Arthropod Saliva: the Missing Piece of the Hygiene/Health/Disease Puzzle

John Santiago Mejia MD MSc

Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, USA

KEY WORDS: arthropod, saliva, immunomodulation, hygiene, disease

IMAJ 2014; 16: 73–77

Hygiene represents one of the most successful health intervention measures in the history of medicine and is responsible for a significant improvement in human well-being and life expectancy [1]. However, as suggested by several epidemiological studies, it might render humans at risk for the development of autoimmune and allergic diseases [2]. It has been postulated that the modifications that hygiene introduces into the microbial ecosystems to which humans are exposed on a daily basis trigger a pathological process that may lead to tissue injury and disease [2,3]. The significance of this immunopathological mechanism in human health is reflected in the number of chronic non-communicable diseases associated with hygiene, such as inflammatory bowel diseases [4], multiple sclerosis [5], type I diabetes [2], asthma, and allergic diseases [6]. Furthermore, this pathological process might play a role in the etiopathogenesis of other NCDs associated with chronic inflammation such as atherosclerosis, type 2 diabetes, cancer, and neurodegenerative disorders [7]. In order to delineate the health policies required to control infectious diseases without increasing the risk of chronic NCDs, it is essential to determine the boundaries between beneficial and deleterious hygiene and to identify the molecular mechanisms that cause this dichotomy.

This review examines the role of parasitic helminths and hematophagous arthropods, two major sources of immunomodulatory molecules [8-11], in this hygiene-disease association and analyzes their potential role as tools to control the epidemic of chronic NCDs that is spreading around the world.

NCD = non-communicable disease

Eliminating the influence of pathogen/vector-derived immunomodulatory molecules on human tissues induces a homeostatic imbalance of the immune system that is associated with an increased risk of chronic non-communicable disease

EXOGENOUS IMMUNOMODULATORY MOLECULES AND HYGIENE-ASSOCIATED DISEASES

The immune system of vertebrates evolved under the selective pressure of microorganisms that deploy immune deactivation mechanisms to colonize and thrive in host tissues [3,12]. The immunomodulatory molecules that these microorganisms produce mitigate the intensity of the immune response mounted against them and facilitate their multiplication and dispersion. From an evolutionary perspective, this strategy ensures the survival of both hosts and microorganisms while shaping the reactivity profile of the immune system and its activation threshold [13]. Since the biological effect of these immunomodulatory molecules is non-specific in nature, a great deal of functional synergy and redundancy can be expected. This implies that for residents of low hygiene environments, where multiple infections are the rule rather than the exception, the physiological response to tissue injury is heavily influenced by these immunomodulatory factors. The importance of the imbalance generated by interventions that reduce the influence of these molecules in the immune system lies in its capacity to amplify tissue damage caused by etiopathogenic factors of disease. The fact that hygiene has been associated with several autoimmune, inflammatory and allergic diseases illustrates the non-specific nature of this pathogenesis-amplifying effect.

HYGIENE, HELMINTHS AND DISEASE

The role that hygiene plays in the etiopathogenesis of human disease is best characterized by Crohn's disease and ulcerative colitis – inflammatory pathologies of the intestinal tract that are more prevalent in industrialized nations with high hygiene standards [14,15]. A disruption in the intestine-associated microbial ecosystem triggers an abnormal inflammatory response to intestinal flora that is responsible for tissue injury and disease [15]. A variety of pathogenic microorganisms (virus, bacteria, fungi, protozoa) has been implicated as etiologic agents of these inflammatory bowel diseases, but there is no consensus regarding their pathogenic role [15]. In contrast, parasitic intestinal helminths appear to play a protective role [15]. This conclusion is supported by epidemiological

evidence of an inverse correlation between the prevalence rates of IBD and intestinal helminthiasis, and evidence showing that experimental infection with helminths ameliorates intestinal pathology in animals with chemically induced IBD [16]. Furthermore, it has been shown that patients with Crohn's disease who do not respond to conventional therapy with anti-inflammatory drugs enter clinical remission following experimental therapy with parasitic helminths [17]. The mediators of this therapeutic effect are the immunomodulatory molecules that parasitic helminths secrete in order to deactivate the defense systems of the vertebrate host [8,9]. These molecules break the vicious cycle of tissue injury and inflammation occurring inside active lesions and operate by changing the proliferation and activation profile of regulatory T lymphocytes, dendritic cells and macrophages [8,9]. The double biological function of parasitic helminths as promoters of anti-inflammatory Th2 immunes responses, and down-regulators of pathogenic Th1/Th17 immune responses, has transformed them into a potential new weapon for treating chronic NCD [18].

ARTHROPOD SALIVA AND HYGIENE

An additional source of immunomodulatory molecules to which humans can be exposed on a regular basis is the saliva that hematophagous arthropods deliver at the blood site while feeding [10,11]. The immunomodulatory activity of arthropod saliva is similar to that described for parasitic helminths and involves promotion of Th2-type immune responses and down-regulation of pathogenic Th1/Th17-type responses [10,11,19]. A prominent histopathologic feature of the inflammatory and immune response that vertebrates mount against arthropod saliva, eosinophilia [20], is also characteristic of the response to parasitic helminths [21]. The overall similarity of the tissue response to helminths and arthropods is an example of convergent evolution and indicates that these parasites have been exploiting a fundamental vulnerability of the defense systems of vertebrates in order to thrive in a parasitic relationship. The structural similarity in immunomodulatory glycans of hematophagous sand flies and parasitic trematodes illustrates the convergent nature of this evolutionary process [20]. The functional redundancy that characterizes these parasite-derived molecules can have significant effects on human health because the immunomodulation caused by helminth-derived molecules can be amplified by those present in arthropod saliva, and vice versa. This is of particular significance for residents of poor and neglected areas of the world that are frequently exposed to both groups of parasites, their immune response being modulated by a parasite-derived immunomodulatory cloud that affects the way they respond to self and foreign antigens alike. An additional consequence of this functional redundancy is that the pathological consequences of hygiene-promoting measures are most apparent when both parasitic helminths and hematophagous arthropods are eliminated from human dwellings.

IBD = inflammatory bowel disease

EXPOSURE TO ARTHROPOD SALIVA AS A RISK FACTOR OF DISEASE

Exposure to the immunomodulatory factors present in arthropod saliva plays a dual role in the immunobiology of arthropod-borne diseases: a) it facilitates pathogen multiplication, dispersion and transmission, and b) it protects infected individual from the immunopathological events triggered by arthropod-borne pathogens. The fact that asymptomatic individuals with very high parasitemias can be found in malaria hyper-endemic areas [22] is an indication of the role that this duality plays in the transmission dynamics of arthropod-borne pathogens. Due to the non-specificity of the saliva-induced immunomodulation, chronic exposure to arthropod saliva can modulate the immune response to a wide variety of microbial pathogens, including some that are not transmitted by hematophagous arthropods. This might be of significance for individuals with diseases like leprosy or tuberculosis who benefit from therapeutic interventions designed to reduce exposure to parasite-derived immunomodulators [23]. The combined effect of helminth- and arthropod-derived immunomodulatory molecules, to which residents of endemic areas are exposed constantly, is a critical component of the complex pathological process that compromises their ability to respond adequately to microbial pathogens and vaccines. This parasite-driven immunomodulation can be further amplified by undernourishment, concomitant diseases, and other factors prevalent in endemic areas. While the immune response phenotype conditioned by these factors places individuals at a higher risk of infectious diseases, it may also protect them from unregulated inflammatory and autoimmune responses. The fact that some of the geo-epidemiological characteristics of autoimmune disease [24] can be explained by this environmentally conditioned phenotype is an indicator of the possible role of helminth/arthropod-free habitats in the etiopathogenesis of autoimmune diseases.

Exposure to arthropod saliva is, simultaneously, an environmental risk factor for arthropod-borne diseases and a protective factor for chronic NCDs

EXPOSURE TO ARTHROPOD SALIVA AS A DISEASE-PROTECTION FACTOR

Arthropod saliva is a complex cocktail of anticoagulants, neuropeptides and vasodilatory and immunomodulatory molecules with powerful effects on the defense mechanisms that vertebrates deploy to prevent blood loss and parasitism [25,26]. It is surprising, given the myriad of biological effects mediated by arthropod saliva, that it has not been considered before as

a major environmental variable in epidemiological studies of chronic NCDs. Although the association of hygiene, helminthiasis and chronic NCDs has been extensively documented [2-7], it is not clear whether exposure to arthropod saliva plays a protective role in the pathological process that causes these diseases. Evaluating this possibility is complicated by the fact that residents of endemic areas for arthropod-borne diseases are frequently co-infected with parasitic helminths. Since evidence indicates that parasitic helminths and hematophagous arthropods use similar mechanisms to deactivate the immune system of vertebrates [8-11,20], dissecting out the precise contribution of either one to the postulated disease-protection effect may not be possible. As a result, epidemiological evidence indicating that parasitic infections confer protection from inflammatory and autoimmune diseases [2-7] may be partly attributed to the arthropod salivary immunomodulators to which these individuals are exposed on a daily basis. Accordingly, the low incidence of autoimmune diseases in malaria-endemic areas, which has been attributed to repeated exposure to Plasmodium parasites [27], might be explained instead by repeated exposure to the immunomodulatory molecules present in the saliva of the mosquito vectors that transmit them. This environmental factor may also help explain why the incidence of systemic lupus erythematosus is lower in black people residing in sub-Saharan Africa than in people of African descent residing in the United States and Europe [24]. Finally, repeated exposure to arthropod saliva could prove to be a protective factor for breast cancer, a disease more frequent in African-American women than in their counterparts residing in Africa [28]. This cancer protection effect could be due to repeated exposure to salivary molecules that counter the cancer-promoting properties of chronic inflammation [29] or to protective immunity developed overtime towards two cancer-associated antigens, T and Tn, that are expressed by cancer cells [30] and arthropod glycoproteins [31].

Exposure to helminth-derived and arthropod-derived immunomodulatory molecules offers a promising alternative in the treatment and prevention of chronic NCDs

PARASITIC HELMINTHS AND ARTHROPOD SALIVA AS THERAPEUTIC TOOLS

Whereas treatment with living helminths is an emerging field in medical therapeutics [18], it has yet to be determined whether exposure to arthropod saliva has a similar therapeutic potential. Both of these sources of immunomodulatory molecules down-regulate the pathogenic Th1 and Th17-type immune responses that play a prominent role in the immunopathogenesis of autoimmune diseases [32] and generate the anti-inflammatory phenotype that is required for wound healing [8-11]. The main risk of using living helminths in human therapeutics is the erratic tissue migration that these parasites can display, especially in immunocompromised individuals. This risk has been minimized by the use of parasites, such as the porcine whipworm, that produce a short-term and self-limited colonization of the human intestine [17]. By comparison, exposure to the bite of hematophagous arthropods would be a safer alternative since their interaction with human tissues is relatively brief and they can be reared in laboratories under pathogen-free conditions. The availability of two different mechanisms to deliver therapeutic immunomodulation would be useful in medical practice because their therapeutic range is quite different: parasitic helminths being ideal delivery vehicles of therapeutic immunomodulation to the intestinal tract, while hematophagous arthropods are better vehicles for dermal and systemic delivery of immunomodulators. Therapeutic exposure to the bite of hematophagous arthropods, for example, would be a better alternative for patients with diseases such as multiple sclerosis, psoriasis, asthma or preeclampsia that are associated with pathogenic Th1/Th17-type immune responses [33-36] and who are not eligible for helminth therapy. Finally, a major advantage of hematophagous arthropods as therapeutic tools is that they can be reared easily, at low cost and without the need of refrigeration. These features could prove useful for the implementation of therapeutic programs in developing countries with limited resources that face a growing epidemic of chronic NCDs [38].

ARTHROPOD SALIVA AS A DISEASE-PREVENTION TOOL

In addition to its promising role in medical therapeutics, periodic exposure to the saliva of non-infected arthropods has another potential application of great significance in public health: the prevention of chronic NCDs. Unfortunately, the vector-control programs implemented worldwide to protect against malaria, leishmaniasis, dengue fever and other arthropod-borne diseases do not discriminate between infected and non-infected arthropods. As a result, residents of endemic areas are deprived of any potential benefit from exposure to the saliva of healthy arthropods. In the absence of any practical mechanism to selectively eliminate infected arthropods from human dwellings, a reasonable alternative would be to provide access to arthropod saliva in a clinical setting under conditions that prevent the inadvertent transmission of arthropod-borne pathogens.

While it remains to be determined whether repeated exposure to the bite of non-infected arthropods can modify the immunopathological processes associated with chronic NCDs, several factors need to be determined before it is considered a viable alternative in preventive medicine: a) do the anti-saliva antibodies generated after repeated exposure to saliva interfere with the desired disease-preventive effect, b) how does the allergenicity of some salivary glycoproteins restrict its potential use

in allergic and atopic individuals [11], and c) what is the minimal age at which this disease-prevention approach can be safely initiated to provide the best protection possible. The latter is a critical consideration because, with mounting evidence indicating the prominent role of fetal programming in the etiopathogenesis of chronic NCDs [37], it is theoretically possible that the best disease-preventing effect from exposure to arthropod saliva occurs during fetal development. Experimental models could be used to clarify whether exposure of pregnant animals to the bite of hematophagous arthropods causes changes in the vascular and immune phenotype of the placenta or in the development of fetal organs.

PERSPECTIVES ON HYGIENE, HEALTH AND DISEASE

Preventing contact with parasitic helminths and hematophagous arthropods has been an essential element of health policies designed to control infectious diseases, improve living standards, and increase longevity in human populations worldwide. The potential role that these interventions might have played in the current epidemic of chronic NCDs around the world [38] must be analyzed in the context of the transformation of human habitats following the adoption of westernized lifestyles, modernity and globalization. Increased exposure to a variety of environmental pollutants (heavy metals, toxins, antibiotics, hormones), and unhealthy behaviors (stress, addictions, overeating, sedentarism) are among the many changes that this habitat transformation imposes on its residents. Decreased exposure to helminths and arthropods, on the other hand, plays a pathogenic role because it deprives human tissues of the anti-inflammatory and immunomodulatory molecules that these parasites provide. Thus, the overall contribution of hygiene to the epidemic of chronic NCDs can be attributed to its effect as passive amplifier of tissue damage caused by other bona fide etiopathogenic factors of disease. The relevance of this differentiation is that in order to construct disease-free habitats, it will be necessary to eliminate all etiopathogenic factors of disease and restore the ecological and immunological balance that was compromised by hygiene-promoting interventions.

CONCLUSIONS

The recognition that hygiene has both beneficial and deleterious effects on human health challenges the prevailing paradigm on determinants of health and disease. A new perspective, based on ecological harmony, is already providing valuable information on how environmental perturbations that modify the human microbiome become etiopathogenic factors of disease [39]. The complex microbial ecosystems that are associated with every exposed surface of the human body respond to environmental changes in ways that can prevent or promote disease [39,40]. With this perspective in mind, the removal of parasitic helminths from the intestinal ecosystem, and of hematophagous arthropods from the skin ecosystem, can be seen as pathogenic imbalances of the human microbiome. In this context, therapeutic and preventive measures that use helminths and arthropods are likely to work because they tend to restitute the original balance. Since it is not possible to consider a reintroduction of helminths and arthropods into human communities, and there is no mechanism available to replace their function in microbial ecosystems, health authorities will need to rely on helminth/arthropod-exposure programs as the best way to treat and prevent chronic NCDs. The implementation of these programs will pose an unprecedented challenge to health authorities worldwide because of the degree of coordination required to ensure that every human being on the planet has access to immunomodulatory molecules derived from parasitic helminths and hematophagous arthropods.

Acknowledgments

The author wishes to thank L. Soon, A. Pérez de León and C. Bernal, for useful suggestions and critical reviews of the manuscript
Dedicated to the memory of Dr. Richard G. Titus, a friend, a mentor, and a collaborator of this manuscript

Corresponding author:
Dr. J.S. Mejia
Boulder Diagnostics US LLC, 5401 Western Avenue, Boulder, CO 80301, USA
email: smejia@boulderdiagnostics.com

References

1. Prüss A, Kay D, Fewtrell L, Bartram J. Estimating the burden of disease from water, sanitation, and hygiene at a global level. *Environ Health Perspect* 2002; 110: 537-42.
2. Okada H, Kuhn C, Feillet H, Bach JF. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. *Clin Exp Immunol* 2010; 160: 1-9.
3. Jackson JA, Friberg IM, Little S, Bradley JE. Review series on helminths, immune modulation and the hygiene hypothesis: immunity against helminths and immunological phenomena in modern human populations: coevolutionary legacies? *Immunology* 2009; 126: 18-27.
4. Weinstock JV, Elliott DE. Helminths and the IBD hygiene hypothesis. *Inflamm Bowel Dis* 2009; 15: 128-33.
5. Fleming JO. Helminths and multiple sclerosis: will old friends give us new treatments for MS? *J Neuroimmunol* 2011; 233: 3-5.
6. Smits HH, Everts B, Hartgers FC, Yazdanbakhsh M. Chronic helminth infections protect against allergic diseases by active regulatory processes. *Curr Allergy Asthma Rep* 2010; 10: 3-12.
7. Rook GA. 99th Dahlem Conference on Infection, Inflammation and Chronic Inflammatory Disorders: Darwinian medicine and the 'hygiene' or 'old friends' hypothesis. *Clin Exp Immunol* 2010; 160: 70-9.
8. Harn DA, McDonald J, Atochina O, Da'dara AA. Modulation of host immune responses by helminth glycans. *Immunol Rev* 2009; 230: 247-57.
9. Maizels RM, Yazdanbakhsh M. T-cell regulation in helminth parasite infections: implications for inflammatory diseases. *Chem Immunol Allergy* 2008; 94: 112-23.
10. Titus RG, Bishop JV, Mejia JS. The immunomodulatory factors of arthropod saliva and the potential for these factors to serve as vaccine targets to prevent pathogen transmission. *Parasite Immunol* 2006; 28: 131-41.
11. Fontaine A, Diouf I, Bakkali N, et al. Implication of haematophagous arthropod salivary proteins in host-vector interactions. *Parasit Vectors* 2011; 4: 187.
12. Donaldson DS, Williams NA. Bacterial toxins as immunomodulators. *Adv Exp Med Biol* 2009; 666: 1-18.
13. Sears BF, Rohr JR, Allen JE, Martin LB. The economy of inflammation: when is less more? *Trends Parasitol* 2011; 27: 382-7.
14. Economou M, Pappas G. New global map of Crohn's disease: genetic, environ-

mental, and socioeconomic correlations. *Inflamm Bowel Dis* 2008; 14: 709-20.

15. Lidar M, Langevitz P, Shoenfeld Y. The role of infection in inflammatory bowel disease: initiation, exacerbation and protection. *IMAJ* 2009; 11: 558-63.
16. McKay DM. The therapeutic helminth? *Trends Parasitol* 2009; 25: 109-14.
17. Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV. *Trichuris suis* therapy in Crohn's disease. *Gut* 2005; 54: 87-90.
18. Osada Y, Kanazawa T. Parasitic helminths: new weapons against immunological disorders. *J Biomed Biotechnol* 2010; 2010: 743-58.
19. Skallová A, Iezzi G, Ampenberger F, Kopf M, Kopecky J. Tick saliva inhibits dendritic cell migration, maturation, and function while promoting development of Th2 responses. *J Immunol* 2008; 180: 6186-92.
20. Mejia JS, Toot-Zimmer AL, Schultheiss PC, Beaty BJ, Titus RG. BluePort: a platform to study the eosinophilic response of mice to the bite of a vector of Leishmania parasites, *Lutzomyia longipalpis* sand flies. *PLoS One* 2010; 5: e13546.
21. Makepeace BL, Martin C, Turner JD, Specht S. Granulocytes in helminth infection – who is calling the shots? *Curr Med Chem* 2012; 19: 1567-86.
22. Laishram DD, Sutton PL, Nanda N, et al. The complexities of malaria disease manifestations with a focus on asymptomatic malaria. *Malar J* 2012; 11: 29.
23. Resende Co T, Hirsch CS, Toossi Z, Dietze R, Ribeiro-Rodrigues R. Intestinal helminth co-infection has a negative impact on both anti-*Mycobacterium tuberculosis* immunity and clinical response to tuberculosis therapy. *Clin Exp Immunol* 2007; 147: 45-52.
24. Carmi G, Amital H. The geoepidemiology of autoimmunity: capsules from the 7th international congress on autoimmunity, Ljubljana, Slovenia, May 2010. *IMAJ* 2011; 13: 121-7.
25. Ribeiro JM, Mans BJ, Arcà B. An insight into the sialome of blood-feeding Nematocera. *Insect Biochem Mol Biol* 2010; 40: 767-84.
26. Andersen JF. Structure and mechanism in salivary proteins from blood-feeding arthropods. *Toxicon* 2010; 56: 1120-9.
27. Butcher G. Autoimmunity and malaria. *Trends Parasitol* 2008; 24: 291-2.
28. Fregene A, Newman LA. Breast cancer in sub-Saharan Africa: how does it relate to breast cancer in African-American women? *Cancer* 2005; 103: 1540-50.
29. Multhoff G, Molls M, Radons J. Chronic inflammation in cancer development. *Front Immunol* 2011; 2: 98.
30. Brockhausen I. Mucin-type O-glycans in human colon and breast cancer: glycodynamics and functions. *EMBO Rep* 2006; 7: 599-604.
31. ten Hagen KG, Zhang L, Tian E, Zhang Y. Glycobiology on the fly: developmental and mechanistic insights from Drosophila. *Glycobiology* 2009; 19: 102-11.
32. Dardalhon V, Korn T, Kuchroo VK, Anderson AC. Role of Th1 and Th17 cells in organ-specific autoimmunity. *J Autoimmun* 2008; 31: 252-6.
33. Fletcher JM, Lalor SJ, Sweeney CM, Tubridy N, Mills KH. T cells in multiple sclerosis and experimental autoimmune encephalomyelitis. *Clin Exp Immunol* 2010; 162: 1-11.
34. Jacob SE, Nassiri M, Kerdel FA, Vincek V. Simultaneous measurement of multiple Th1 and Th2 serum cytokines in psoriasis and correlation with disease severity. *Mediat Inflamm* 2003; 12: 309-13.
35. Langier S, Sade K, Kivity S. Regulatory T cells in allergic asthma. *IMAJ* 2012; 14: 180-3.
36. Saito S, Nakashima A, Shima T, Ito M. Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. *Am J Reprod Immunol* 2010; 63: 601-10.
37. Hanson M, Godfrey KM, Lillycrop KA, Burdge GC, Gluckman PD. Developmental plasticity and developmental origins of non-communicable disease: theoretical considerations and epigenetic mechanisms. *Prog Biophys Mol Biol* 2011; 106: 272-80.
38. Boutayeb A. The double burden of communicable and non-communicable diseases in developing countries. *Trans R Soc Trop Med Hyg* 2006; 100: 191-9.
39. Cho I, Blaser MJ. The human microbiome: at the interface of health and disease. *Nat Rev Genet* 2012; 13: 260-70.
40. Hemarajata P, Versalovic J. Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. *Ther Adv Gastroenterol* 2013; 6: 39-51.

Capsule

Rare coding variants in the phospholipase D3 gene confer risk for Alzheimer's disease

Genome-wide association studies (GWAS) have identified several risk variants for late-onset Alzheimer's disease (LOAD). These common variants have replicable but small effects on LOAD risk and generally do not have obvious functional effects. Low frequency coding variants, not detected by GWAS, are predicted to include functional variants with larger effects on risk. To identify low frequency coding variants with large effects on LOAD risk, Cruchaga and fellow authors carried out whole-exome sequencing (WES) in 14 large LOAD families and follow-up analyses of the candidate variants in several large LOAD case-control data sets. A rare variant in *PLD3* (phospholipase D3; Val232Met) segregated with disease status in two independent families and doubled risk for Alzheimer's disease in seven independent case-control series with a total of more than 11,000 cases and controls of European descent. Gene-based burden analyses in 4387 cases and controls of European descent and 302 African American cases and controls, with complete sequence data for *PLD3*, reveal that several variants in this gene increase risk for Alzheimer's disease in both populations. *PLD3* is highly expressed in brain regions that are vulnerable to Alzheimer's disease pathology, including hippocampus and cortex, and is expressed at significantly lower levels in neurons from Alzheimer's disease brains compared to control brains. Overexpression of PLD3 leads to a significant decrease in intracellular amyloid-β precursor protein (APP) and extracellular Aβ42 and Aβ40 (the 42- and 40-residue isoforms of the amyloid-β peptide), and knockdown of PLD3 leads to a significant increase in extracellular Aβ42 and Aβ40. Together, our genetic and functional data indicate that carriers of *PLD3* coding variants have a twofold increased risk for LOAD and that PLD3 influences APP processing. This study provides an example of how densely affected families may help to identify rare variants with large effects on risk for disease or other complex traits.

Nature 2014; 505: 550

Eitan Israeli

"The luck of having talent is not enough; one must also have a talent for luck"

Hector Berlioz (1803-1869), French composer