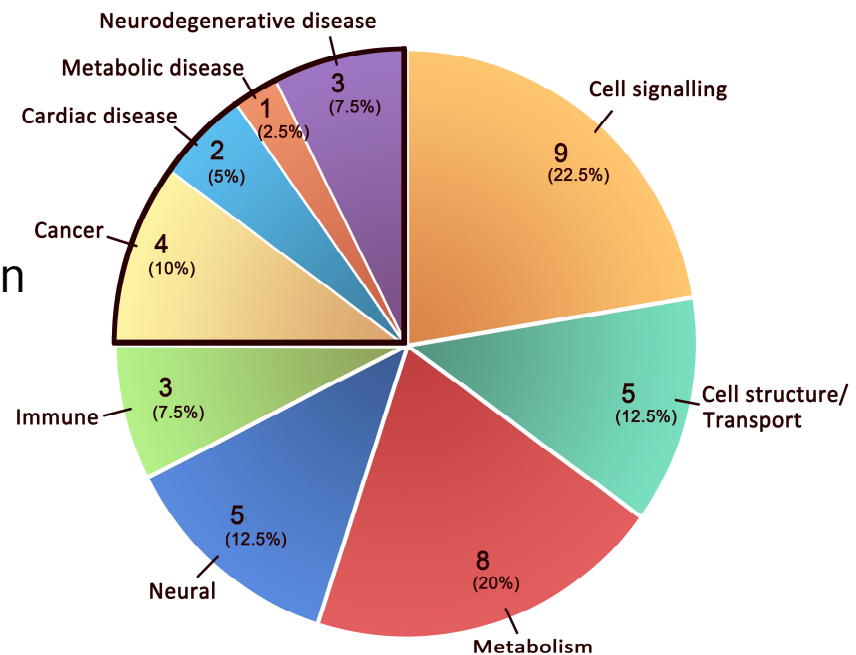
Calcium signaling & ASDs

Calcium signaling & cardiac disease

ASDs & increased risk of cardiac disease

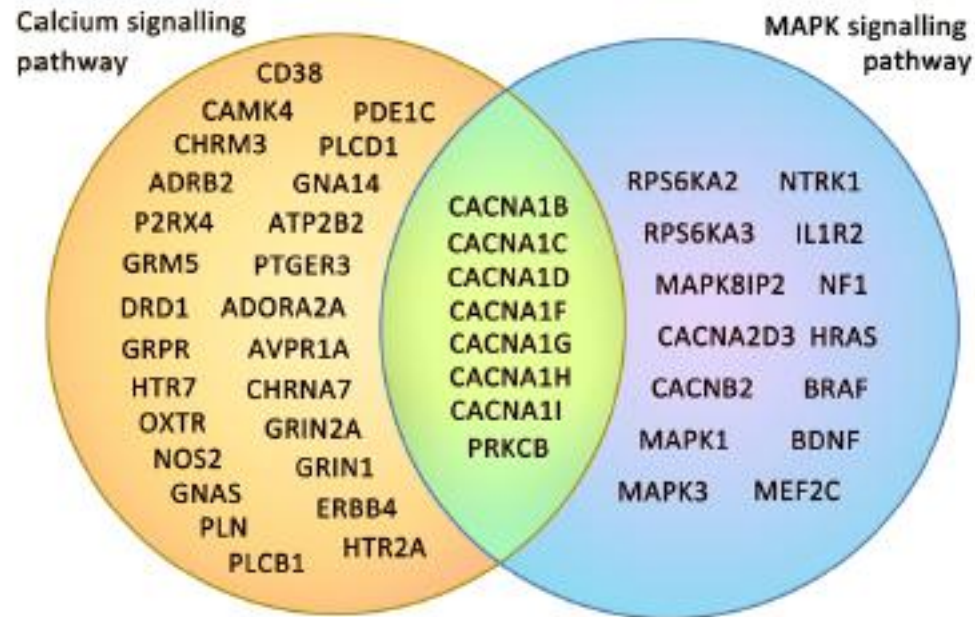
Hypothesis:

Common vulnerability to calcium signaling abnormalities are a potential link between ASDs and cardiac diseases



# Integrated MAPK and calcium signaling

Overlapping genes: MAPK and calcium signaling pathways overlapped via 8 ASD genes: CACNA1H, CACNA1G, CACNA1I, CACNA1D, CACNA1B, CACNA1C, CACNA1F, and PRKCB



## Looking at the ASD genes in the network

Most involved genes: MAPK1, MAPK3, HRAS, PRKCB and BRAF are the most involved genes, in that they participate in 14–23 out of 40 enriched pathways/collections.

| Gene Symbol | Gene Description                                   | # Pathways |
|-------------|--|------------|
| MAPK1       | mitogen-activated protein kinase 1                 | 23         |
| MAPK3       | mitogen-activated protein kinase 3                 | 23         |
| HRAS        | v-Ha-ras Harvey rat sarcoma viral oncogene homolog | 18         |
| PRKCB       | protein kinase C, beta                             | 17         |
| BRAF        | v-raf murine sarcoma viral oncogene homolog B1     | 14         |

\*These top involved genes all participate in MAPK signaling pathway\*

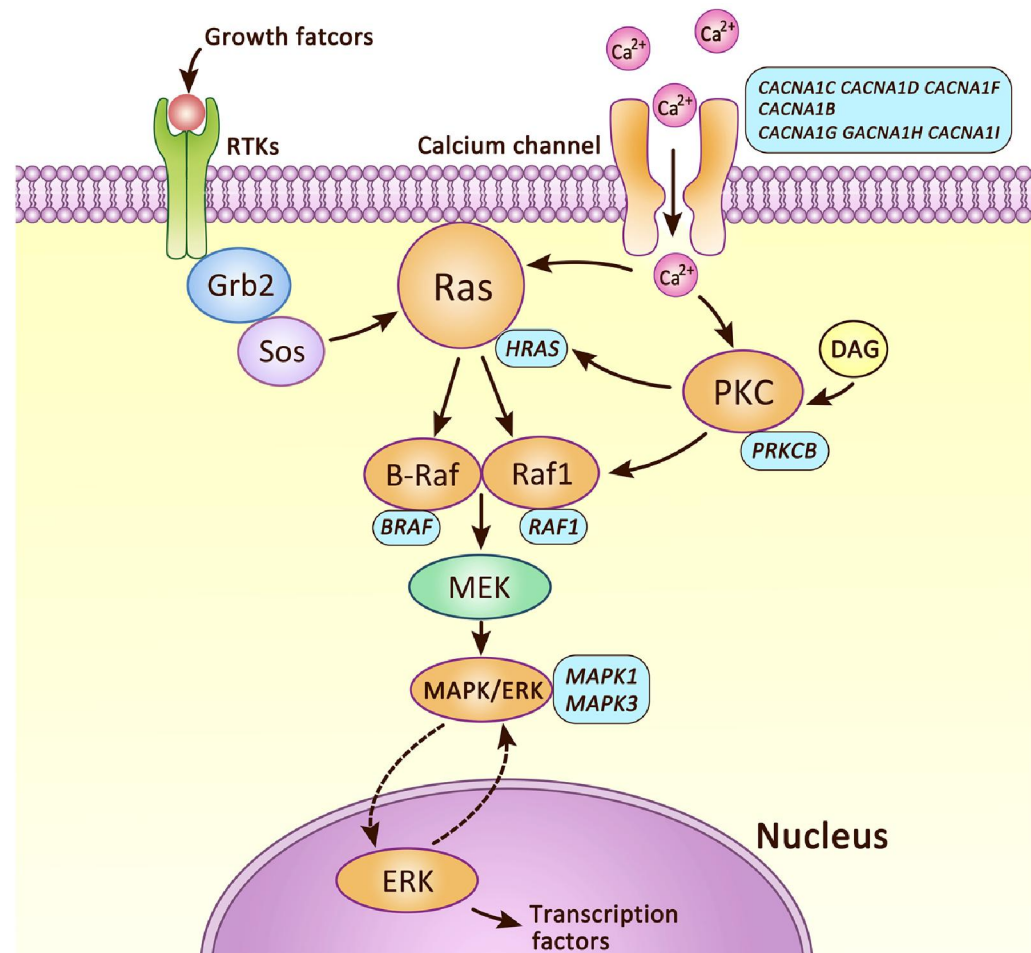
# Identify a possible key process in the network: calcium-PKC-Ras-Raf-MAPK

Most involved genes and MAPK calcium signaling overlapping genes together function in the process:

calcium-PKC-Ras-Raf-MAPK

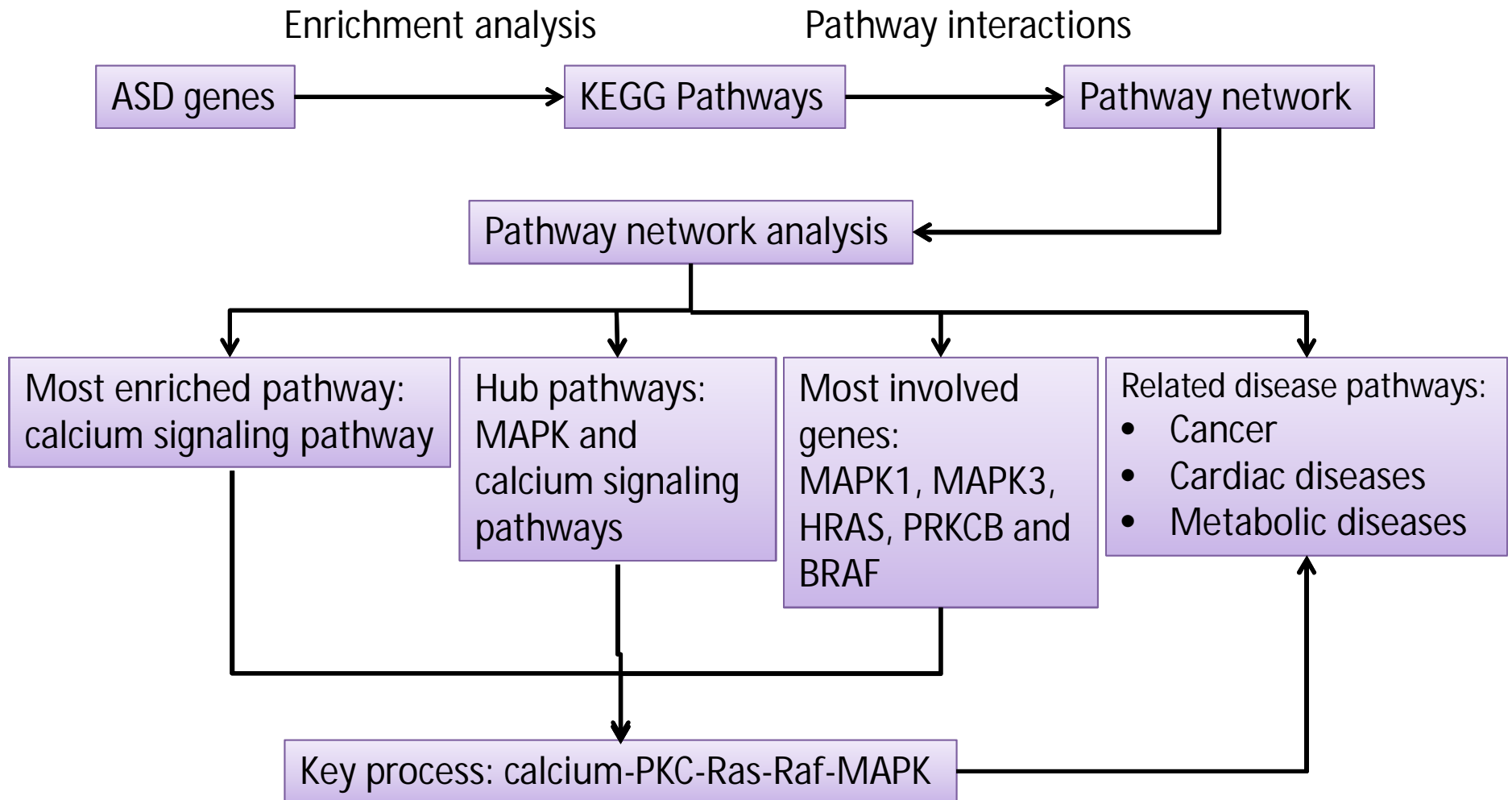
This might be a key process that contribute to ASD pathophysiology.

This potential key process plays a central role in a large range of biological processes, whose activities, when abnormal, may compromise biological output and contribute not only to features of ASD themselves but also to a range of other vulnerabilities





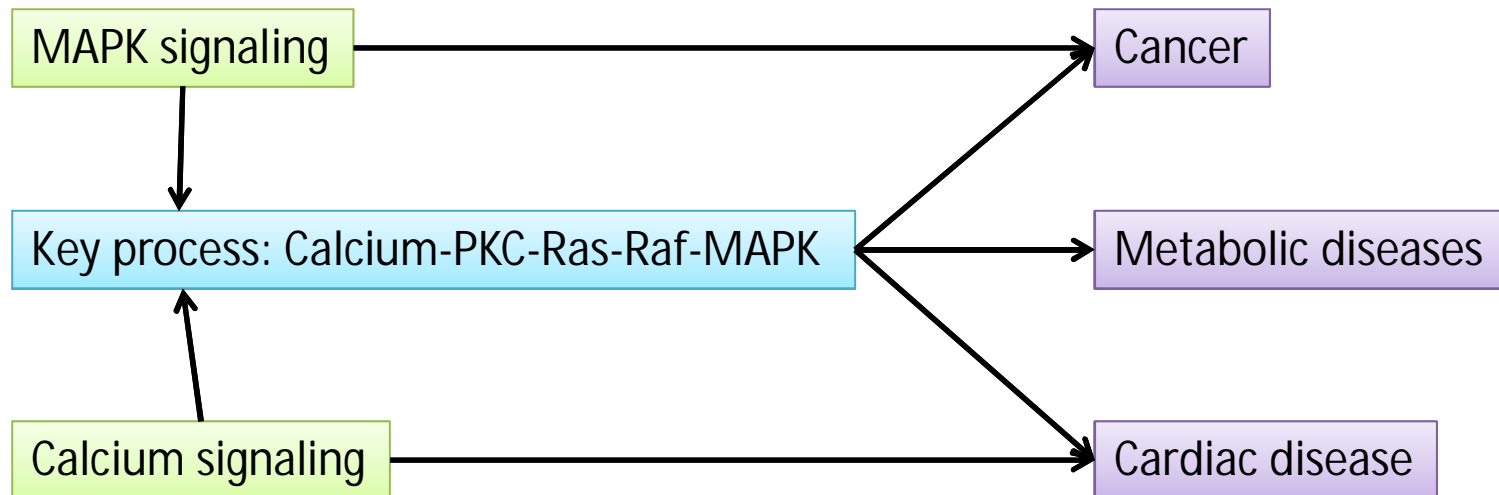
# Network analysis summary: What we did and what we found



# Functional and disease pathways connections in the ASD pathway network

Functional pathways

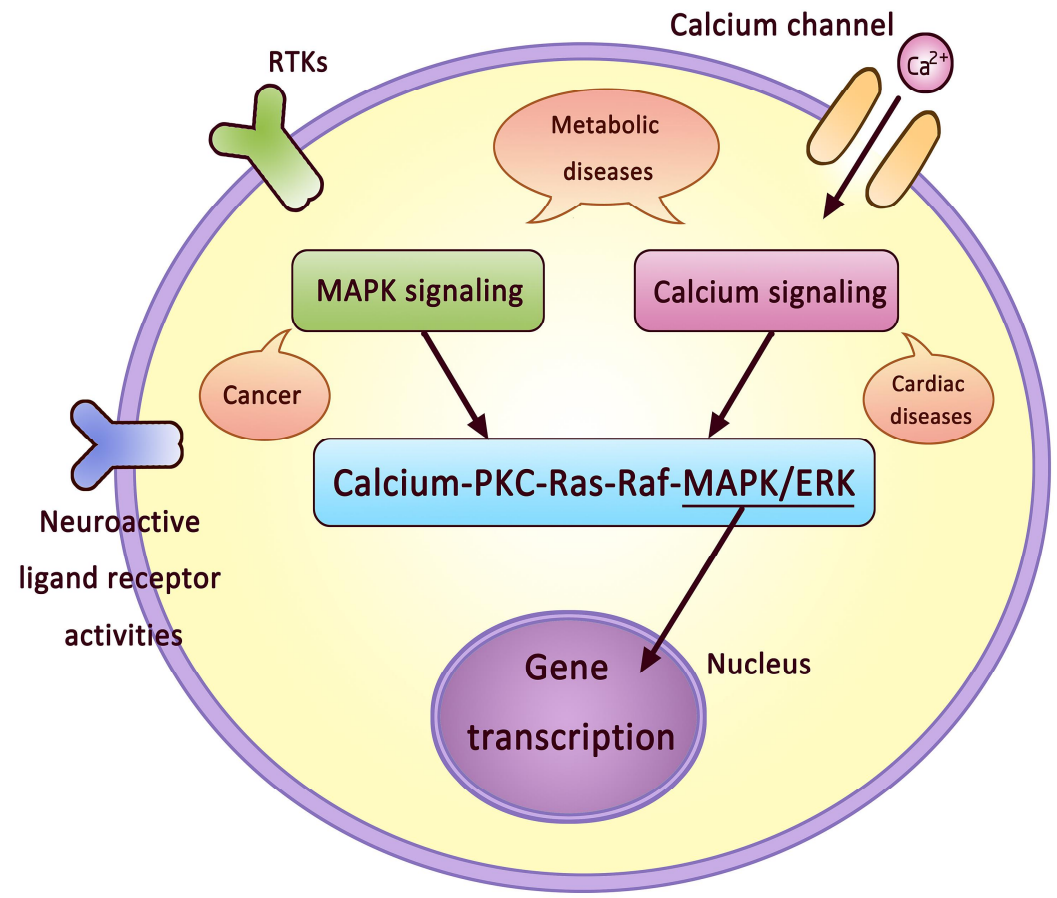
Disease pathways



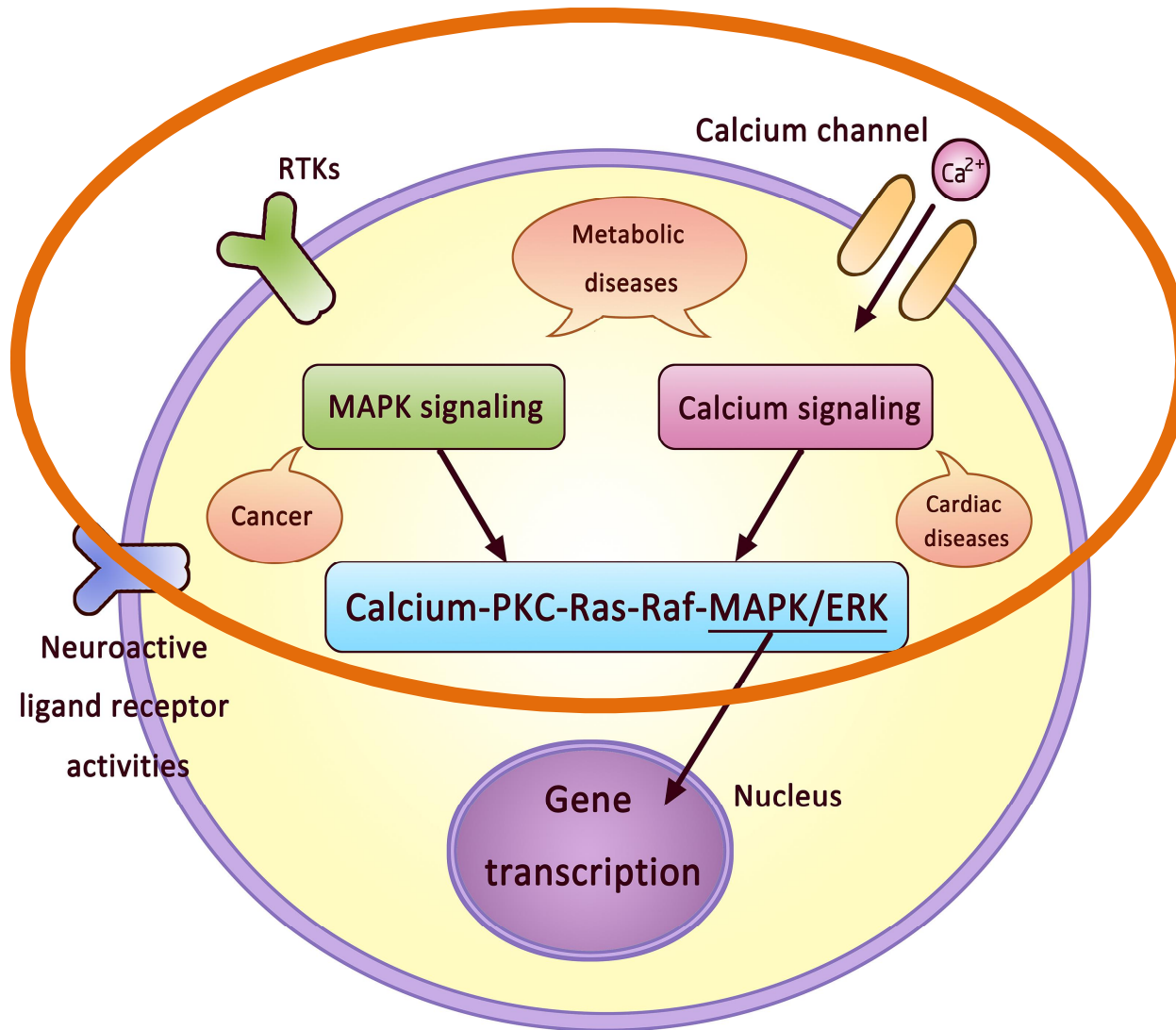
# We integrated our top findings into a cellular model

## A cellular model:

MAPK and calcium signaling pathways respond to signals from outside the cell and converge on the process calcium-PKC-Ras-Raf-MAPK, which lead to a series of reactions including gene transcription modification.



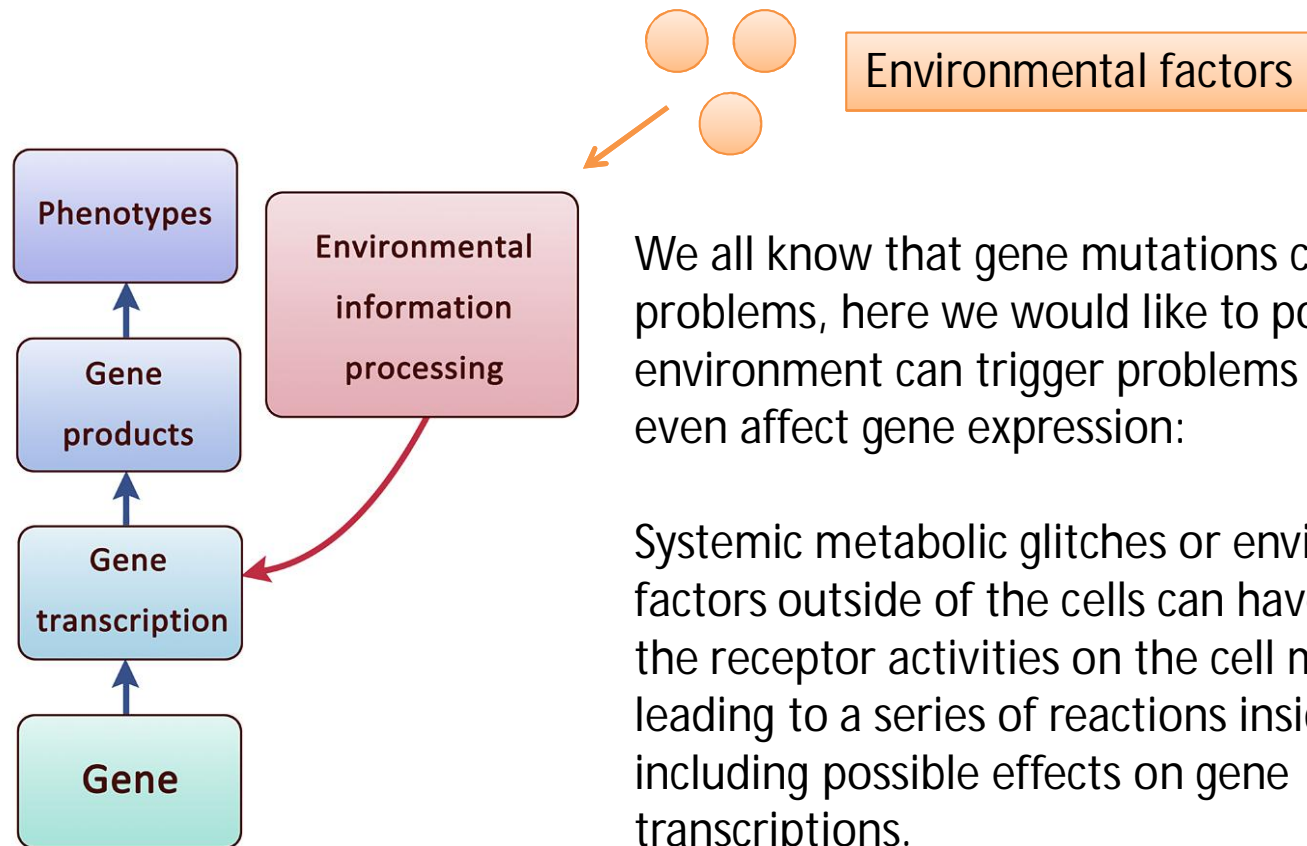
# Environmental information processing (EIP) might be important in ASDs



EIP: the procedures that cells utilize to react to environmental information and conduct consequent signal transductions.

For example, the calcium signaling, MAPK signaling and receptor activities we have been discussing constantly react to signal molecules and changes in the environment outside of cells, with processing of that information leading to a series of reactions.

# Environmental factors may trigger intracellular changes and eventually affect gene expression



We all know that gene mutations can cause problems, here we would like to point out that environment can trigger problems too and even affect gene expression:

Systemic metabolic glitches or environmental factors outside of the cells can have impacts on the receptor activities on the cell membrane, leading to a series of reactions inside the cells, including possible effects on gene transcriptions.

## Summary: our key findings

- We first found the relatively most important pathways, and then we generated a pathway network by mapping the pathway-pathway interactions into an ASD Pathway Network.
- Our systems analyses of this network converged upon an important role in autism pathophysiology for two pathways: MAPK signaling and calcium signaling, and specifically the process where they overlap, “calcium-protein kinase C-Ras-Raf-MAPK”.
- This study also illuminated genetic relationships between autism and several other kinds of illness, including cancer, metabolic and heart diseases.
- Many of the significant genes and pathways were associated with vulnerability in the processing of challenging environmental influences.



# The big picture

Our findings support the idea that autism may emerge from underlying vulnerabilities related to pleiotropic (multipurpose) genes associated with pervasively important molecular mechanisms and multiple systemic comorbidities.

We think that the behaviors we label “autism” are not directly determined by genes, but instead are “emergent properties” that are produced by a brain whose functioning is altered by these underlying mechanism.

This may help us understand how autism can have so much heterogeneity and yet still have common features.

# Summary: what makes our study different

We did not introduce any biases from favoring certain types of biological processes or domains over others. We simply let the data speak for itself.

To our knowledge this is the first study that look at pathway-pathway interactions at the functional level in ASDs.

With the functional pathway network we were able to expand our understanding to include not only genes but also the function of gene products, as well as the networking of reactions and interactions. We provide a framework and clues for further investigations.



We are here



## Discussion: key points

Genes do not function alone, but in groups (pathways). Also, we need to remember that it is not just individual genes on their own but also the function or teamwork of the gene pathways that shapes our vulnerabilities and strengths. So we need to be mindful of the whole picture, the whole map.

Pathways are like streets in a city (with each cell being like a little city in itself). Molecules and other gene products move around the "city" in the "streets". A traffic jam in key processes can easily extend to many places - not just to one or two genes or "intersections", but to many activities (or locations) quite a distance away.

On the other hand, if we can overcome this "traffic jam", we might fix many problems all at the same time. The "hubs" or core processes we identified can be thought of as major intersections or traffic circles that connect many different processes—and these other processes can get jammed up when the hubs are not working right. It can also work the other way around: traffic jams near the hubs may also slow down the hubs, with big effects. This is why we say that when you address a core process that generates a spectrum of symptoms you have a shot at affecting all of these symptoms at once. For patients this means making as many healthy choices as you can, because so many of your core processes are interconnected.

## Further potential

This method of investigating pathway-pathway interactions, overlaps between different diseases and shared functional mechanisms has potential to illuminate much more than autism.

It may help us find or understand potent ways to help people with many complicated chronic illnesses with multiple triggers.

Further studies that investigate environmental information processing would help understand more about environmentally modulated chronic illnesses and their relationships with each other, and some common leverage points.