NATURE, NURTURE AND VITAMIN K: SPECULATIONS REGARDING NEURODEVELOPMENTAL EFFECTS OF VK GENOTYPE

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Key Points and a Question

- Humans vary in the gene that encodes for Vitamin K epoxide reductase complex (VKORC1).
- Vitamin K (VK) promotes growth and health of neural cells.
- VK protects against oxidative stress associated with toxic exposure.
- Some neural effects of VK paucity overlap with key brain development aberrations.

- Could VK gene/environment interaction play a role in problems of neural development?

VKORC1

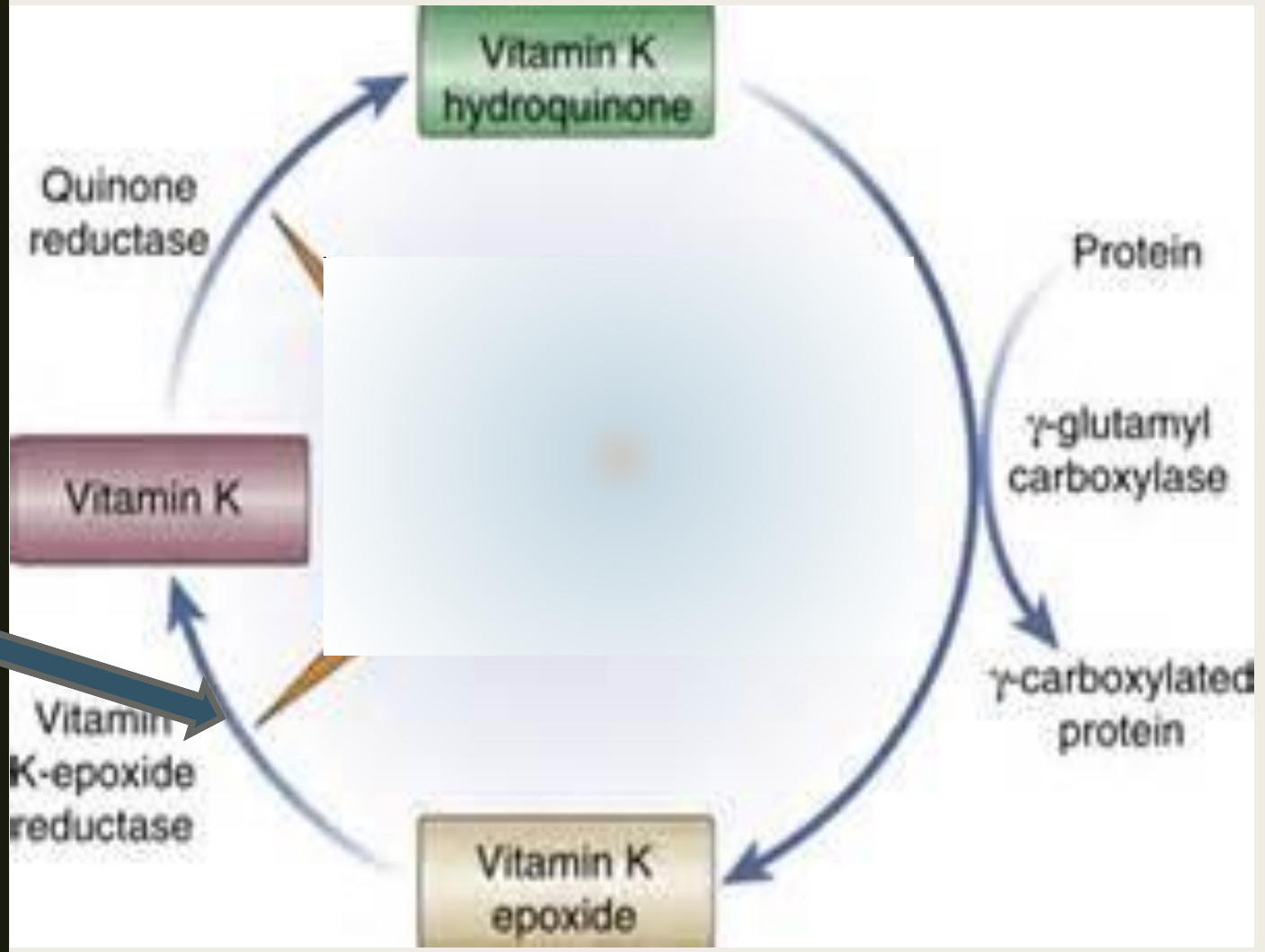
- A key polymorphism has been widely studied because it causes clinically important differences in response to common blood thinning agents
  - *A to G sub at 3673 reduces the efficiency of the VK cycle.*
- Ethnicity
  - *more than 35% of Caucasians*
  - *About 10% for persons of African descent*

VK Cycle

VK is recycled, allowing small amounts to be reused, thus decreasing the dietary requirement.

VKORC1 makes VK epoxide reductase enzyme.

VKORC1 comes in a genotype that lessens VK enzyme action here….



VK and nervous system: Effects are well documented, if not widely known

- Affects neurite growth

- Key players in neurodevelopment require adequate VK
  - *Protein S is VK dependent*
  - *Protein S is critical for proper neurodevelopment. i.e., sub-ventricular zone (SVZ)*

  - *Gas6 is VK dependent*
  - *inhibition of Tumor Necrosis Factor toxicity on oligodendrocytes*
  - *anti-inflammatory effects on nervous system*

Why was Protein S role in SVZ interesting?

- Abnormalities in SVZ cell division are associated with autism.
- Persons with autism have been shown to have various abnormalities of the SVZ.
- Recently, cellular differences in specific regions of the SVZ have been characterized in persons with autism (Kotagiri et. al., 2014).

Affects Neurite Growth

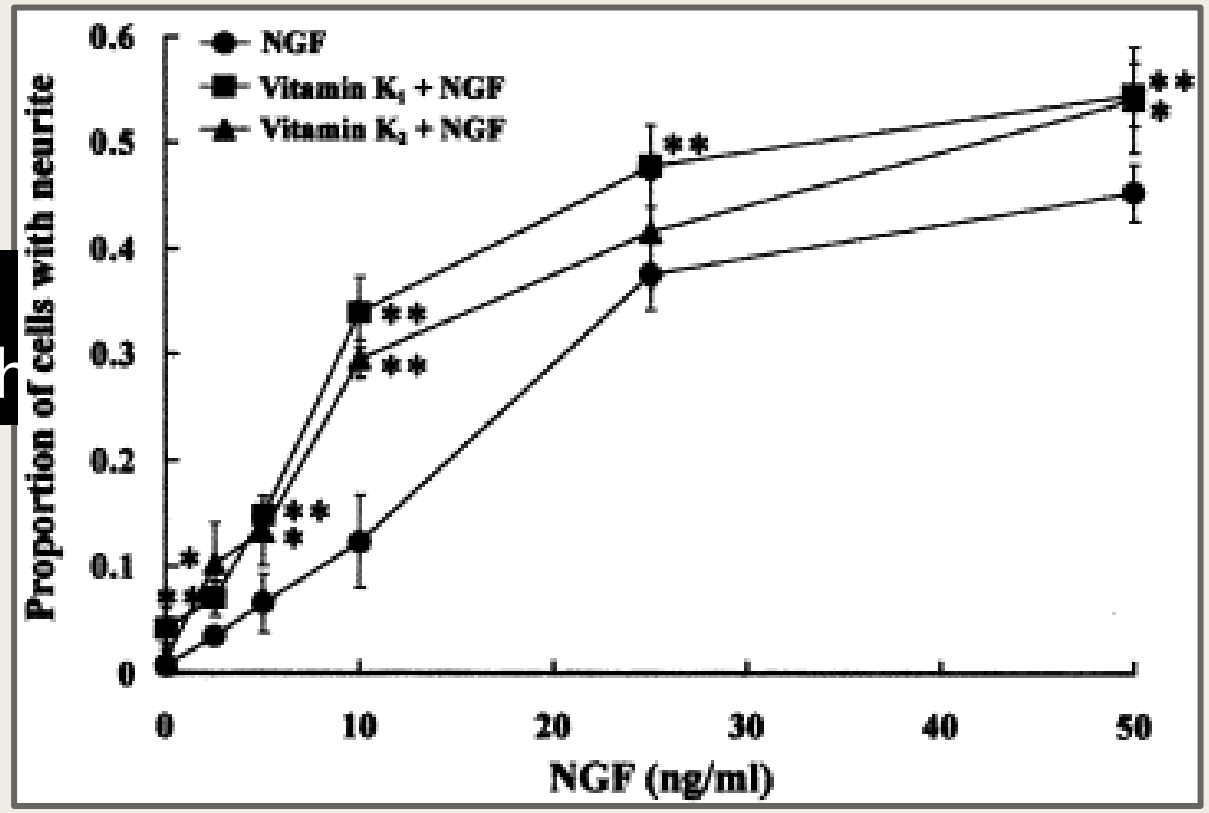


Fig. 1. Dose–response curve of the neurite outgrowth from PC12D cells for NGF, NGF plus 100 μg/ml vitamin K1 and NGF plus 100 μg/ml vitamin K2. Each point represents the mean±SD (n=6) from two replicate experiments. Significant difference from the NGF-only con...

Chi Kwan Tsang, Yuto Kamei

Novel effect of vitamin K1 (phylloquinone) and vitamin K2 (menaquinone) on promoting nerve growth factor-mediated neurite outgrowth from PC12D cells

Neuroscience Letters, Volume 323, Issue 1, 2002, 9–12

http://dx.doi.org/10.1016/S0304-3940(01)02550-2

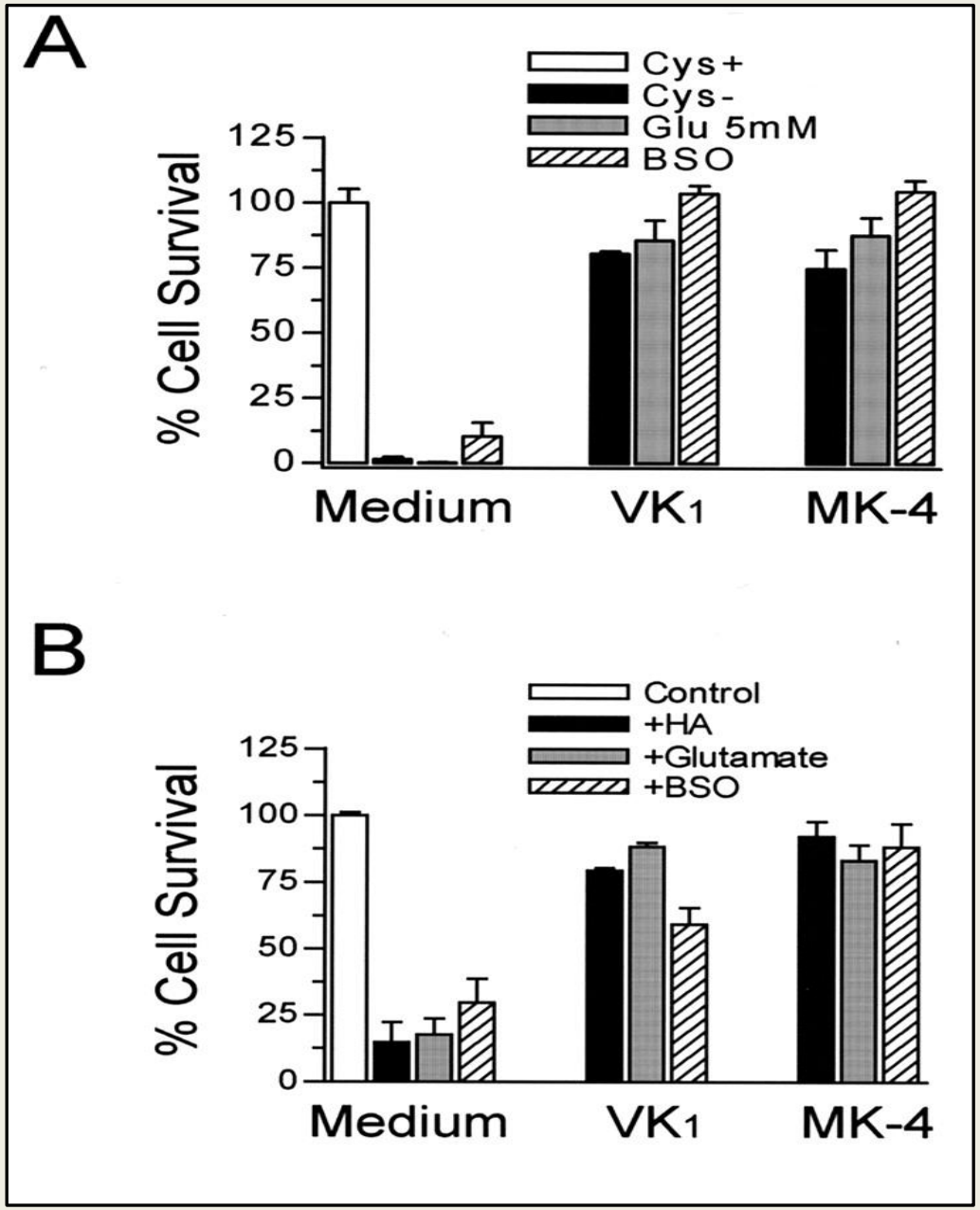
VK and oxidative stress

- VK prevents neuronal death during exposure to neurotoxins

Vitamin K1 and MK-4 protected both OL precursors and neurons from cell death induced by various GSH depletion methods.

Primary cells were incubated with indicated agents for 24 hr in the presence or absence of K1 (0.1μm) or MK-4 (0.1μm), and cell viability was analyzed after 24 hr.

A. Vitamin K prevented OL cell death induced by cystine depletion, by glutamate (5 mm), and by BSO (1 mm).
B. Vitamin K1 and MK-4 prevented immaturecorticalneuronaldeathinducedbyhomocysteicacid(HA,2.5mm),glutamate(5mm), or BSO (1 mm).



Jianrong Li et al. J. Neurosci. 2003;23:5816-5826

- Li et. Al., 2003: "…vitamin K_1 and K_2 (menaquinone-4) potently inhibit glutathione depletion-mediated oxidative cell death in primary cultures of oligodendrocyte precursors and immature fetal cortical neurons.."
  - *independent of its function as a cofactor for γ-glutamylcarboxylase*

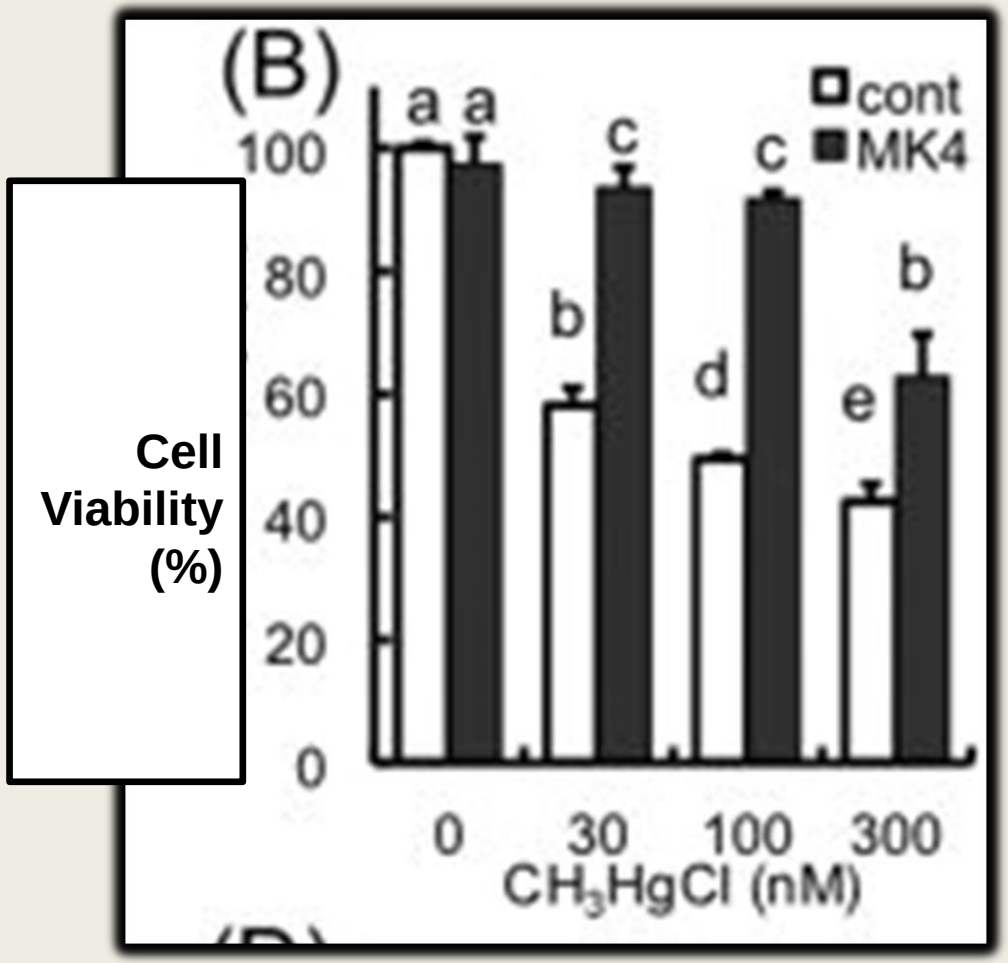
Several follow up papers (e.g., 2005, 2009) have elucidated the process -- which likely is via blocking the generation of reactive oxygen species.

Sakaue et al., 2011

Vitamin K has the potential to protect neurons from methylmercury-induced cell death *In Vitro*

- Human neuroblastoma IMR-32 cells
- cells were treated with vitamin K_2 (MK-4; **B**) at 1 μM 1 hr before methylmercury (CH_3HgCl) incubation for 48 hr.

Journal of Neuroscience Research
Volume 89, Issue 7, pages 1052-1058, 12 APR 2011 DOI: 10.1002/jnr.22630
http://onlinelibrary.wiley.com/doi/10.1002/jnr.22630/full#fig1



Lipids: phospholipid bilayer and myelin

- Mechanism whereby MK-4 impacts the formation of these lipids was uncovered and detailed in the 1970s and 1980s
- BUT past decade…. (wow this matters)
  - *sphingolipids have been shown to directly affect VK neural proliferation, migration and apoptosis*

Sex Differences in VK

- Animal research has shown that VK levels in the brain are higher in females, even when other variables are held constant
- Males are more readily adversely impacted by low VK levels

COULD VK GENE/ENVIRONMENT INTERACTION MATTER FOR PROBLEMS OF NEURAL DEVELOPMENT?

Existing research that has gotten little attention

- reports of un-hypothesized relationships between VK and autism symptoms
  - *levels of VK were found to be the strongest predictor of post-intervention improvement in autism symptoms*

"Vitamin K had the strongest correlation with the Average Change of the PGI-R, followed by biotin and lipoic acid…"

"The primary role of vitamin K is in blood coagulation, which is not reported as a common problem in autism, which is why it was not included…"

Parent Global Impressions & Pervasive Development Disorder Behavior Inventory

Adams JB, Audhya T, McDonough-Means S, et al. Effect of vitamin/mineral supplement on children and adults with autism. BMC Pediatrics. 2011

Vitamin K was extracted from plasma by methylene chloride in a monophasic design, purified on a C-18 cartridge, separated on a reversed-phase column, and t detected fluorometrically.

WHAT DID I DO BESIDES REVIEW PRE-EXISTING DATA?

Autism

- The disorder is more common in boys and current diagnostic practice considers autism as a spectrum, with mild and severe cases.
- The more severe cases often feature little to no language and low measured IQ.
- It appears that there may be pockets of high incidence or at least higher diagnosis.
- Minnesota is a state with a high rate of diagnosis within the USA school system, and the Somali population within Minnesota has been reported to have an usually high prevalence.
- Children with autism in Minnesota that are of Somali descent have been shown to be far more likely to have a more severe form and/or to have more intellectual disability.
- **Overall 33% of children on the autistic spectrum also had a concurrent low IQ (measured below 70), whereas vast majority (~100%) of the Somali children diagnosed as being on the spectrum did so.**

An idea untested

- A diet (or maternal diet) low in VK and the child (or fetus) carries the VKORC1 substitution, then there would be low vitamin K
- Leading to
  - *neural development effects ranging from proper migration to apoptosis to poor handling of toxic exposures…..*

….Stop thinking about <u>The</u> Cause

…and start thinking of threshold of risks getting crossed.

VKORC1 gene may play role in autism?

- Few reports of the effects of low levels of VK during fetal development
  - *Effects of prenatal Warfarin exposure appear to be dependent on the timing of exposure (e.g. skeletal defects seem more associated with first trimester exposure).*
  - *Negative outcomes are heterogeneous, but can include seizures and mental retardation of various degrees.*
  - *Experimentally controlled animal research has shown that postnatal VK deficiency causes less movement and exploration, and slower maze learning ability*

VKORC1 genotype may play an etiological role in a subset of children with a high incidence of severe autism

- children of Somali descent would be expected to have a low frequency of the specific VKORC1 G to A substitution that results in less VK
- FIVE n = 5
- The binomial distribution was utilized to estimate the probability of selecting five children and finding three have the uncommon substitution.
  - *The probability was $p < .05$, specifically $p = .01$.*

caveats

- only one SNP on the VK gene was genotyped -- there are several that matter
  - *the SNPs -1877G/A and -4931T/C were not genotyped in any of the participants. If a lack of available VK at key times in development are in fact playing an etiological role in the development of autism, it is probable that more than one genotypic vulnerability may matter.*

Nature via Nurture

- Finding genotypic variations does not imply that environmental exposures do not play a role.
- Other than for purely genetic outcomes with a perfect 100% concordance for identical twins, understanding how genes and environment are interacting is the way to move forward.

Nature via Nurture

- Is it possible for a trait to have a heritability estimate that increases over time and have ALL of the increase be due to environmental change?
- Can a highly heritable trait increase in frequency but have all the increase be due to environmental changes?
- Can gene expression change due to environmental experience and be passed on to offspring?

Hypothetical Example of Heritability Drift

1970

H=.2

- Main Cause of a Disease is Exposure X prenatally
- Gene A doesn't influence how Exposure A is handled.
- Genetic differences (H') do not make a big difference in the Disease risk

H=.7

If one estimates Heritability using twin data, the H' statistic will be higher in 1990. This is not an illusion-- ---the importance of genetic variation regarding who does and does not have Disease really did increase.

Mice fear of cherry smell

- Developed more M71 receptors and detected smell at much lower levels
- So did their offspring, and THEIR offspring.
- Learned from dads somehow…? No way.
- DNA sequencing of sperm from the grandfather mice and their sons also revealed epigenetic marks on the gene encoding M71 that weren't seen in control mice.
- Lamarck rises from the grave
- Marcus Pembrey at the University of Bristol: "It is high time public-health researchers took human transgenerational responses seriously," he says. "I suspect we will not understand the rise in neuropsychiatric disorders or obesity, diabetes and metabolic disruptions generally, without taking a multigenerational approach."

Lamarck rises from his grave: parental environment-induced epigenetic inheritance in model organisms and humans

(Wang et al., 2017)

"Emerging evidence indicates that both ancestral and parental experiences, including nutrition, environmental toxins, nurturing behavior, and social stress, can have powerful effects on the physiological, metabolic and cellular functions in an organism. In certain circumstances, these effects can be transmitted across several generations through epigenetic (i.e. non-DNA sequence-based rather than mutational) modifications.

In this review, we summarize recent evidence on epigenetic inheritance from parental environment-induced developmental and physiological alterations in nematodes, fruit flies, zebrafish, rodents, and humans…."

Not Nature Vs Nurture

- Diseases and disorders and traits that have increased over the past generation do not have **A Cause**.
- Individual genes matter.
- Individual exposures matter.
- Mom or dad's experiences matter.

VKORC1, by increasing the importance of adequate vitamin K in the diet for some individuals, MIGHT be one of several genes that can bring an individual closer to some unknown threshold of multiple risks – that if met -- can lead to autism.

Take home message is how genetic susceptibility really works :

Nature VIA Nurture

NATURE VS NURTURE