# Literature Review on nuclear factors NRf2/NFE2L2, Erf/ETS2 repressor factor, and ELK1

Krishyra Mitchell | Neurobiology | McNair Scholar

Faculty Mentor: Ronald Waclaw | CCHMC Divisions of Experimental Hematology and Cancer Biology

# Abstract

Genes regulating distinct pathways are of particular interest in relation to their function in organ systems of the human body. In the last several years, an increase of data from the human genetics field has identified mutations in key genes, which may result in their gain or loss of function, resulting in various diseases, syndromes, or cancers. This review provides an overview for following 3 nuclear factors genes: NFE2L2, Erf/ETS2 repressor factor, and the ETS transcription factor ELK-1. NFE2L2 is a transcription factor that regulates the response to chemical and radiation induced oxidative stress in cells. The ERF/ETS2 repressor factor is an ETS family member gene that's been suggested to act as a negative regulator by competing with other ETS-family members for DNA binding. ELK-1 is a transcription factor and member of the ETS oncogene family of transcription factors that includes nuclear phosphoproteins. These genes are involved in many biological processes that contribute to them being prominent factors in many central nervous system diseases, disorders and human cancers. ERF and ELK1 are effectors of the MAPK pathway and NFE2L2 is a stress response transcription factor. These genes play a variety of roles in the cell, but ERK and ELK are directly associated with the MAPK pathway. The MAPK pathway is the key pathway that increased in RASopathy syndromes. These nuclear factors may be involved in response to elevated MAPK signaling through RASopathy mutations, which could affect distinct cellular phenotypes. An increased understanding of how these genes work is crucial to revealing their roles in certain diseases or cancers.

# Methodology and Individual Role

- Due to this only being a background literature review without any actual experiments being performed there wasn't a set "research question" or "hypothesis" other than aiming to acquire more recent information on these genes for future research.

- This review was performed remotely from home. Due to the ongoing Covid-19 epidemic, all none employees of Cincinnati Children's Hospital (i.e. undergraduate student trainees)and some University of Cincinnati students were not allowed in the hospital's lab as well as lab teams having to downsize the number of people in the lab at all times and rotating who would be present on each coming day. My scheduled time in the lab was Spring/Summer 2020 during the early months of the evolving epidemic. To overcome this unusual situation, this review was assigned on 3 genes that the Waclaw lab was interested in studying related to a project focused on the response to elevated MAPK signaling in the cell. I was able to do these tasks remotely with a laptop and internet connection. To collect data, I reviewed scholarly journals and articles, made note of functions, signaling pathways, mutations and their effects, etc, form outlines of this literature review on the 3 novel genes , and wrote and revised the review as new information came in.

# Erf/ETS2 repressor factor

- ERF is a gene for a ubiquitously expressed ETS family gene
  - It's been suggested to act as a negative regulator by competing with other ETS-family members for DNA binding or through unique targets.
  - Enhances erythroid differentiation and regulates cell proliferation (Bose et al., 2017).
- ERF is regulated by extracellular signal-related kinase (Erk1/2), a key protein in mediating mitogen activated protein kinases signaling downstream of RAS phosphorylation
  - Leads to nuclear uptake and modulation of multiple targets and nucleocytoplasmic shuttling (Twigg et al 2013).
- In its nuclear form, ERF blocks cell proliferation-arresting cells at the $G_0/G_1$ phase in a cell-type-specific manner
  - It suppresses ETS- and RAS-induced tumorigenicity in fibroblasts (Peraki et al., 2017).
- The ETS family, once activated, participates in cell proliferation, differentiation, migration, apoptosis, and metastasis.
  - Studies show that the ETS family could regulate angiogenesis in normal and cancer tissues and ETS2's role as an oncogene in different cancers (Liu X et al 2017).
- A study was done on the role of ETS2 as an oncogene and ETS2 in hypopharyngeal cancer pathogenesis in hypopharyngeal cancer tissues and corresponding non-neoplastic tissues
  - Expression of ETS2 was shown to be increased in cancer tissues as compared with the expression in corresponding non-neoplastic tissues(Liu X et al 2017).
  - It was concluded that ETS2 functions as an oncogene and plays a key role in the progression of hypopharyngeal cancer and inhibition of ETS2 (Liu X et al 2017).

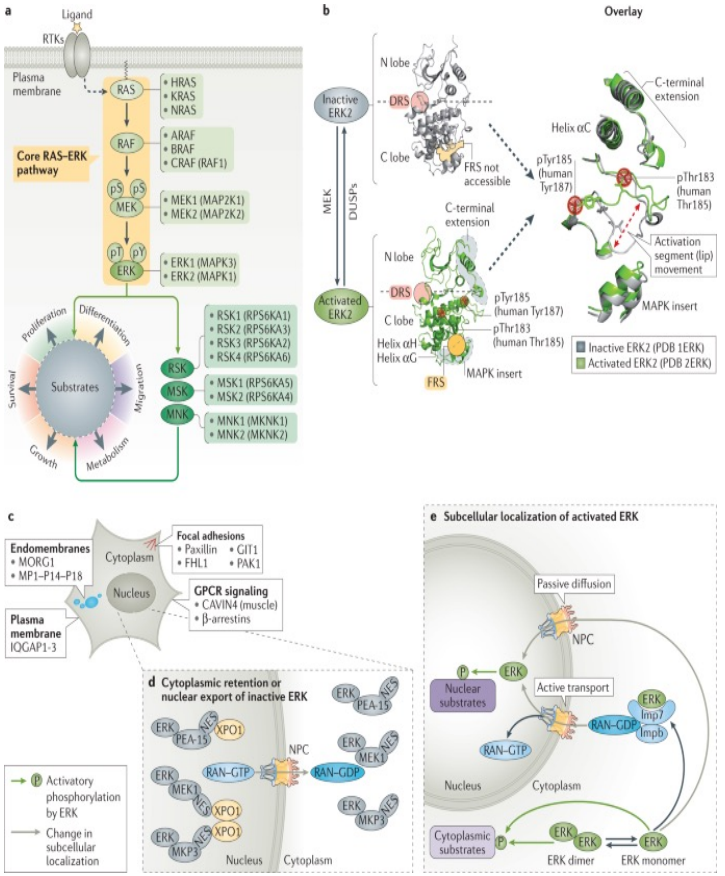



Figure modified Lavoie et al., 2020. Nat Rev Mol Cell Biol 21, 607-632

# Role in Human Disease/Cancer

- ERF mutations that cause disease
  - Mutations in the ERF gene result in reduced protein expression and causes craniosynostosis in both humans and mice via haplo sufficiency
    - This identifies ERF as a novel regulator of osteogenesis within the RAS-ERK signaling pathway (Peraki et al., 2017).
  - Features of this clinical disorder include multiple suture synostosis, craniofacial dysmorphism, Chiari malformation, poor gross and/or fine motor control, hyperactivity, poor concentration and language delay.
- ERF mutations associated with cancer
  - Mice with functional ERF reduced to ~30% of normal exhibit postnatal multisuture synostosis
    - By contrast, embryonic calvarial development appears mildly delayed (Glass et al., 2019).
  - An ERF-null embryo leads to failed chorioallantoic fusion and labyrinth development due to a block in chorion trophoblast stem cell (Ch-TSC) differentiation
    - Results in death at embryonic day 10.5 in mice (Peraki et al., 2017).

- In summary, these data show that the level of ERF is absolutely crucial for normal biological process. Moreover, complete loss of ERF is catastrophic for early embryonic growth.

# Animal Model and Pathway

- In a study, ERF is shown to be required in all three waves of embryonic hematopoiesis.
- In ERF epiblast-specific knockout embryos, it was shown to have reduced numbers of circulating blood cells from E9.5 onwards (Peraki et al., 2017).
  - Indicates that ERF is necessary for hematopoietic stem cell maintenance or differentiation
  - Required for both primitive and erythromyeloid progenitor waves of hematopoietic stem cell -independent hematopoiesis and for the normal function of HSCs (Peraki et al., 2017).
- ERF/ETS2 being a member of the ETS family of transcription factors as well as a downstream effector of the RAS/RAF/ERK pathway, have been shown that both are involved in diverse facets of hemopoiesis. (Peraki et al., 2017).
- ERF acting as a transcriptional repressor can help ensure the absence of spurious activation by other ETS family proteins
  - Led to correct differentiation programs and homeostasis(Peraki et al., 2017).
  - Thus, eliminating ERF can affect the entire regulatory of ETS factors in hemopoiesis (Peraki et al., 2017).

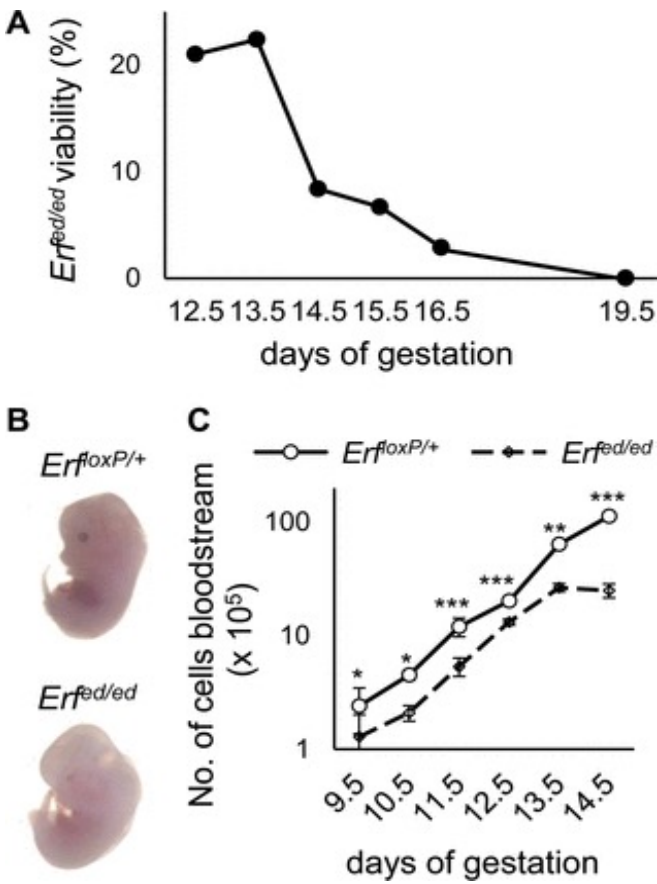



Figure from Peraki et al., 2020. Molecular and Cellular Biology, 37(19)

# ELK-1

- ELK-1 is a transcription factor and member of the ETS oncogene family of transcription factors
  - Includes nuclear phosphoproteins involved in biological processes such as cell growth, differentiation, and survival, hematopoiesis, angiogenesis, wound healing, cancer, and inflammation (Arnaud et al., 2007).
- ELK-1 is also a part of the ternary complex factor (TCF) subfamily (Arnaud et al., 2007).
  - Activated by phosphorylation, the TCFs form a ternary complex with two molecules of serum response element on the DNA consensus site while additionally recruiting coactivators (Arnaud et al., 2007).
- ELK-1 has multiple isoforms, but two known variants
  - Elk-1 and sElk-1(Cotterill 2019).
    - Elk-1 variant formed from alternative splicing that lacks the SRF interaction domain and part of the Elk-1 DNA-binding domain (DBD).
    - sElk-1 is a neuronal specific isoform of Elk-1 that arises from an internal translation start site in the ELK-1 sequence and corresponds to a protein lacking the first 54 amino acids of the DBD (Vanhoutte et al. 2001).
      - It potentiates NGF-driven PC12 neuronal differentiation.
      - Overexpression of sElk-1 increases neurite extension (Vanhoutte et al. 2001).
- In summary, the different variants of Elk1 might provide a mechanism for maintaining the appropriate "balance" of ELK1 gene function.

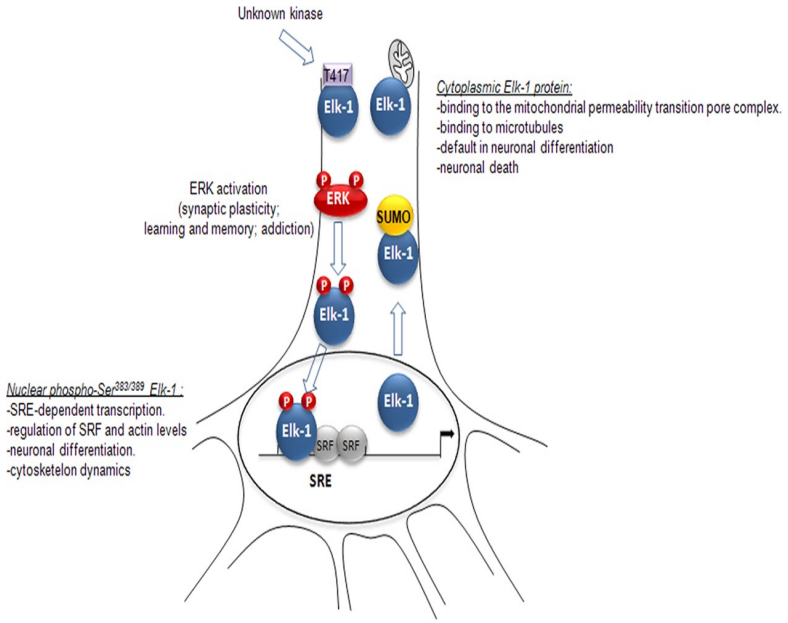



Figure from Besnard et al., 2011. Frontiers in Neuroscience, 5.

# Roles in Human Disease/Cancer

- ELK-1 plays an important role in controlling gene expression in human embryonic stem cells (hESCs) (Prise and Sharrocks AD 2019).
  - Its phosphorylation has been shown to be modulated in various CNS diseases (i.e. Alzheimer's disease (AD), Huntington's Disease (HD), and Synucleinopathies )
- Sublethal concentrations of Aβ appear to interfere with BDNF-induced activation of Elk-1 in cultured cortical neurons and results in an altered SRE-driven gene regulation,
  - likely accounts for increased neuronal vulnerability (Yildirim et al., 2019).
- In various synucleinopathies, the α-Synuclein forms aggregate in neurons in Parkinson's disease (PD) and mainly in oligodendrocytes in multiple system atrophy (MSA).
- The common theme of AD, HD, and (PD), is their shared aspect of the accumulation of a cytoplasmic and toxic form of Elk-1 observed in post-mortem brain (Besnard et al., 2011).
- Therefore, showing that gene therapy for ELK-1, inhibition, or the pharmacological activation has the potential of being a possible therapeutic approach for targeting transcriptional dysregulation in HD, and other neuropsychiatric conditions with similar molecular pathologies (Yildrim et al., 2019).

# Animal Model and Pathway

- A study on mice about HD, a chronic neurodegenerative disorder characterized by a late clinical onset despite ubiquitous expression of the mutant Huntington gene (HTT) from birth, was done.
  - Transcriptional dysregulation was shown to be an important aspect of HD while the altered genes in the prodromal period and their regulators are not yet clear (Besnard et al., 2011).
    - Transcriptional and chromatin profiling was used and aberrant transcription and changes in histone H3K27acetylation in the striatum of R6/1mice during presymptomatic disease stages were found.
- Therapies that promote the expression and activation of ELK-1 and its transcriptional activity showed beneficial effects in a primary striatal cell culture model of HD.
  - Seemed to alleviate aspects of HD progression (Yildrim et al., 2019).
    - Versus its inhibition which caused apoptosis in neuronal cultures stimulated with nerve growth factor.
- This study collectively demonstrates that aberrant gene expression precedes overt disease onset in HD, identifies ELK-1 transcription factor as a key regulator linked to early epigenetic and transcriptional changes in HD, and presents evidence for ELK-1 as a target for alleviating molecular pathology (Yildrim et al., 2019).

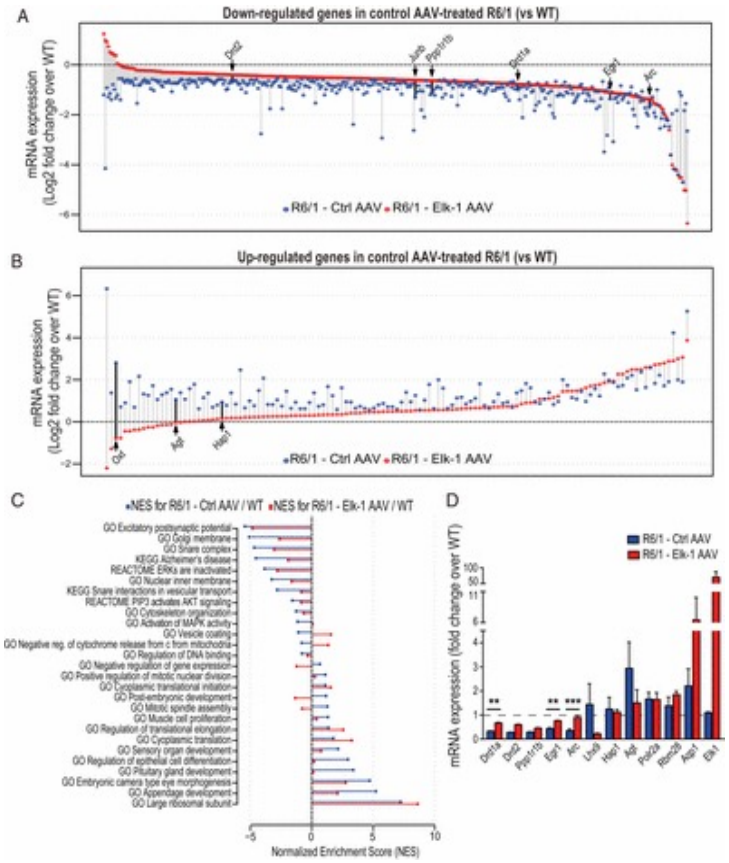



Figure from Yildrm et al., 2019. Proceedings of the National Academy of Sciences, 116(49), 23840-24851

# NRf2/NFE2L2

- NRF2 is a transcription factor protein product encoded by the nuclear factor NFE2L2 gene
  - Regulates the response to chemical and radiation induced oxidative and electrophilic stress in mammalian cells. (Huppke et al., 2017).
- The NRF2 pathway responds to oxidative stress by inducing the transcriptional upregulation of a broad range of cytoprotective genes (Stavroula et al., 2015).
  - This system responds to endogenous reactive molecules, reactive nitrogen species, and to exogenous substances (Stavroula et al., 2015).
- The sensing mechanisms comprise oxidation or alkylation of critical KEAP-1 cysteine residues, and phosphorylation of NRF2 on amino acids Ser40 and Tyr568 .
  - When redox homeostasis is restored, NRF2 activity is repressed via export from the nucleus back into the cytoplasm and degradation via a Cullin-RING ligase 3 -KEAP-1 complex (Stavroula et al., 2015).
- NRF2 can trigger a feedback loop of increased expression of ARE-dependent genes (i.e. KEAP-1 and Cul3)
  - Promotes NRF2 degradation
  - Participates in resetting NRF2 activity at its basal level (Stavroula et al., 2015).

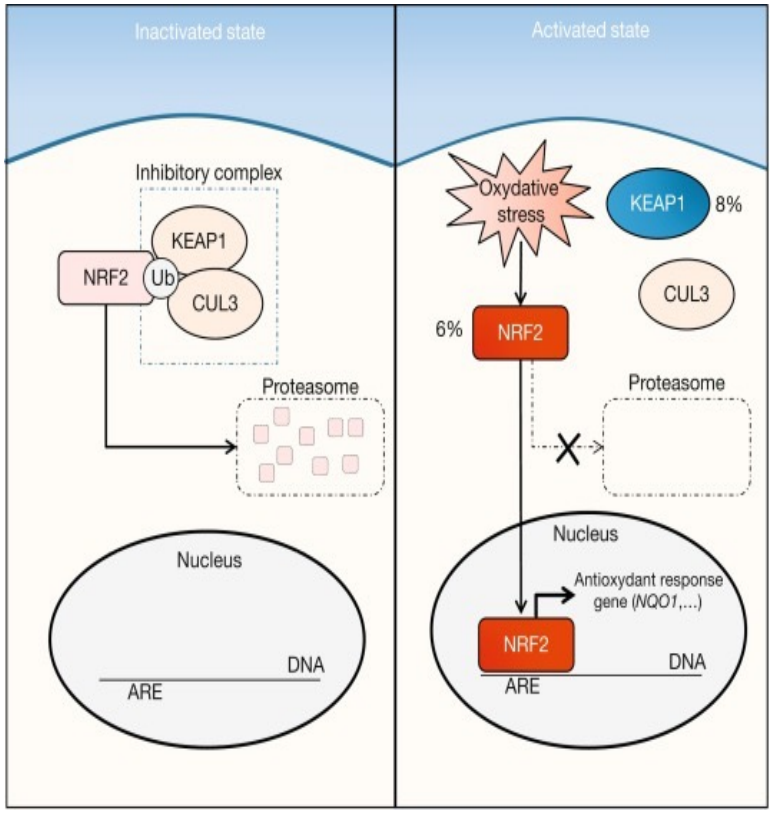



Figure from Nault & Zucman-Rossi, 2014

# Role in Human Disease/Cancer

- What is known
  - It has been demonstrated that oncogene-directed increased expression of the NFE2L2 gene can be an alternative mechanism of NRF2 activation.
  - K-Ras and B-Raf, which operate in the MAPK pathway, have been shown to increase NFE2L2 transcription via activation of Jun or/and Myc.

- Where is it expressed?
  - It's been shown to be expressed in various cell types (i.e., lung, liver, kidney, stomach, small intestine, neurons, astrocytes etc)
    - Considered a multi-organ protector
      - Enhances cellular resistance to harmful insults that occur during cells' normal activities and during environmental exposures
    - It has also been found to be overexpressed in various human cancers (Yoo et al., 2012; Lee and Surh, 2005; Stavroula et al., 2015).

# Implications of Mutations in this Gene

- Somatic mutations of NFE2L2 found have mainly been missense mutations and in frame insertions or deletions of NFE2L2
  - Localized in the DLG and ETGE motifs of the Neh2 domain
    - These are present in many types of cancer and are associated with a poor prognosis (Huppke et al., 2017).
    - Causes modifications and accumulation in the NRF2 protein
      - Leads to an impaired interaction of NRF2 with KEAP1
        - » Promotes cell survival and drug resistance in cancer (Huppke et al., 2017).
    - An NRF2 protein with a mutation in the DLG motif retains binding to KEAP-1
      - This causes NRF2 ubiquitination.
        - » Accumulation in the nucleus leads to widespread misregulation of gene expression, increased expression of stress response genes and an imbalance in cytosolic redox balance (Huppke et al., 2017).

# Animal Model and Pathways

- In a mouse model of AD, crossbreeding of NRF2 knockout mice (NRF2 -/-) with APP/PS1 mice (APP/PS1 +/0) were used to study the role of NRF2 in its pathogenesis (Caterina et al., 2017).
  - Four groups of mice: APP/PS1 having both endogenous copies of the NRF2 gene, APP/PS1 without both copies of the NRF2 gene (APP/PS1;NRF2 -/-), wildtype mice with both copies of the gene (WT) and wildtype mice lacking both copies of the gene (NRF2 -/-), (Caterina et al., 2017).
  - NRF2 was clearly detected only in those genotypes with both copies of the NRF2 gene (Caterina et al., 2017).
- In conclusion, it was found that decreasing NRF2 levels or a lack of NRF2 exacerbates cognitive deficits (i.e., spatial learning and memory, working and associative memory) in APP/PS1 mice.
  - Associated memory function related to the amygdala-hippocampus axis was also found to be unaltered in these mice.
  - Indicates that the lack of NRF2 has an overall effect on brain function (Caterina et al., 2017).

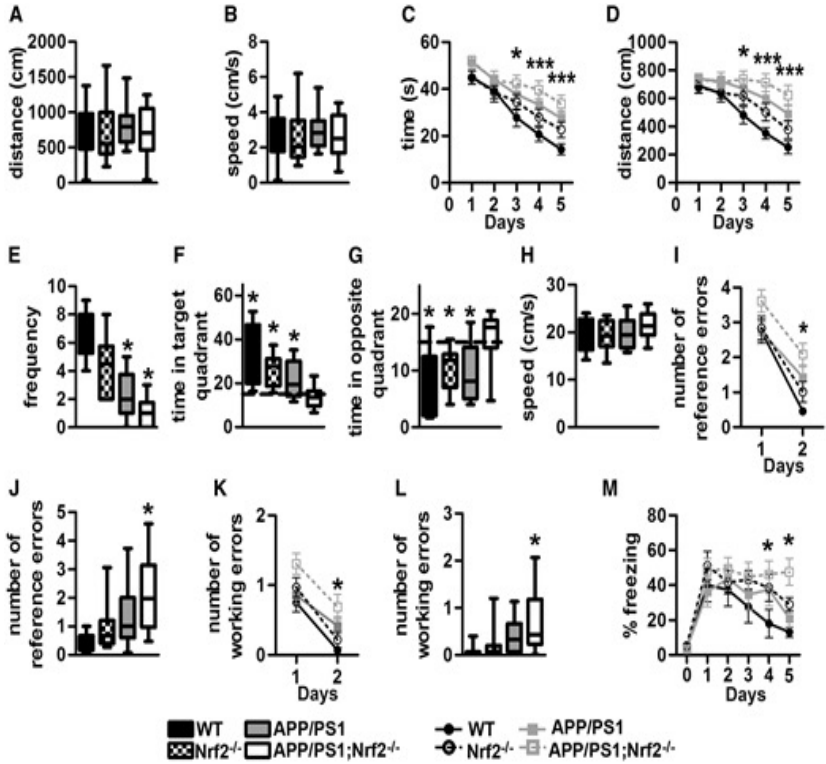
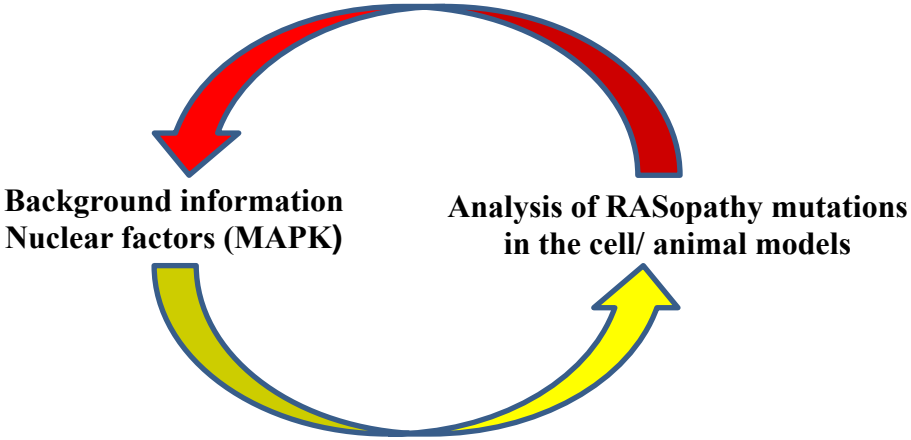



Figure from Caterina et al., 2017. Human Molecular Genetics, 26(24), 4823-4835

# Conclusion

These three genes are all involved in many biological processes such as cell growth, proliferation, differentiation, wound healing, inflammation etc., that contribute to them being prominent factors in many central nervous system diseases, disorders and human cancers. Their promotion or inhibition of expression of genes and pathways play an important role in how these affect the normal or mutated tissues in the human body. ERF and ELK1 are downstream of the MAPK pathway. The MAPK pathway. NFE2L2 is a transcription factor involved in cellular response to stress and candidate gene downstream of ectopic MAPK pathway activation. The MAPK pathway is known to be altered in RASopathy syndromes. These three nuclear factors may be involved in output or response to elevated MAPK signaling which could affect the way these factors are transcribed and/or affect target genes. Improved understanding of how these genes work and their roles in certain diseases is crucial to understanding the biology and to inform future strategy for treatment.




**Nuclear Factors are key to understanding transcriptional impact of RASopathy muations**

# Lessons and Takeaways

- Communication
  - Talking between teammates ensures a cohesive flow within the lab and that everyone knows their position and does their part.
- Every role is important
  - Everything from background research, to redoing the same exact protocol when encountering errors, specific equipment, and grant writing, etc., are all important pieces in doing research and nothing works well alone without the others
- Patience Patience Patience
  - Research is all about trial and error. Nothing will ever be discovered all on the first try.

Picture from PMG Admin, 2019. Manufacturing Services

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