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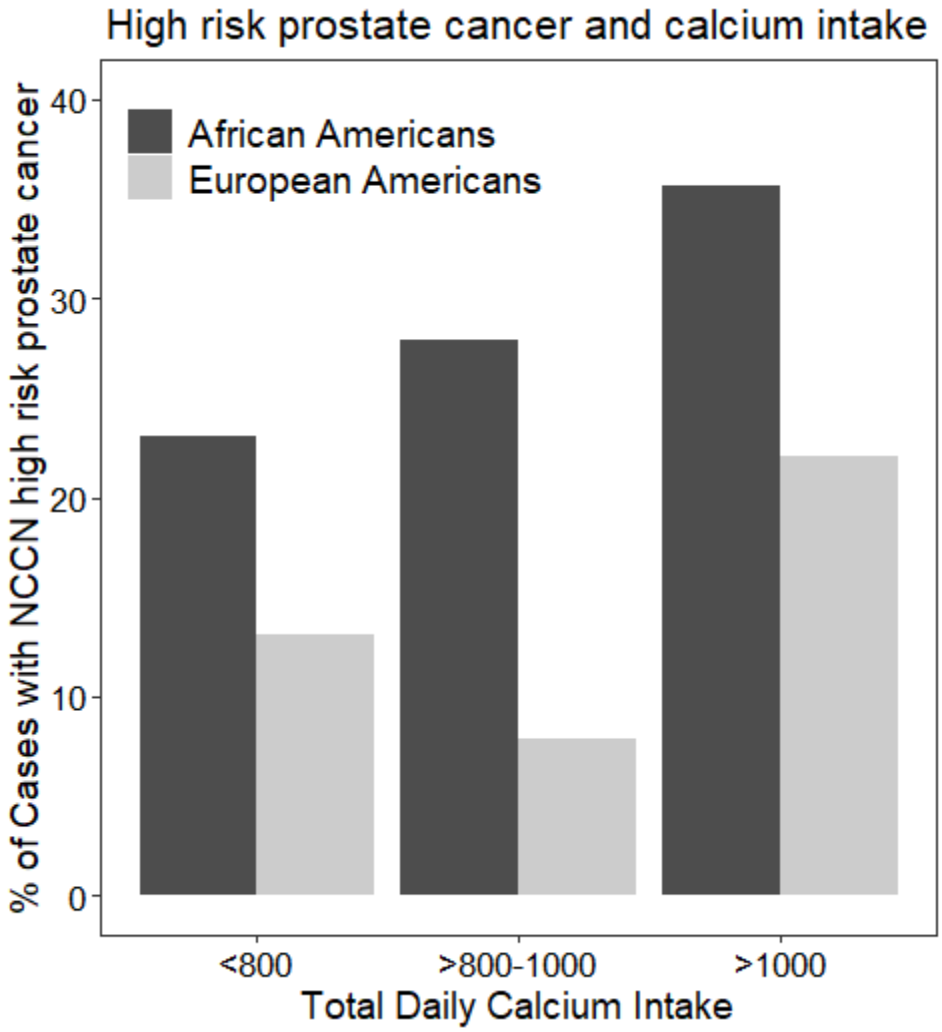
Abstract:
It is well established that African Americans exhibit higher incidence, higher mortality, and more aggressive forms of some cancers, including those of breast, prostate, colon, stomach, and cervix. Here we examine the ancestral haplotype of the TRPV6 calcium channel as a putative genomic factor in this racial divide. The minor (ancestral) allele frequency is 60% in people of African ancestry, but between 1 and 11% in all other populations. Research on TRPV6 structure/function, its association with specific cancers, and the evolutionary-ecological conditions which impacted selection of its haplotypes are synthesized to provide evidence for TRPV6 as a germline susceptibility locus in cancer. Recently elucidated mechanisms of TRPV6 channel deactivation are discussed in relation to the location of the allele favored in selection, suggesting a reduced capacity to inactivate the channel in those who have the ancestral haplotype. This could result in an excessively high cellular Ca^{2+}, which has been implicated in cancer, for those in settings where calcium intake is far higher than in their ancestral environment. A recent report associating increasing calcium intake with a pattern of increase in aggressive prostate cancer in African-American but not European-American men may be related. If TRPV6 is found to be associated with cancer, further research would be warranted to improve risk assessment and examine interventions with the aim of improving cancer outcomes for people of African ancestry.

Figure 1:
Proportion of AA and EA cases having NCCN high risk prostate cancer (%) vs. calcium intake. The proportion of AA cases with high risk prostate cancer displays a pattern of increase with total calcium intake for AA men. Multiethnic study of 1,657 cases and 1,657 controls. Study by K. Batai *et al.* Figure reformatted.



Racial disparities in cancer

African Americans (AAs) are residents of the United States of America, typically with an admixture of Sub-Saharan African and European ancestry [1]. The disparity in cancer outcomes between AAs and Americans of European ancestry (EAs) has been a cause for concern in recent years. Centers for Disease Control and Prevention age-adjusted death rates per 100,000 for AAs from prostate cancer (14.3) indicate mortality twice as high as for EAs (7.4). The breast cancer death rate for AAs (16.4) is high compared with that of EAs (11.1) [2] and they exhibit twice the proportion of triple negative breast cancer, which is more aggressive, more deadly, and typically strikes women of younger age than other molecular subtypes. Colorectal cancer mortality in AAs (18.9) is alarmingly high compared with that of EAs (13.8). and pancreatic cancer is also disproportionately higher (13.3) than in EAs (10.9) [2]. In these cancers TRPV6 is upregulated – highly so for breast and prostate cancers, where TRPV6 inhibition is under study as a potential therapeutic strategy. In prostate cancer, TRPV6 expression level has been linked to tumor progression and has been proposed as a prognostic marker [3].

Non-genomic factors in racial disparity

It has been suggested that a combination of factors including social determinants of health, access to health care, biological factors including genomics, and patterns of research lead to racial disparities in cancer outcomes [4]. Socioeconomic factors that impact health include education status, level of insurance, access to medical care. Factors rooted in stressors such as childhood trauma and also historical and societal inequalities that impact attitudes regarding medical care are implicated. Furthermore, therapies and guidelines are typically based on research that is overwhelmingly conducted on subjects of European descent. This has led to a recent call to address health disparities through the redesign of clinical trials [5].

Culturally influenced factors such as exercise, lifestyle and diet are also under study. Recently, Batai et al (Kittles lab) investigated the association of calcium and vitamin D intake with prostate cancer in a study of a multiethnic group of 1,657 cases and 1,657controls [6]. The proportion of cases diagnosed with aggressive prostate cancer (as determined using the National Comprehensive Cancer Network (NCCN) risk stratification scheme) was found to increase with increasing total calcium intake in AA men but not in EA men (See figure 1). Also, in AA men there was an inverse relationship between vitamin D intake and prostate cancer that was not seen in EA men.

Search for genomic factors in racial disparity

Possible hereditary factors in the higher cancer burden observed in AAs are confounded by socioeconomic and cultural factors, such as those discussed above, that impact health and access to care. Studies that normalize socioeconomic factors still detect racial disparities, providing evidence that genomic factors, possibly in interaction with environmental factors [5], for example calcium intake, may impact cancer burden.

However, discovery of genomic factors in racial cancer disparities is greatly hampered by racial disparities in genome-wide association studies (GWAS), from which such associations are typically inferred. People of African ancestry continue to be vastly under-represented in GWAS. As recently as 2009, 96% of GWAS samples were conducted on subjects of European descent.

The subsequent 7 years saw a rise in GWAS samples from Asian populations, however, less than 4% of all samples analyzed were from subjects of either African or Latino ancestry [7].

When conclusions are drawn from such studies, they may not be pertinent to AAs. We are aware of only one study that aimed to investigate the association of TRPV6 with prostate cancer progression, and the finding was that TRPV6 alleles do not influence prostate cancer progression [8]. However, the study included only Caucasians as controls; case samples were 11.3% heterozygous; only 1 sample (0.7%) was homozygous for the ancestral allele. Arguably, the study did not have the statistical power to detect an association if it is specific to the ancestral haplotype.

Attempts have been made to elucidate genomic underpinnings of racial disparities in cancer, however, a genomic explanation remains elusive. Some studies have investigated germline susceptibility loci that were previously implicated in specific cancers of subjects who were predominantly EAs [9], finding some that are and some that are not also implicated in cancer of AAs. However, most breast and prostate cancer GWAS hits have not been replicated in people of African descent. Some whole-genome investigations of germline variants for association with cancer in AAs have been conducted from an agnostic viewpoint, without preselecting the single nucleotide polymorphisms (SNPs) under investigation [10]. The statistical power of such studies is generally sufficient to detect large effect sizes, and TRPV6 has not been implicated. Approaches such as admixture mapping have been used to narrow the field of germline candidates. Mutation profiling has been undertaken to investigate exclusively somatic variants in breast cancer samples. To our knowledge, however, an investigation of association between the germline TRPV6 and cancer in subjects of African ancestry has not been conducted.

Recently, the African American Breast Cancer Epidemiology and Risk (AMBER) [11] and African Ancestry Prostate Cancer GWAS (AAPC) [10] Consortiums were created to conduct studies with greater statistical power to detect genomic and environmental factors that are specific to AAs. The AMBER consortium has produced a dataset to investigate breast cancer in AAs by combining biospecimens from 7,500 AA cases and 17,000 healthy controls. This consortium has investigated germline microRNAs, rare exome variants, specific genes, and pathways, preferential linkage disequilibrium and admixture associated regions for association with breast cancer in AAs. The AAPC consortium has produced a dataset to investigate prostate cancer in AAs that currently contains over 10,000 cases and 10,000 controls [12] It has conducted genome-wide association meta-analyses, and studies of specific regions. The H3Africa Consortium [13] and RESPOND study are newer initiatives. The latter, with over 20,000 cases, is currently recruiting AA men with prostate cancer [14]. These consortiums are widely expected to contribute further to understanding of genomic factors influencing racial disparity in breast and prostate cancers [15]. Neither consortium has yet, to our knowledge, investigated the TRPV6 region (7q34-35) specifically.

M681T identified as carrier of the favored allele in positive selection

The TRPV6 haplotype is a coupled polymorphism consisting of three nonsynonymous SNPs (C157R, M378 V and M681T) in humans on chromosome 7q34. Accelerated evolution is demonstrated for the TRPV6 protein, but only in this region [16]. The ancestral allele is in this case the minor allele. It has a frequency of 60% in people of African ancestry but is between 1 and 11% in all other populations listed in 1000 Genomes [17]. All non-African populations carry

a signature of selection on the derived haplotype and their nucleotide diversity is an order of magnitude lower than those of African populations [16]. This and other evidence supports the hypothesis that the derived haplotype results from widespread parallel selection acting on a genetic variation present in the ancestral African populations that became fixed in a soft sweep [18]. In a hard sweep, a population adapts to an environmental change through a new advantageous mutation. In a soft sweep, however, adaptation arises from standing genetic variation that pre-exists in the population. The derived haplotype results in a protein variant that is thought to have conferred evolutionary advantage and become fixed in populations outside of Africa about 7-10,000 years ago [19]. It is the M681T SNP of this haplotype that is hypothesized as the carrier of the favored allele in positive selection [19]. This has been confirmed by recent research [20], suggesting that differences between the ancestral and derived protein variants coded by this region impact function and confer evolutionary advantage in non-African populations.

TRPV6, calcium, and cancer

TRPV6 and its closest relative, TRPV5, with which it shares 75% amino acid identity, are genes that code for calcium channels in epithelial cells [3] such as in breast, prostate, colon, lung and pancreas. These proteins permit and regulate calcium (Ca^{2+}) entry into these cells. They are also important in Ca^{2+} homeostasis, with TRPV6 facilitating Ca^{2+} absorption in the intestines and TRPV5 involved in Ca^{2+} reabsorption in the kidneys [21].

Intracellular (ie: cytosolic) Ca^{2+} concentration is typically prevented from reaching toxic levels by a feedback inhibition mechanism that inactivates the TRPV6 Ca^{2+} channel. In contrast, Ca^{2+} level in extracellular fluids, including blood, is much higher [3]. Ca^{2+} signaling is central to many cellular processes, including balanced cell proliferation, cell motility, and apoptosis. Remodeling of this signaling and the attendant changes in Ca^{2+} concentration have been implicated in cancer [22]. Carcinomas, which are cancers originating from epithelial cells, characteristically exhibit altered Ca homeostasis [3]. Research has revealed upregulation of TRPV6 in some carcinomas, particularly of the prostate, breast, colon and pancreatic: cancers from which AAs suffer higher mortality. It has been proposed that people with the ancestral haplotype may have dysregulated Ca^{2+} homeostasis when their calcium consumptions is far higher than in the ancestral environment [23].

M681T located in region of Ca^{2+}-dependent inactivation of TRPV6 channel

Investigations into the structure and function of TRPV5 and TRPV6 have described channel gating mechanisms, mediated by a calmodulin (CaM) ligand, whereby Ca^{2+} may enter the cell (open channel) or is blocked by closure of the channel [24], [25]. Mutation of a key residue in the gating region induced cell death due to Ca^{2+} overload [26], highlighting the importance of the inactivation mechanism to health. Calmodulin (CaM) binding enables the TRPV6 channel to be in an open state, but subject to fast inactivation by a Ca^{2+} feedback mechanism [24].

Recent research places M681T, the allele favored in selection, in the region of influence over the CaM binding that is necessary for the TRPV6 protein to inactivate the channel, protecting the cell from Ca^{2+} overload. Structural studies of TRPV6 place M681T at a distance of 5 amino acids from a key CaM interface region [24]. Additionally, new research has extended the region of influence on CaM binding to include M681T [25].

Hughes et al investigated TRPV6 channel function in derived vs. ancestral TRPV6 protein variants that were cloned into expression vectors and transfected into a human cell line. The percentage drop in Ca^{2+} current density was measured when Ca^{2+} extracellular concentration became high and activated gate closure. The channel of the derived variant exhibited a greater (48.9 +/− 5%) drop in Ca^{2+} current density than the ancestral (34.9 +/− 6%). This difference was not statistically significant ($p=0.09$) at alpha cutoff of 0.05. However, due to the importance of the M681T region to CaM binding, the authors suggested that the difference in channel inactivation could be biologically relevant, and the derived allele more sensitive to Ca^{2+} feedback [19].

An evolutionary-ecological model

The typical admixture in AAs is approximately 75% Niger-Kordofanian West African and 25% Northern European genetic ancestry [23]. It has been postulated that TRPV6 sequence variation among populations may have been influenced by domestication of milk-producing animals, beginning ~10 000 years ago [27]. The dairy farming that swept through Europe, parts of Africa, and much of the rest of the world did not take hold in West Africa due to the tsetse fly, thus calcium consumption in this region remained low (~200–400 mg per day) [28]. Surprisingly, West Africans do not develop osteoporosis, as do East Africans and others from dairy farming regions characterized by far higher calcium consumption [29]. It is thought that the TRPV5 and TRPV6 ancestral haplotypes play a role in greater Ca^{2+} absorption and reabsorption, explaining higher bone density in populations of West African descent [19].

Although not as high as the AAs who share much of their ancestry, high prevalence of prostate cancer has also been found in West African men [30]. It has been recently hypothesized by Hilliard [23] that the TRPV6 ancestral haplotype plays a role in both high bone density and the racial cancer disparity in AAs and others of West African descent. While conferring advantage in calcium-poor ancestral environments, this haplotype may be maladaptive in the US and other regions where calcium dietary and supplemental intake is far higher.

Synthesis of research

People of African descent suffer worse outcomes from common carcinomas in which TRPV6 is upregulated. The ancestral allele for the TRPV6 epithelial CA channel has a frequency of 60% in people of African ancestry, yet all non-African populations carry a signature of selection on the derived haplotype. The West African environment, rare in that the derived allele was not selected, was and remains to some extent a calcium-poor environment, yet West Africans do not develop osteoporosis. The association of ancestral TRPV6 and TRPV5 with higher calcium absorption and reabsorption has been previously established. However, an explanatory mechanism for evolutionary advantage of the allele that is thought to be the target of selection in evolution (M681T) is not agreed upon.

Recent research supports a hypothesis that translation of the derived transcript results in TRPV6 protein structure that blocks the passage of Ca^{2+} into the cell when cytosolic levels become excessively high, while the ancestral variant of the protein may not as effectively protect the cell from Ca^{2+} overload. M681T is in close proximity to an important binding site in TRPV6-CaM inactivation of the Ca^{2+} channel. The deactivation region of the TRPV6 channel has not itself

been resolved by x-ray crystallography. However, atomic-resolution of other regions and related structures [25], along with biophysical techniques have been used to investigate channel deactivation, implicating the M681T region as crucial to the channel deactivation that is required to protect the cell from Ca^{2+} overload when plasma levels become too high.

The above evidence, taken together with Batai et al research associating increasing Ca^{2+} intake with a pattern of increase in aggressive prostate cancer in AA men leads us to hypothesize a mechanism for Hilliard's evolutionary-ecological model: that when calcium consumption is high, binding differences in the M681T region of the ancestral TRPV6 haplotype do not as effectively inactivate the epithelial Ca^{2+} channel, leading to dysregulation of Ca^{2+} homeostasis. This could potentially worsen outcomes for carcinomas of the prostate, breast, and colon. Investigation of the TRPV6 ancestral haplotype as a germline susceptibility locus in cancer, both as main effect and in interaction with TRPV5 and with calcium consumption, could lead to actionable discoveries including preventive lifestyle changes for people having the ancestral allele.

Future Directions:

Racial disparities in cancer present a serious problem, and an opportunity for the research community to improve cancer outcomes for people of African ancestry. Addressing genomic factors along with patterns of care is expected to have a positive impact. A research challenge will be to uncover genomic underpinnings of the disparity, including complex factors such as epistatic effects. This could lead to inclusion of implicated SNPs in hereditary cancer screens designed to be relevant to people of African descent. Additionally, greater understanding of genomic factors could pave the way for investigations into gene-environment interactions, with attendant improvements in lifestyle advice delivered to this population. To accomplish these goals, research on genomic samples from subjects of African ancestry should be dramatically increased.

We have examined Hilliard's recently proposed hypothesis of ancestral TRPV6 as a genetic factor in racial cancer disparities due to excessive cytosolic Ca^{2+} when calcium consumption is high. A synthesis of recent discoveries in TRPV6 structure/function/evolution and association of increasing calcium intake with a pattern of increase in high risk prostate cancer in AA men supports her hypothesis and leads us to suggest a mechanism for it. Hilliard's hypothesis, if true, would imply a rethinking of current dietary advice delivered to people of African ancestry, and should be investigated via research into the impact of TRPV6 haplotype on prostate, triple negative breast and other carcinomas from which people of African ancestry suffer disproportionately. However, to our knowledge, no studies have been conducted to investigate the association of the TRPV6 ancestral haplotype with cancer, whether as a main effect or in interaction with another gene or an environmental factor, such as calcium intake. If such research were to uncover an association of the ancestral TRPV6 haplotype with cancer in people of African descent, this could lead to precision medicine inclusive of precision prevention: delivered through improved cancer risk assessment and dietary recommendations specific to the ancestral genotype.

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